**CYP2A6 and CYP2E1 polymorphisms in a Brazilian population living in Rio de Janeiro**


**Abstract**

Cytochrome P450 (CYP) is a superfamily of enzymes involved in the metabolism of endogenous compounds and xenobiotics. CYP2A6 catalyzes the oxidation of nicotine and the activation of carcinogens such as aflatoxin B1 and nitrosamines. CYP2E1 metabolizes ethanol and other low-molecular weight compounds and can also activate nitrosamines. The CYP2A6 and CYP2E1 genes are polymorphic, altering their catalytic activities and susceptibility to cancer and other diseases. A number of polymorphisms described are ethnic-dependent. In the present study, we determined the genotype and allele frequencies of the main CYP2A6 and CYP2E1 polymorphisms in a group of 289 volunteers recruited at the Central Laboratory of Hospital Universitário Pedro Ernesto. They had been residing in the city of Rio de Janeiro for at least 6 months and were divided into two groups according to skin color (white and non-white). The alleles were determined by allele specific PCR (CYP2A6) or by PCR-RFLP (CYP2E1). The frequencies of the CYP2A6*1B and CYP2A6*2 alleles were 0.29 and 0.02 for white individuals and 0.24 and 0.01 for non-white individuals, respectively. The CYP2A6*5 allele was not found in the population studied. Regarding the CYP2E1*5B allele, we found a frequency of 0.07 in white individuals, which was statistically different (P < 0.05) from that present in non-white individuals (0.03). CYP2E1*6 allele frequency was the same (0.08) in both groups. The frequencies of CYP2A6*1B, CYP2A6*2 and CYP2E1*6 alleles in Brazilians are similar to those found in Caucasians and African-Americans, but the frequency of the CYP2E1*5B allele is higher in Brazilians.

**Introduction**

Cytochrome P450 (CYP) is a superfamily of hemoproteins that plays an important role in the biotransformation of various xenobiotics and endogenous compounds (1). Several CYP enzymes can activate procarcinogens to genotoxic intermediates (2). Genetic polymorphisms in CYP genes have been described (3), and some of the polymorphic alleles encode proteins with altered catalytic activities or show differences in gene expression (3). CYP genetic polymorphisms could be associated with the high degree of individual variability in the susceptibility to developing cancer and other diseases related...
to environmental carcinogens or endogenous compounds (4-7).

CYP2A6 is involved in the metabolism of several nitrosamines, particularly those present in tobacco smoke (1), and is also responsible for most of the metabolism of nicotine in humans (6). The CYP2A6 gene is located on chromosome 19, at 19q13.2, in a cluster with other genes of the CYP2A sub-family (CYP2A7 and CYP2A13) and genes from other CYP2 sub-families (CYP2B6, CYP2F1) (7). Within the CYP2A6 gene, more than 20 alleles have been described (3). The CYP2A6 wild-type allele was termed CYP2A6*1A. The CYP2A6*1B allele presents a gene conversion with CYP2A7 in the 3'-untranslated region and its effect on CYP2A6 activity is not known. The CYP2A6*2 allele has a single point mutation (1799T > A) leading to a single amino acid substitution (L160H) (8-10) and the CYP2A6*5 allele has a point mutation in exon 9 (6582G > T) also leading to a single amino acid substitution (G479V), as well as a gene conversion in the 3'-untranslated region (11). The CYP2A6*2 and CYP2A6*5 alleles encode unstable and catalytically inactive enzymes.

CYP2E1 is an ethanol-inducible enzyme that metabolizes some low-molecular weight compounds such as ethanol, acetone, and nitrosamines (12). The CYP2E1 gene is located on the long arm of chromosome 10, at 10q24.3-qter (13) and several CYP2E1 polymorphisms have been identified (3). The wild-type allele (CYP2E1*1A) has an RsaI restriction site in the regulatory region of the gene, at -1053, and a restriction site for DraI in exon 6, at 7632. The CYP2E1*5B allele, detected by resistance to digestion by RsaI, seems to be associated with changes at the transcriptional level, resulting in altered catalytic rates (14,15). This allele shows a restriction site for PstI, characterized by a G > C substitution at -1293. A second allele, CYP2E1*6, shows a T > A substitution at 7632 and is not digested by DraI (16). However, its effect on CYP2E1 activity remains unclear (15,16).

CYP2A6 and CYP2E1 genetic polymorphisms are ethnic dependent (17), and the polymorphic alleles can be more than ten times more frequent in Asians than in Caucasians (5,6,17). However, little information is available about these genetic polymorphisms in multi-ethnic populations.

In the present study, we analyzed the frequency of CYP2A6 and CYP2E1 polymorphisms in individuals living in the city of Rio de Janeiro, a highly admixed population, because these polymorphisms can be involved in the susceptibility to various diseases related to tobacco smoking and alcohol drinking.

