*Biomarkers*, 2009; 14(2): 111–117

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RESEARCH ARTICLE

# Polymorphisms in genes encoding drugs and xenobiotic metabolizing enzymes in a Brazilian population

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**Abstract**

Polymorphic variations of several genes associated with drugs and xenobiotic metabolism have been linked to the factors that predispose to the carcinogenesis process. As considerable interindividual and interethnic variation in metabolizing enzyme activity has been associated with polymorphic alleles, we evaluated the frequency of the polymorphisms of *CYP2D6*, *EPHX1* and *NQO1* genes in 361 Brazilian individuals separated by ethnicity (European and African ancestry), using the polymerase chain reaction-restriction fragment length (PCR-RFLP) method. The allele frequencies of the variants *3 and *4 for the gene *CYP2D6* were 0.04 and 0.14 for white subjects and 0.03 and 0.10 for black individuals, respectively. For the both variants of the gene *EPHX1*, we found higher allele frequencies among white individuals compared with mulatto subjects (0.62 vs 0.54 and 0.18 vs 0.14, respectively); however, these differences were not statistically significant ($p = 0.39$ and 0.56, respectively). For the *NQO1* gene we observed a higher frequency of the homozygous genotype among black individuals (7.9%) compared with white subjects (6.3%) ($p = 0.003$). The genotype frequencies were within the Hardy–Weinberg equilibrium. We concluded that the allele frequencies of *CYP2D6*, *EPHX1* and *NQO1* gene polymorphisms in this Brazilian population showed ethnic variability when compared with those observed in other populations.

**Keywords:** *Genetic polymorphisms; metabolizing enzymes; Brazilian population*

## Introduction

CYP super family gene products convert procarcinogens into DNA-reactive electrophilic forms by phase I reactions (Ingelman-Sundberg 2001, Raucy & Allen 2001). CYP enzyme variants are involved in the metabolism of many different potential carcinogens and drugs. The *CYP2D6* gene encodes for debrisoquine hydroxylase, which mediates the metabolism of drugs and carcinogenic substances (Bradford 2002). The most common polymorphisms are the variants *3 and *4. *CYP2D6**3 represents a one base pair deletion in position 2673 in exon 5 (del A2673). The variant *4 is characterized by a single nucleotide polymorphism at position G1934A, which causes a disruption of the splice site at the junction of the third intron and fourth exon (Gough et al. 1990). The *CYP2D6**4 polymorphism usually leads to the absence or a decrease in the amount and activity of the CYP2D6 protein. The total loss of its function leads to a poor metabolizer (PM) phenotype (polymorphic homozygous) and the duplication or amplification of an active gene leads to an ultrarapid metabolizer (UM). The extensive metabolizer (EM) and the intermediate metabolizer (IM) are characterized by the wild-type homozygous genotype and by the heterozygous genotype, respectively (Lemos et al. 1999). The *CYP2D6**3 and *4 polymorphisms has been reported to be a major cause of the *CYP2D6* PM

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*(Received 16 September 2008; revised 20 January 2009; accepted 21 January 2009)*

ISSN 1354-750X print/ISSN 1366-5804 online © 2009 Informa UK Ltd
DOI: 10.1080/13547500902767294



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phenotype, which represents 5–10% of white individuals (Steijins & Van Der Weide 1998). Molecular epidemiological studies have recently shown associations between these enzyme variants and altered risk of a variety of cancers, including colorectal, ovarian, bladder and breast cancer (Sachse et al. 1997, Taningher et al. 1999).

Quinone-oxoreductase, NQO1, is a two-electron reducing enzyme that is important for the detoxification of some chemotherapy metabolites and is an activator of bioreductive antitumour agents, such as mitomycin-C (Traver et al. 1997). The *NQO1**2 polymorphism occurs in the *NQO1* gene and consists of a C609T substitution, which results in P187S acid replacement and causes a complete loss of enzyme activity (Krajinovic et al. 2002). Microsomal epoxide hydrolase (EPHX1) is generally considered to be a protective enzyme involved in the defence against oxidative damage (Vodicka et al. 2001). Two common polymorphic sites in the *EPHX1* gene that influence the enzyme activity, can be detected. An exon 3 thymine-to-cytosine mutation changes Tyr residue 113 to His (variant *2), thus reducing the enzyme activity by about 50%. The second mutation, an adenine-to-guanine transition in exon 4 of the gene, changes His residue 139 to Arg (variant *3) and results in the production of EPHX1 with the activity increased by about 25% (Hassett et al. 1997). The combination of these polymorphisms leads to the formation of several functional phenotypes of *EPHX1*. Microsomal epoxide hydrolase metabolizes a broad array of epoxide substrates, including polycyclic aromatic hydrocarbons (PAH), which are carcinogens found in cigarette smoke (Fretland & Omiecinski 2000). Several studies conducted in different populations have suggested that the *EPHX1* genotype may influence individual susceptibility to a variety of diseases (Lancaster et al. 1996; Park et al. 2003, Wang et al. 2003).

