Genetic variability of *CYP3A4* in a heterogeneous Brazilian population from Maranhão

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ABSTRACT. Inter-individual variability in drug metabolism may result in adverse drug responses. Pharmacogenetic studies have shown that polymorphisms in drug metabolizing enzymes may contribute to this variability. Among these enzymes, *CYP3A4* is responsible for metabolizing over 50% of the clinically used drugs. The Brazilian population is composed of people with Native American, European, and African ancestries, and is therefore considered as one of the most intermixed populations in the world. A thorough knowledge of the genetic frequencies of *CYP3A4*

allelic variants is useful for the establishment of better pharmacological therapies; therefore, the aim of this study was to describe the polymorphic frequencies for *CYP3A4* -392A>G (rs2740574) in a sample population from Maranhão, Brazil. Our results showed that 75.1, 21.9, and 3.0% of the individuals expressed the -392AA, -392AG, and -392GG genotypes, respectively. The -392A and -392G alleles were observed in 86.1 and 13.9% of the population, respectively. Our results reiterate the need for a better understanding of the variations in the genotype and allele frequencies of *CYP3A4* -392A>G polymorphisms in various Brazilian regions, in order to elucidate the variability in drug response.

Key words: *CYP3A4**1B; Polymorphism; Genetic variability; Drug metabolism; Pharmacogenetics

INTRODUCTION

The new genetic pool contributed to a high degree of variability, directly affecting most of the genetic polymorphic traits, such as the genes of the P450 cytochrome superfamily. The P450 cytochromes are heme proteins that catalyze the metabolism of a large number of xenobiotics and endobiotics, which are largely responsible for the clearance of therapeutic drugs (Deenen et al., 2011). Among these, special importance has been attributed to the *CYP3A4* gene by previous researchers.

CYP3A4 is highly expressed in the liver and small intestine (Shimada et al., 1994), and metabolizes >50% of the clinically administered drugs (Guengerich, 1999; Rodriguez-Antona et al., 2005). The enzyme activity of *CYP3A4* has a wide subject-specific range; this activity can be affected by non-genetic factors, such as the age, endogenous hormone levels, health status, and environmental stimuli, in addition to genetic polymorphism (Ozdemir et al., 2000).

To date, approximately 40 allelic variants have been described for *CYP3A4*, some of which are responsible for a reduction in human cytochrome P450 activity. In addition, a common polymorphism located in the promoter region of the gene (*CYP3A4* -392A>G rs2740574; the -392G allele also known as CYP3A4*1B) influences *CYP3A4* expression as a result of altered nuclear protein binding affinity to the polymorphic element (Rodriguez-Antona and Ingelman-Sundberg, 2006).

The genotyping of *CYP3A4* might help predict the treatment outcome of several drugs (Evans and Relling, 1999). However, because of the high cost of testing, the population frequencies of its various genotypes in a target population must be determined, to facilitate the design of an appropriate strategy for clinical application. Therefore, the aim of this study was to identify the genotypic and allelic frequencies of the *CYP3A4* -392A>G (rs2740574) polymorphism in the São Luís population in Maranhão, Brazil.

MATERIAL AND METHODS

Ethics statement

This study was approved by the Committee of Ethics in Research of the University Hospital President Dutra of Universidade Federal do Maranhão on April 30, 2012 (CEP 007/2012). Informed consent was obtained from all enrolled patients prior to the study.

Samples

In this study, 201 DNA samples were obtained from unrelated individuals from São Luís, Maranhão, Brazil, between April 2012 and April 2013 for polymorphism genotyping. The following exclusion criteria were applied for the selection of patients: no history of cancer, no alcohol or tobacco addiction, and no occupational diseases, immunodeficiencies, mental disorders, or neurological diseases. Additionally, an inability to answer the questionnaire was considered as an exclusion criterion.

The age of the included individuals varied from 18 to 91 years, with a mean of 61.8 years (standard deviation ±15.6 years); among these, 92 (45.8%) were men and 109 (54.2%) were women (Table 1).

Table 1. Gender, age, and CYP3A4 -392A>G polymorphism.

Features	Frequencies	
	No.	(%)
Gender		
Female	109	(54.2)
Male	92	(45.8)
Age, years (mean ± SD, 61.8 ± 15.6 years)		
≤40	21	(10.5)
41-61	61	(30.3)
61-80	97	(48.3)
>80	22	(10.9)
Genotypic frequencies of CYP3A4 -392A>G		
AA	151	(75.1)
AG	44	(21.9)
GG	6	(3.0)
Allelic frequencies of CYP3A4 -392A>G		
A	-	(86.1)
G	-	(13.9)
Hardy-Weinberg equilibrium		
Chi square	1.526	
P value[a]	0.217	
Total	201	(100.0)

[a]Equilibrium was assumed when P > 0.05.