Material and Methods

Study group

A total of 289 individuals were included in the present study. The group was composed of outpatients recruited at Hospital Universitário Pedro Ernesto (HUPE, UERJ) during 1999, who had already been included in a previously published study (18). All volunteers had been residents of the metropolitan area of Rio de Janeiro for at least 6 months. To avoid selecting individuals presenting a specific pathology, the subjects were recruited at the central laboratory of the hospital, and those with a previous history of cancer were not included in the study. Information was obtained using a standardized questionnaire, including data on social habits and health problems. The group eligible for the study was classified as white (151 individuals) or non-white (138 individuals) according to skin color. The group of non-white individuals included 63 mulattos and 75 black individuals. The mean age of the individuals that took part in this study was 54.2 years (range: 20 to 85 years) (Table 1). There was no significant difference in mean age between white and non-white individuals. The study proposal and all ethical
proceedings were approved by the Ethics Committee of HUPE, UERJ. All of the participants signed an informed consent form.

Genotyping

DNA extraction. Blood was collected into EDTA-containing tubes and DNA was extracted from blood lymphocytes by proteinase K/SDS digestion as described (19).

CYP2A6 polymorphisms. Genotyping for the CYP2A6*1B, CYP2A6*2, and CYP2A6*5 alleles were performed as described by Os- carson et al. (10,11). Briefly, a two-step PCR method was used to detect CYP2A6*1B and CYP2A6*5 alleles. In the first PCR reaction (PCR I), a region from exon 8 to the 3' flanking region of CYP2A6 was specifically amplified. This PCR product was subsequently used as a template in the second allele-specific PCR reaction (PCR II). The PCR I was identical for each allele, and PCR II was carried out using specific primers for the amplification of CYP2A6*1B or CYP2A6*5 in separated reaction tubes. For CYP2A6*2 a similar approach was conducted. In the PCR I a region from exon 1 to exon 4 was amplified, followed by PCR II, with specific primers for the amplification of CYP2A6*2. The PCR II for CYP2A6*1B, CYP2A6*2 or CYP2A6*5 always had a primer that amplified the wild-type or the polymorphic allele. The genotype was determined according to the amplification products in PCR II.

CYP2E1 polymorphisms. The genotyping assays for the CYP2E1 polymorphisms were performed by PCR-RFLP analysis. The *5B allele was detected as described by Hayashi et al. (15), with modifications. After initial denaturation at 94°C for 4 min, amplification was carried out for 35 cycles at 94°C for 60 s, 53°C for 80 s, 72°C for 90 s, followed by a final extension at 72°C for 5 min. Ten microliters of the PCR product (410 bp) was incubated with 2.5 U *5B allele was detected as described by Hayashi et al. (15), with modifications. After initial denaturation at 94°C for 4 min, amplification was carried out for 35 cycles at 94°C for 60 s, 53°C for 80 s, 72°C for 90 s, followed by a final extension at 72°C for 5 min. Ten microliters of the PCR product (410 bp) was incubated with 2.5 U Rsal (Life Technologies, Carlsbad, CA, USA) and 8 µL was incubated with 3.0 U PstI (Life Technologies), and the resulting fragments were separated on 2.5% agarose gel stained with ethidium bromide. The digestion products according to the different genotypes are described in Table 2. For allele *6 determination, the PCR conditions were those previously described (15,20). The amplification product (10 µL) was incubated with 2.5 U DraI (Life Technologies), and the products were separated on 10% polyacrylamide gel and stained with silver (21). Table 2 shows the genotypes, the restriction enzymes used and the corresponding sizes of the digestion products.

Statistical analysis

Data were analyzed statistically using the GraphPad Instat (GraphPad Software, Inc., San Diego, CA, USA) and Genepop (Genepop web version 3.4) software (22). Differences were considered to be significant at P < 0.05.

Table 1. Characteristics of the study population.

<table>
<thead>
<tr>
<th>Color</th>
<th>Men</th>
<th>Women</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Age</td>
<td>N</td>
</tr>
<tr>
<td>Black</td>
<td>21</td>
<td>55.9</td>
<td>54</td>
</tr>
<tr>
<td>Mulatto</td>
<td>21</td>
<td>53.5</td>
<td>42</td>
</tr>
<tr>
<td>Non-white*</td>
<td>42</td>
<td>54.7</td>
<td>96</td>
</tr>
<tr>
<td>White</td>
<td>64</td>
<td>49.8</td>
<td>87</td>
</tr>
<tr>
<td>Total</td>
<td>106</td>
<td>51.7</td>
<td>183</td>
</tr>
</tbody>
</table>

*aNon-white includes mulatto and black skin-colored individuals. *bNon-white (black + mulatto) and white individuals.