Hence, determining the role of genetic polymorphisms as a cancer risk factor requires studies aimed at the integrated analysis of many genes involved in the carcinogenesis process, which despite several investigations, still presents questions that remains poorly understood. In this study, we estimated the frequencies of polymorphisms in the genes encoding metabolic enzymes involved in drugs and xenobiotic activation and detoxification processes (*CYP2D6*, *EPHX1* and *NQO1*) in healthy individuals in order to determine their prevalence in a mixed Brazilian population.

## Materials and methods

### *Study subjects*

The study population comprised a total of 364 unrelated individuals (168 males and 196 females; mean age 89.54 months; range 4–240 months). This group was composed of patients recruited at the University Hospital of the Faculty of Medicine of Ribeirão Preto, University of São Paulo (HC-FMRP-USP), Brazil, from 2003 to 2005. The subjects reflected the general population seeking medical attention at University Hospital. To avoid selecting individuals with a specific pathology, subjects were enrolled randomly at the central laboratory of the Hospital. The subjects' medical conditions included common infections, anaemia, obesity, diabetes and hormone disorders; those with a previous history or diagnosis of cancer or genetic disease were excluded from the analysis. Epidemiological data for the study population were obtained by a standard interviewer-administered questionnaire that gathered data on social habits, health problems, family history of cancer and ancestry. Based on phenotypic characteristics and questions about the origins of their parents, 223 individuals were white (European descendants) and 141 were non-white (African descendants). The group of non-whites included 102 mulatto and 39 black subjects. The human subject's protocol was approved by the Ethics Committee (Proc. No. 4273/2003) of the HC-FMRP-USP and written informed consent was obtained from all subjects or their parents.

### *Genotype analysis*

#### DNA extraction

Blood was collected into EDTA-containing tubes and genomic DNA was extracted by the conventional phenol–chloroform method and by the TRizol® protocol. Isolated DNA was re-suspended in Tris–EDTA buffer, pH 8.0, and stored at –20°C until use. The number of individuals genotyped varied according to the genes analyzed, depending on DNA availability (see Table 2).

Allelic variants of the *CYP2D6*, *EPHX1* and *NQO1* genes were differentiated from the wild-type alleles by the polymerase chain reaction-restriction fragment length (PCR-RFLP) method based on previously described methods (Table 1) (Gough et al. 1990, Smith & Harrison 1997, Smith et al. 1992, Wiemels et al. 1999). PCR amplification was performed in a total reaction volume of 25 µl containing 200 ng of genomic DNA, 100 ng each of primer, 200 µM dNTPs, 2.5 µl of 10x PCR buffer (1x: 200 mM Tris–HCl, 500 mM KCl, pH 8.4), 1.5 mM $MgCl_2$, 5% dimethylsulfoxide (DMSO), and 1.25 U Taq DNA polymerase (Invitrogen, Carlsbad, CA, USA). The PCR-amplified products of *CYP2D6*, *EPHX1* and *NQO1* were digested with the restriction enzymes according to the manufacturers' instructions (Fermentas, St. Leon-Rot, Germany or New England Biolabs, Frankfurt, Germany) and analyzed after agarose gel electrophoresis.

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**Table 1.** Primers, PCR products and the restriction enzymes used in the characterization of polymorphic sites of the studied genes.

| Gene | Polymorphism | Variant | Primers | PCR product (bp) | Restriction enzyme | Reference |
|---|---|---|---|---|---|---|
| *CYP2D6* | del A2637 | *3 | (F) 5′- GATGAGCTGCTAACTGAGCCC-3′<br>(R) 5′- CCGAGAGCATACTCGGGAC-3′ | 270 | *Msp*I | Gough et al. 1990; Smith et al. 1992 |
| | G1934A | *4 | (F) 5′-GCCTTCGCCAACCACTCCG-3′<br>(R) 5′-AAATCCTGCTCTTCCGAGGC-3′ | 334 | *Bst*N1 | |
| *EPHX1* | T339C | *3 | (F) 5′-GATCGATAAGTTCCGTTTCACC-3′ | 162 | *EcoRV* | Smith & Harrison 1997 |
| | A418G | *4 | (R) 5′-ATCCTTAGTCTTGAAGTGAGGAT-3′<br>(F) 5′-ACATCCACTTCATCCACGT-3′ (R) 5′-ATGCCTCTGAGAAGCCAT-3′ | 210 | *Rsa*I | |
| *NQO1* | C609T | *2 | (F) 5′-CCTCTCTGTGCTTTCTGTATCC-3′<br>(R) 5′-GATGGACTTGCCCAAGTGATG-3′ | 298 | *Hin*F1 | Wiemels et al. 1999 |

**Table 2.** Genotypes and allele frequencies for *CYP2D3*, *EPHX1*, *MPO* and *NQO1* polymorphisms in healthy Brazilians of different ethnic ancestry.

| Reference ID | Polymorphism | Black | Mulatto | White | Total |
|---|---|---|---|---|---|
| rs5030656 | *CYP2D6*3* | | | | |
| | *1/*1 | 31/33 (93.9) | 86/89 (96.6) | 167/178 (93.8) | 284/300 (94.7) |
| | *1/*3 | 2/33 (6.1) | 3/89 (3.4) | 11/178 (6.2) | 16/300 (5.3) |
| | *3/*3 | 0/33 (0.0) | 0/89 (0.0) | 0/178 (0.0) | 0/300 (0.0) |
| | *1/*3 + *3/*3 | 2/33 (6.1) | 3/89 (3.4) | 11/178 (0.0) | 16/300 (5.3) |
| | Alleles | | | | |
| | *1 | 0.97 | 0.98 | 0.96 | 0.97 |
| | *3 | 0.03 | 0.02 | 0.04 | 0.03 |
| rs5030866 | *CYP2D6*4* | | | | |
| | *1/*1 | 27/33 (81.8) | 73/89 (82.0) | 133/178 (74.7) | 233/300 (77.6) |
| | *1/*4 | 6/33 (18.2) | 15/89 (16.9) | 40/178 (22.5) | 61/300 (20.3) |
| | *4/*4 | 0/33 (0.0) | 1/89 (1.1) | 5/178 (2.8) | 6/300 (2.0) |
| | *1/*4+ *4/*4 | 6/33 (18.2) | 16/89 (17.9) | 45/178 (25.3) | 67/300 (22.3) |
| | Alleles | | | | |
| | *1 | 0.90 | 0.90 | 0.86 | 0.88 |
| | *4 | 0.10 | 0.10 | 0.14 | 0.12 |
| rs1051740 | *EPHX1* (E3) | | | | |
| | *1/*1 | 6/33 (18.2) | 19/89 (21.4) | 26/178 (14.6) | 51/300 (17.0) |
| | *1/*2 | 27/33 (81.8) | 69/89 (77.5) | 147/178 (82.6) | 243/300 (81.0) |
| | *2/*2 | 0/33 (0.0) | 1/89 (1.1) | 5/178 (2.8) | 6/300 (2.0) |
| | *1/*2 + *2/*2 | 27/33 (81.8) | 70/89 (78.6) | 152/178 (85.4) | 249/300 (83.0) |
| | Alleles | | | | |
| | *1 | 0.42 | 0.46 | 0.38 | 0.58 |
| | *2 | 0.58 | 0.54 | 0.62 | 0.42 |
| rs2234922 | *EPHX1* (E4) | | | | |
| | *1/*1 | 22/33 (66.7) | 66/89 (74.1) | 122/178 (68.54) | 210/300 (70.0) |
| | *1/*3 | 8/33 (24.2) | 20/89 (22.5) | 49/178 (27.53) | 77/300 (25.7) |
| | *3/*3 | 3/33 (9.1) | 3/89 (3.4) | 7/178 (3.93) | 13/300 (4.3) |
| | *1/*3 + *3/*3 | 11/33 (33.3) | 23/89 (25.8) | 56/178 (31.4) | 90/300 (30.0) |
| | Alleles | | | | |
| | *1 | 0.81 | 0.86 | 0.82 | 0.83 |
| | *3 | 0.19 | 0.14 | 0.18 | 0.17 |
| rs1800566 | *NQO1* | | | | |
| | *1/*1 | 22/38 (57.9) | 47/101 (46.5) | 13/223 (50.7) | 82/361 (22.7) |
| | *1/*2 | 13/38 (34.2) | 47/101 (46.5) | 96/223 (43.0) | 156/361 (43.2) |
| | *2/*2 | 3/38 (7.9)[a] | 7/101 (6.9) | 14/223 (6.3)[a] | 24/361 (6.6) |
| | *1/*2 + *2/*2 | 16/38 (42.1)[b] | 54/101 (53.5) | 110/223 (49.3)[b] | 180/361 (49.8) |
| | Alleles | | | | |
| | *1 | 0.76 | 0.68 | 0.71 | 0.72 |
| | *2 | 0.24 | 0.32 | 0.29 | 0.28 |

All values are number (%) of individuals. *1/*1, homozygous for the wild-type allele; *1/*2, *1/*3, *1/*4, heterozygous; *2/*2, *3/*3, *4/*4, homozygous for the mutant allele.

[a] The homozygous polymorphic genotype frequency was higher in black than in white subjects ($p = 0.003$).