Table 2. Size of the digestion products according to CYP2E1 genotype and different restriction enzymes used in their analysis.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Rsal digestion</th>
<th>PstI digestion</th>
<th>DraI digestion</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1/C1</td>
<td>360 and 50 bp</td>
<td>410 bp</td>
<td>--</td>
</tr>
<tr>
<td>C2/C2</td>
<td>410 bp</td>
<td>290 and 120 bp</td>
<td>--</td>
</tr>
<tr>
<td>D/D</td>
<td>410, 360 and 50 bp</td>
<td>410, 290 and 120 bp</td>
<td>--</td>
</tr>
<tr>
<td>D/D</td>
<td>--</td>
<td>--</td>
<td>572, 302 and 121 bp</td>
</tr>
<tr>
<td>C/C</td>
<td>874 and 121 bp</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>D/D</td>
<td>--</td>
<td>--</td>
<td>874, 572, 302 and 121 bp</td>
</tr>
</tbody>
</table>
Results

Table 3 shows the genotype frequencies of CYP2A6. The most frequent genotype was *1A/*1A, followed by *1A/*1B. The *2/*2 genotype was found in only 1 volunteer, corresponding to a frequency of 0.3% in the study group. The frequency of the wild-type allele was 0.72 in the study population as a whole (Table 4), and 0.75 and 0.69 in non-white and white individuals, respectively. The CYP2A6*5 allele was absent in the individuals analyzed.

The frequencies of RsaI genotype polymorphism of CYP2E1 in the population were 91% for the *1A/*1A genotype, 8% for the *1A/*5B genotype, and 1% for *5B/*5B genotype (Table 3). Among non-white individuals, the respective frequencies were 94.9, 4.4, and 0.7%, whereas among white individuals the frequencies were 87.4% for *1A/*1A, 11.3% for *1A/*5B, and 1.3% for *5B/*5B. When comparing white and non-white individuals we found a significant difference regarding these genotype frequencies (P < 0.05), and the polymorphic allele (*5B) was more frequent in white (7%) than in non-white individuals (3%; Table 5). As shown in Table 5, the population analyzed is not in Hardy-Weinberg equilibrium.

The genotype frequencies of the DraI polymorphism of the CYP2E1 gene were 84.8, 14.5, and 0.7% for the *1A/*1A, *1A/*6 and *6/*6 genotypes, respectively (Table 3). There was no difference in genotype frequencies between white and non-white individuals, and the frequency of CYP2E1*6 was 8% in the population studied (Table 6).

Discussion

In Western countries, carcinogens present in tobacco smoke are the main substances involved in the development of tumors such as those of the lung (23) and the esophagus (24), among others. In most cases, there is a synergistic effect between tobacco smoking and alcohol drinking for the risk of tumor development (24,25). Pro-carcinogens in tobacco smoke such as nitrosamines and ethanol are metabolized by CYP enzymes (1). CYP2A6 and CYP2E1 are the most important enzymes involved in nitrosamine
CYP2A6 and CYP2E1 polymorphisms

activation in human tissues (1), and ethanol is metabolized by CYP2E1 and induces this enzyme. Therefore, polymorphisms in low penetrance genes like CYP2A6 and CYP2E1 can have an impact on cancer susceptibility (4), since polymorphic alleles encode proteins with altered catalytic activities or cause alterations in the regulation of the gene.

CYP2A6 genetic polymorphisms are also involved in the interindividual differences in nicotine disposition. Hepatic CYP2A6 catalyzes the C-oxidation of nicotine to cotinine and the subsequent hydroxylation of cotinine to trans-3'-hydroxycotinine, the major route of nicotine metabolism (6). Thus, individuals showing reduced CYP2A6 activity determined by CYP2A6 genetic polymorphisms have impaired nicotine metabolism and reduced smoking behavior (6). In addition, these individuals may be less exposed to other tobacco compounds (6).

The Brazilian population consists of the admixture of Africans, Europeans and Amérindians, with a high degree of miscegenation (26,27). This characteristic makes the Brazilian population different from other less admixed populations. Since it has been reported that CYP polymorphisms are ethnic dependent, in this study we determined the genotype and allele frequencies of CYP2A6 and CYP2E1 polymorphisms in a group of 289 volunteers from Rio de Janeiro.