[b]The combination of heterozygous genotype and homozygous polymorphic genotype was significantly higher in white than in black individuals ($p = 0.0001$).

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### *Statistical analysis*

Statistical analyses were done using the GraphPad InStat software (GraphPad Software Inc., San Diego, CA, USA). The Hardy–Weinberg equilibrium was tested by the $\chi^2$ test to compare the observed genotype frequencies within groups stratified by ethnicity. The statistical significance of the differences between groups was calculated by the $\chi^2$ test or Fisher's exact test (two sided). Differences were considered to be significant at $p \leq 0.05$.

## Results

The present study evaluated the genotype distribution for the drug metabolizing enzymes CYP2D6, EPHX1 and NQO1 in 364 healthy individuals and the distributions of the genotypes and the allele frequencies in this Brazilian population of mixed ethnic ancestry are reported in Table 2. We found that the allele frequency of the gene *CYP2D6* for the polymorphism *3 was lower in black (0.03) and mulatto subjects (0.02) compared with white individuals (0.04), but the differences were not statistically significant. Analyzing the *CYP2D6**3 we found that none of the individuals were homozygous for the rare *3 allele. In the Brazilian population the most prevalent variant allele was the functional *4 allele (0.12) (Table 2). The distributions of the alleles in the studied individuals were analyzed and all of them were found to be in Hardy–Weinberg equilibrium ($\chi^2 = 1.503$; $p > 0.05$). Comparing the *3 and *4, polymorphisms in the Brazilian population with those in other major populations, significant differences were seen in the observed frequencies and were also found between the frequencies of the PM among white individuals in the Brazilian population (2.8%) compared with the worldwide white population (5–10%). The most common *CYP2D6* variant genotypes were *1/*4, representing 20.3% of the Brazilian population (Table 2). For the *EPHX1* gene we analyzed two variant forms: *2 and *3. We observed higher frequencies of the heterozygous genotype *1/*2 among white individuals (82.6%) compared with the mulatto group (77.5%); however this difference did not represent a statistical significance ($p = 0.22$) (Table 2). The frequency of the polymorphic allele *2 was lower among the mulatto group compared with the white group (0.54 vs 0.62). When analyzing the polymorphic variant *3 we observed similar frequencies of the wild-type genotype among the ethnic groups. The allele frequency of variant *3 did not differ significantly among black, mulatto and white groups (0.19, 0.14 and 0.18, respectively). The distributions for the combined population was in Hardy–Weinberg equilibrium ($\chi^2 = 3.182$ for *2 and $\chi^2 = 2.901$ for *3; $p > 0.05$); however the distribution of the allele *2 for the black group was not ($\chi^2 = 9.69$; $p < 0.05$). We also did a combined genotype analysis in order to examine the variant forms of EPHX1 as a surrogate of enzyme activity. The population was divided into four different enzyme-activity groups (very low, low, intermediate and high) according to the combined genotype of polymorphisms in variants *2 and *3. The very low group consisted of individuals with His/His in both *2 and *3. The low-activity genotype group consisted of His/His in *2 and either His/Arg or Arg/Arg in *3 or Tyr/His in *2 and His/His in *3. The intermediate-activity genotype group consisted of Tyr/His in *2 and His/Arg in *3 or Tyr/Tyr in *2 and His/His in *3, and the high-activity genotype group consisted of individuals with Tyr/Tyr in *3 and either His/Arg or Arg/Arg in *3 or Tyr/His in *2 and Arg/Arg in *3. However no overall differences among very low, low, intermediate or high genotypes and the ethnic groups were found. We also considered the combined genotypes regarding the genes *EPHX1* and *CYP2D6*. In this analysis, we have taken the presence of either homozygous or heterozygous *CYP2D6* and *EPHX1* variants as a single variable. The subjects with at least one variant allele were grouped together. Among the entire possible combinations only two genotypes were observed in the total population. The frequencies of the *CYP2D6* PM phenotype combined with the very low and with the low phenotypes for the gene *EPHX1,* among the total Brazilian population, were 9.3% and 6.3%, respectively. However, when the analysis of the combined genotype was stratified regarding the different ethnic groups, we did not observe any significant difference among the frequencies. The *NQO1* allele frequency was lower in black individuals (0.24) compared with white (0.29) and mulatto subjects (0.32), but this difference was not statistically significant ($p = 0.52$ and $p = 0.27$, respectively). The gene frequency of the polymorphic homozygous genotype in *NQO1*C609T was significantly higher in the black individuals compared with the white subjects (7.9% vs 6.3%; $p = 0.003$). However, considering the T allele-containing genotypes (genotype *1/*2 and *2/*2), the frequency was significantly reduced among black individuals (42.1%) compared to white subjects (49.3%) ($p = 0.0001$). This distribution was in Hardy–Weinberg equilibrium (1.972; $p > 0.05$).

## Discussion

Polymorphisms in genes coding for drug and xenobiotic metabolizing enzymes are largely responsible for interindividual differences in the metabolism process. Biotransformation of xenobiotics by phase I and phase II enzymes is an important process in initiating carcinogenesis and therefore the individual susceptibility to cancer may be influenced by these polymorphisms (Garte 1998). The inhabitants of Brazil have highly heterogeneous ethnic origins, consisting of indigenous

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Amerindian populations and immigrants from Europe, Africa and Asia (Arruda et al. 1998, Alves-Silva et al. 2000, Carvalho-Silva et al. 2001). This characteristic makes the Brazilian population different from other less mixed populations, and Brazilians can have unique frequencies of genetic polymorphisms in metabolizing enzyme genes. As it has been reported that these polymorphisms are dependent upon ethnicity, in this study we determined the genotype and allele frequencies of *CYP2D6*, *EPHX1* and *NQO1* polymorphisms in a group of 361 healthy individuals from São Paulo State. Although the subjects presented medical conditions, we avoided selecting those subjects with specific pathologies as genetic disorders and diagnosis or previous history of cancer in order to minimize a bias in the study.

In our study population, the allele frequency of the *CYP2D6**3 polymorphism was 0.04 among individuals of European descendants and 0.03 among black individuals and 0.02 among the mulatto group. Although these differences were not statistically significant, there was a trend to a lower frequency of this allele among non-white individuals. These results are in agreement with the allele frequencies reported by Leathart et al. (1998) among African-American populations and also with the allele frequency described for the Turkish population (both presented allele frequencies of 0.02) (Aydin et al. 2005). Similar results were described in studies conducted in the German/European population and in the Faroese population which found an allele frequency for the *CYP2D6**3 of 0.02 and 0.002, respectively (Sachse et al. 1997, Halling et al. 2005). The *CYP2D6**4 allele frequency was higher among individuals of European descendant (0.14) than among the individuals of African descendant, (0.10); however, this difference was not statistically significant. The same frequency (0.14) was described in the Turkish population (Aydin et al. 2005). These results differ from others reported by the literature which described frequencies of 0.08, 0.21 and 0.18 for African-American, German/Europe and USA populations, respectively (Meyer & Zanger 1997, Leathart et al. 1998). The reason for the contradictions between our results and those described by others is not clear; but possible explanations may include ethnic and geographical differences. Furthermore, when we grouped the population by ethnicity we observed a small number of black subjects, possibly because of the mixture of the African descendants with Caucasian individuals and consequently the increased number of mulatto individuals. The analysis of *CYP2D6**4 polymorphism in our Brazilian population revealed that genotype distributions were within Hardy–Weinberg equilibrium when the two ethnic subgroups (white and black) were considered separately, or grouped.

Analyzing the study population by the phenotype's frequency, we only considered the PM phenotype regarding the variant *4. As we did not observe any homozygous individual for the variant *3, we were not able to make a combined analysis between both variants. We observed that the PM frequency among European descendants (2.8%) was significant lower than the frequencies reported in other Caucasian populations, which represents 5–10% of the European population (Taningher et al. 1999). This deviation from PM frequencies between the Brazilian population and the other worldwide populations may be explained by the racial admixture in our population. The potential bias from population stratification has raised concerns in population studies involving mixed ethnicities. For example, the Brazilian population is an ethnically mixed group consisting of individuals of native indigenous Amerindian populations and immigrants from Europe, Africa and Asia. Thus, future studies with a large sample size and/or a homogeneous ethnic background can minimize or avoid selection biases and prevent confounding from ethnicity for *CYP2D6* genotypes.

The data on the *EPHX1* gene polymorphism revealed a higher allele frequency for the *2 variant among individuals of European descent compared with those of African descent (0.62 and 0.58, respectively). Our results differ from others reported in the literature which describe frequencies for the variant *2 of 0.30 in Caucasians of Dutch origin (van der Logt et al. 2006), 0.31 in a non-Hispanic white population (Huang et al. 2005) and of 0.37 in white Americans (Tranah et al. 2004). For the variant *3, the frequencies for the black group and the white group were similar (0.19 and 0.18, respectively). Concordant results were described in other studies (van der Logt et al. 2005, Tranah et al. 2004, Yoshimura et al. 2003) which described allele frequencies of 0.20 for Caucasians (Dutch and Americans) and 0.18 for a Japanese population. Considering the total Brazilian population, we observed allele frequencies of 0.42 and 0.17 for the variants *2 and *3, respectively. Similar results were also found in Japanese populations which presented frequencies of 0.40 and 0.18 for the variants *2 and *3, respectively (Yoshimura et al. 2003) and in a non-Hispanic white population, which presents an allele frequency of variant *3 of 0.19 (Huang et al. 2005). We also performed an analysis associating both variants (*2 and *3 alleles) to determine the enzyme activity phenotype and the combined genotypes resulted in four classes of enzyme activity: very low, low intermediate and rapid (Kelsey et al. 1997). We observed a higher frequency of low phenotype among the total population (57.7%) compared with the others phenotypes; however we did not observe any difference regarding the ethnic groups. These results differ from those reported by Židzik et al. (2008) which described a frequency of 27.5% of the low phenotype among the Slovak population, which might be due to the geographical isolation, giving rise to

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a genetically homogenous population. All the frequencies were in Hardy–Weinberg equilibrium except for the black group; this deviation can be explained by the small number of blacks individuals analyzed.