The frequencies of the CYP2A6*1B and CYP2A6*2 alleles were 0.29 and 0.02 among white individuals and 0.24 and 0.01 among non-white individuals, respectively. Although not statistically significant, there was a trend to a lower frequency of these alleles among non-white individuals. These results agree with other studies that showed that these alleles seem to be less frequent among individuals of African ancestry (6,28,29). Similar results were obtained in another study on Brazilian individuals (30), which found allele frequencies of 0.38 and 0.24 for CYP2A6*1B and 0.02 and 0.01 for CYP2A6*2 among white and non-white Brazilians, respectively. The results obtained for the population from Rio de Janeiro are similar to those obtained for Europeans and North Americans, whose allelic frequencies are about 0.30 for CYP2A6*1B and 0.01-0.03 for CYP2A6*2 among Caucasians (11, 28,31,32). We did not detect the presence of the CYP2A6*5 allele in the population studied. This allele has been shown to be very rare among Caucasians and Asians, with frequencies of 0-0.01 (6,11,33). Recently, it was shown that CYP2A6*5 is absent in individuals from Africa (29).

In relation to the RsaI polymorphism of CYP2E1, we found genotype frequencies of 91, 8, and 1% for *1A/*1A, *1A/*5B, and

<table>
<thead>
<tr>
<th>Table 5. CYP2E1 genotypes and allele frequencies (allele *6) in white and non-white Brazilian individuals.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Genotype</strong></td>
</tr>
<tr>
<td><strong>Non-White</strong></td>
</tr>
<tr>
<td>*1A/*1A</td>
</tr>
<tr>
<td>*1A/*5B</td>
</tr>
<tr>
<td>*5B/*5B</td>
</tr>
<tr>
<td><strong>P</strong></td>
</tr>
<tr>
<td><strong>Allele frequency</strong></td>
</tr>
<tr>
<td><strong>Non-White</strong></td>
</tr>
<tr>
<td>*1A</td>
</tr>
<tr>
<td>*5B</td>
</tr>
</tbody>
</table>

*Non-white includes mulatto and black skin-colored individuals. **Non-white (black + mulatto) and white individuals.

<table>
<thead>
<tr>
<th>Table 6. CYP2E1 genotypes and allele frequencies (allele *6) in white and non-white Brazilian individuals.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Genotype</strong></td>
</tr>
<tr>
<td><strong>Non-White</strong></td>
</tr>
<tr>
<td>*1A/*1A</td>
</tr>
<tr>
<td>*1A/*6</td>
</tr>
<tr>
<td>*6/*6</td>
</tr>
<tr>
<td><strong>P</strong></td>
</tr>
<tr>
<td><strong>Allele frequency</strong></td>
</tr>
<tr>
<td><strong>Non-White</strong></td>
</tr>
<tr>
<td>*1A</td>
</tr>
<tr>
<td>*6</td>
</tr>
</tbody>
</table>

*Non-white includes mulatto and black skin-colored individuals. **Non-white (black + mulatto) and white individuals.
*5B/*5B, respectively. Similar results were obtained in studies on volunteers from São Paulo, a State located near Rio de Janeiro. In those studies, the frequencies of the *1A/*1A, *1A/*5B, and *5B/*5B genotypes were 89.1, 10.4 and 0.5% (34), and 90, 9, and 1%, respectively (35). The *5B allele is significantly more frequent among white (0.07) than among non-white individuals (0.03; P < 0.05). Differences in CYP2E1*5B allele frequencies between white and non-white individuals were also shown among North Americans (36,37). The results obtained in the present study suggest that the polymorphic allele CYP2E1*5B is more frequent in Brazilians than in individuals from other Western populations, but less frequent than in Asians. The frequency of CYP2E1*5B was 0.03-0.04 in Germans and in European-Americans (36-38), and 0.003-0.01 in African-Americans (36), and 0.08 and 0.11 among African-Americans and Euro-Americans (36), respectively.

In the present study, we found differences between white and non-white individuals in the genotype and allele frequencies of CYP2A6 (alleles *1B and *2) and of CYP2E1 (allele *5B) gene polymorphisms, but not for the CYP2E1*6 allele. We also observed that the distributions of CYP2A6*1B, CYP2A6*2 and CYP2E1*6 alleles, but not of the CYP2E1*5B allele, among white and non-white individuals were similar to those of Caucasians and African-Americans, respectively.

References


18. Rossini A, Rapozo DCM, Amorim LMF et al. (2001). The phylo-

cemy of Sciences, USA


25. Wynder EL, Mushinski MH & Spivak JC (1977). Tobacco and alco-

chemistry, 27: 9006-9013.

26. Carvalho-Silva DR, Santos FR, Rocha J et al. (2001). The phylo-

cemy of Sciences, USA


41. Wynder EL, Mushinski MH & Spivak JC (1977). Tobacco and alco-

chemistry, 27: 9006-9013.


45. Rossini A, Rapozo DCM, Amorim LMF et al. (2001). The phylo-

chemistry, 27: 9006-9013.

46. Wynder EL, Mushinski MH & Spivak JC (1977). Tobacco and alco-

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