The analysis of combined genotypes of the genes *CYP2D6* and *EPHX1* was done in order to assess the frequency of these combined genotypes on the population, as the combination of these polymorphisms could play a role on the risk of development of various diseases (d'Errico et al. 1996). Evaluation of possible interactions between the *CYP2D6* variants and *EPHX1* genotypes revealed an elevated frequency (9.3%) of the PM (*CYP2D6)* variant combined with the very low enzyme-activity (*EPHX1)* genotype among the total Brazilian population. However, it was not possible to address the combined genotype frequencies of each ethnic group due to the reduced number of samples in each group after the stratification of the data. Despite the impossibility of assessing the frequency of the combined genotypes in the different ethnic groups, these data could be very important in future approaches towards developmental risks of certain diseases in the Brazilian population.

Regarding the polymorphism of the gene *NQO1**2, the obtained data revealed genotype frequencies of 22.7%, 43.2%, and 6.6% for wild-type homozygous, heterozygous and polymorphic homozygous, respectively, in the general population. When we grouped the Brazilian population according to ethnicity, we observed that the genotype frequency for the homozygous polymorphic was higher among those of African descent (7.9%) compared with those of European descent (6.3%) and the same results were found by the wild-type genotype which were more frequent among African descendants (59.7%) compared with the European descendants (50.7%). Other authors (Kiffmeyer et al. 2004, Mitrou et al. 2007) reported a higher frequency of the wild-type genotype among white individuals (67.5% and 69.0%, respectively). The allele frequencies for the variant *2 among the groups were 0.24 for the black subjects and 0.29 for the white subjects. These results differ from other studies that analyzed the allele frequency of the variant *2 in Asian populations such as Chinese (Yin et al. 2001), Japanese (Hori et al. 2003), Korean (Kelsey et al. 1997) and Hmong (Kiffmeyer et al. 2004) populations and reported significantly higher frequencies (0.45, 0.41, 0.43 and 0.61, respectively) compared with American Caucasian populations (0.18) and with a Brazilian Caucasian population (0.29). Our data suggest that the polymorphic *2 allele is more frequent in Brazilians than in Caucasians from North America and Europe (allele frequency of 0.20), but significantly less frequent than in Asians. These frequency variations among the Brazilian populations and the others worldwide could be explained again by the fact that our population is an ethnically mixed group.

In conclusion, the results of the present study indicate that the genotype and allele frequencies for the polymorphisms *CYP2D6*, *EPHX1* and *NQO1* differ hugely among the worldwide population. Whether the differences between ethnic groups may contribute to a predisposition to environmental exposure-related carcinogenesis (chemical, biological and physical agents) in certain populations of Brazil still remains to be determined, which make the studies of polymorphic metabolizing genes a topic that deserves special attention. Variations in cancer risk might be expected as a result of racial and ethnic differences, which may reflect differences in environmental exposure or differences in susceptibility and biological response (Baquet et al. 1991, Trapido et al. 1994, Zahm & Fraumeni 1995). Thus, the influence of ethnicity, geography and other demographic variables should be considered to evaluate fully the variations in cancer risk among particular populations, and to determine the effects of cancer prevention and treatment strategies. Our results indicate that further studies on polymorphisms of metabolizing enzymes genes may yield valuable information on disease susceptibility and treatment outcome in different populations.

## Acknowledgements

This research was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), grant 150163/2004-5, and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), grant 03/02527-4 (Brazil).

***Declaration of interest*:** All authors declared that they have no potential conflicts of interest.

## References

Alves-Silva J, da Silva Santos M, Guimaraes PE, Ferreira AC, Bandelt HJ, Pena SD, Prado VF. (2000). The ancestry of Brazilian mtDNA lineages. *Am J Hum Genet* 67:444–461.

Arruda VR, Grignolli CE, Goncalves MS, Soares MC, Menezes R, Saad ST, Costa FF. (1998). Prevalence of homozygosity for the deleted alleles of glutathione S-transferase l (GSTM1) and u (GSTT1) among distinct ethnic groups from Brazil: relevance to environmental carcinogenesis? *Clin Genet* 54:210–214.

Aydin M, Hatirnaz O, Erensoy N, Ozbek U. (2005). CYP2D6 and CYP1A1 mutations in the Turkish population. *Cell Biochem Funct* 2005;23:133–135.

Baquet CR, Horm JW, Gibbs T, Greenwald P. (1991). Socioeconomic factors and cancer incidence among blacks and whites. *J Natl Cancer Inst* 83:551–557.

Bradford LA. (2002). CYP2D6 allele frequency in European Caucasians, Asians, African and their descendants. *Pharmacogenetics* 3:229–243.

Carvalho-Silva DR, Santos FR, Rocha J, Pena SD. (2001). The phylogeography of Brazilian Y-chromosome lineages. *Am J Hum Genet* 68:281–286.

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d'Errico A, Taioli E, Chen X et al. (1996). Genetic metabolic polymorphisms and the risk of cancer. A review of the literature. *Biomarkers* 1:149–173.

Fretland AJ, Omiecinski CJ. (2000). Epoxide hydrolases: biochemistry and molecular biology. *Chem Biol Interact* 129:41–59.

Garte S. (1998). The role of ethnicity in cancer susceptibility gene polymorphisms: the example of CYP1A1. *Carcinogenesis* 19:1329–1332.

Gough AC, Miles JS, Spurr NK, Moss JE, Gaedigk A, Eichelbaum M, Wolf CR. (1990). Identification of the primary gene defect at the cytochrome P450 CYP2D locus. *Nature* 347:773–776.

Halling J, Petersen MS, Damkier P, Nielsen F, Grandjean P, Weihe P, Lundgren S, Lundblad MS, Brøsen K. (2005). Polymorphism of CYP2D6, CYP2C19, CYP2C9 and CYP2C8 in the Faroese population. *Eur J Clin Pharmacol* 61:491–497.

Hassett C, Lin J, Carty CL, Laurenzana EM, Omiecinski CJ. (1997). Human hepatic microsomal epoxide hydrolase: comparative analysis of polymorphic expression. *Arch Biochem Biophys* 15:275–283.

Hori H, Ohmori O, Matsumoto C, Shinkai T, Nakamura J. (2003). NAD(P)H: quinone oxidoreductase (NQO1) polymorphism and schizophrenia. *Psychiatry Res* 118:235–239.

Huang WY, Chatterjee N, Chanock S, Dean M, Yeager M, Schoen RE, Hou LF, Berndt SI, Yadavalli S, Johnson CC, Hayes RB. (2005). Microsomal epoxide hydrolase polymorphisms and risk for advanced colorectal adenoma. *Cancer Epidemiol Biomarkers Prev* 14:152–157.

Ingelman-Sundberg M. (2001). Genetic susceptibility to adverse effects of drugs and environmental toxicants. The role of the CYP family of enzymes. *Mutat Res* 482:11–19.

Kelsey KT, Ross D, Traver RD et al. (1997). Ethnic variation in the prevalence of a common NAD(P)H quinone oxidoreductase polymorphism and its implications for anti-cancer chemotherapy. *Br J Cancer* 76:852–854.

Kiffmeyer WR, Langer E, Davies SM, Envall J, Robison LL, Ross JA. (2004). Genetics polymorphisms in the Hmong population. *Cancer* 100:411–417.

Krajinovic M, Sinnett H, Richer C, Labuda D, Sinnett D. (2002). Role of NQO1, MPO and CYP2E1 genetic polymorphisms in the susceptibility to childhood acute lymphoblastic leukemia. *Int J Cancer* 97:230–236.

Lancaster JM, Brownlee HA, Bell DA, Futreal PA, Marks JR, Berchuck A, Wiseman RW, Taylor JA. (1996). Microsomal epoxide hydrolase polymorphism as a risk factor for ovarian cancer. *Mol Carcinog* 17:160–162.

Leathart JBS, London SJ, Steward A, Adams JD, Idle JR, Daly AK. (1998). CYP2D6 phenotype-genotype relationships in African-Americans and Caucasians in Los Angeles. *Pharmacogenetics* 8:529–514.

Lemos MC, Cabrita FJ, Silva HA, Vivan M, Placido F, Regateiro FJ. (1999). Genetic polymorphism of CYP2D6, GSTM1 and NAT2 and susceptibility to haematological neoplasias. *Carcinogenesis* 20:1225–1229.

Meyer UA, Zanger UM. (1997). Molecular mechanism of genetic polymorphism of drug metabolism. *Ann Rev Pharmacol Toxicol* 37:269–296.

Mitrou PN, Watson MA, Loktionov AS, Cardwell C, Gunter MJ, Atkin WS, Macklin CP, Cecil T, Bishop DT, Primrose J, Bingham SA. (2007). Role of NQO1C609T and EPHX1 gene polymorphisms in the association of smoking and alcohol with sporadic distal colorectal adenomas: results from the UKFSS Study. *Carcinogenesis* 28:875–882.

Park JY, Schantz SP, Lazarus P. (2003). Epoxide hydrolase genotype and orolaryngeal cancer risk: interaction with GSTM1 genotype. *Oral Oncol* 39:483–490.

Raucy JL, Allen SW. (2001). Recent advances in P450 research. *Pharmacogenomics J* 1:178–186.

Sachse C, Brockmoller J, Bauer S, Roots I. (1997). Cytochrome P450 2D6 variants in a Caucasian population: allele frequencies and phenotypic consequences. *Am J Hum Genet* 60:284–295.

Smith CA, Harrison DJ. (1997). Association between polymorphism in gene for microsomal epoxide hydrolase and susceptibility to emphysema. *Lancet* 350:630–633.

Smith CA, Gough AC, Leigh PN, Summers BA, Harding AE, Maraganore DM, Sturman SG, Schapira AH, Williams AC. (1992). Debrisoquine hydroxylase gene polymorphism and susceptibility to Parkinson's disease. *Lancet* 339:1375–1377.

Steijins L, Van Der Weide J. (1998). Ultrarapid drug metabolism: PCR based detection of CYP2D6 gene duplication. *Clin Chem* 44:914–917.

Taningher M, Malacarne D, Izzotti A, Ugolini D, Parodi S. (1999). Drug metabolism polymorphisms as modulators of cancer susceptibility. *Mutat Res* 436:227–261.

Trapido EJ, Chen F, Davis K, Lewis N, MacKinnon JA. (1994). Cancer among Hispanic males in south Florida. Nine years of incidence data. *Arch Intern Med* 154:177–185.

Tranah GJ, Giovannucci E, Ma J, Fuchs C, Hankinson SE, Hunter DJ. (2004). Epoxide hydrolase polymorphisms, sigarette smoking and risk of colorectal adenoma in the Nurses' Health Study and the Health Professionals Follow-up Study. *Carcinogenesis* 25:1211–1218.

Traver RD, Siegel D, Beall HD, Phillips RM, Gibson NW, Franklin WA, Ross D. (1997). Characterization of a polymorphism in NAD(P) H: quinone oxidoreductase (DT-di aphorase). *Br J Cancer* 75:69–75.

Van der Logt EM, Bergevoet SM, Roelofs HM, Te Morsche RH, Dijk Y, Wobbes T, Nagengast FM, Peters WH. (2006). Role of epoxide hydrolase, NAD(P)H:quinone oxidoreductase, cytochrome P450 2E1 or alcohol dehydrogenase genotypes in susceptibility to colorectal cancer. *Mutat Res* 593:39–49.

Vodicka P, Soucek P, Tates AD, Dusinska M, Sarmanova J, Zamecnikova M, Vodickova L, Koskinen M, de Zwart FA, Natarajan AT, Hemminki K. (2001). Association between genetic polymorphisms and biomarkers in styrene-exposed workers. *Mutat Res* 482:89–103.

Wang LD, Zheng S, Liu B, Zhou JX, Li YJ, Li JX. (2003). CYP1A1, gsts and meh polymorphisms and susceptibility to esophageal carcinoma: study of population from a high-incidence area in north China. *World J Gastroenterol* 9:1394–1397.

Wiemels JL, Pagnamenta A, Taylor GM, Eden OB, Alexander FE, Greaves MF. (1999). A lack of a functional NAD(P)H:quinone oxidoreductase allele is selectively associated with pediatric leukemias that have MLL fusions. *Cancer Res* 59:4095–4099.

Yin L, Pu Y, Liu TY, Tung YH, Chen KW, Lin P. (2001). Genetic polymorphisms of NAD(P)H quinone oxidoreductase, CYP1A1 and microsomal epoxide hydrolase and lung cancer risk in Nanjing, China. *Lung Cancer* 33:133–141.

Yoshimura K, Hanaoka T, Ohnami S, Ohnami S, Kohno T, Liu Y, Yoshida T, Sakamoto H, Tsugane S. (2003). Allele frequencies of single nucleotide polymorphisms (SNPs) in 40 candidate genes for gene-environment studies on cancer: data from population-based Japanese random samples. *J Hum Genet* 48:654–658.

Zahm SH, Fraumeni JF Jr. (1995). Racial, ethnic, and gender variations in cancer risk: considerations for future epidemiologic research. *Environ Health Perspect* 103:283–286.

Zidzik J, Slabá E, Joppa P, Kluchová Z, Dorková Z, Skyba P, Habalová V, Salagovic J, Tkácová R. (2008). Glutathione S-transferase and microsomal epoxide hydrolase gene polymorphisms and risk of chronic obstructive pulmonary disease in Slovak population. *Croat Med J* 49:182–191.

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