Genotyping

Genomic DNA was extracted from blood samples using the Biopur Kit mini spin plus (Biometrix Inc., San Francisco, CA, USA). The total concentration of isolated genomic DNA was determined using a NanoVue UV/VIS spectrophotometer (GE Healthcare, Fairfield, CT, USA). The *CYP3A4**1B polymorphism was genotyped by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP). The following primers were used for the analysis: 5'-GGA CAG CCA TAG AGA CAA CTG CA-3' and 5'-CTT TCC TGC CCT GCA CAG-3', which produce a 334-base pair (bp) fragment. The PCR was performed in a 25-µL reaction mixture containing 50 ng genomic DNA, 1 U HotStarTaq Plus Master Mix (containing the HotStarTaq Plus DNA Polymerase, PCR Buffer with 3 mM $MgCl_2$, and 400 µM dNTP each; Qiagen, Venlo, Netherlands), and 10

pmol each primer. The reaction conditions were set as follows: initial denaturation at 94°C for 5 min, amplification for 40 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min, and a final extension at 72°C for 10 min. The PCR products were digested overnight with a restriction endonuclease *Pst*I (Jena Bioscience, Jena, Germany), following the manufacturer recommendations. The restriction fragments were resolved on a 2% agarose gel. The *CYP3A4**1B allele was characterized by four distinctive fragments of 199, 81, 33, and 21-bp, whereas the *CYP3A4* wild-type allele was identified by three fragments 220, 81, and 33-bp long.

Statistical analysis

Statistical data analysis was done using SPSS Statistics 20.0 (IBM, Armonk, NY, USA). The genotypic frequencies were tested for conformance with the Hardy-Weinberg Equilibrium (HWE). The chi square test was also used to assess deviations of allelic frequencies from the HWE.

RESULTS

The AA (wild type), AG, and GG genotypes of the CYP3A4 -392A>G polymorphism was observed in 151 (75.1%), 44 (21.9%), and 6 (3.0%) of the test subjects, respectively. The allele frequencies of the -392A and -392G alleles were 86.1% and 13.9%, respectively. The genotype frequencies were in line with the HWE (P >0.05). As show in Table 1, the homozygote of the -392A allele of *CYP3A4**B was predominant.

DISCUSSION

Inter-individual variability in drug metabolism, which can result in poor drug response, adverse drug reactions, or unfavorable drug-drug interactions, is a preeminent concern in drug development and treatment. Pharmacogenetic studies have shown that polymorphisms of drug-metabolizing enzymes, transporters, and receptors contribute to variable drug response (Waxman et al., 1988).

CYP3A4 is responsible for the metabolism of over 50% of all clinically used drugs, including commonly used antidepressants, antibiotics, antihypertensives, steroids, and immunosuppressants (Von Hentig and Lötsch, 2009). Thus, polymorphisms in the CYP family may have a majority impact on the fate of these drugs, and other therapeutic drugs whose metabolism they regulate (Evans and Relling, 1999).

CYP3A4 -392A>G has been previously analyzed in a Brazilian population. Jeovanio-Silva et al. (2012), in a study of a sample population in the State of Rio de Janeiro in Brazil, observed that 59.5% of 106 test subjects expressed a homozygous genotype (45.3% -392AA and 14.2% -392GG), while 40.5% displayed a heterozygote genotype; in this study, the -392G and -392A alleles were observed in 34% and 66% of the Brazilian subjects, respectively.

Another related study of the genotype frequency of *CYP3A4* in Rio Grande do Sul (Brazil) revealed that 94.8% of the sample population displayed the -392AA genotype and 5.2% showed the -392AG genotype; however, the -392GG genotype was not observed in these individuals. Consequently, the allelic frequency of -392G was only 2.6% (Fiegenbaum et al., 2005). These

results were very similar to those observed in a Caucasian sample population, wherein 91.9% of the population displayed the -392AA genotype; 7.9% and 0.1% of the total sample population were heterozygous and homozygous -392G carriers, respectively (Becker et al., 2010).

In this study, we observed that 86.1% and 13.9% of the population expressed the -392A and -392G alleles; the genotypic distribution of -392AA, -392AG, and -392GG was 75.1%, 21.9%, and 3.0%, respectively. The allelic and genotypic frequencies of the *CYP3A4* SNP observed in our study was similar to those observed in other studies in Brazilian individuals (Cavalli et al., 2008), while differing from the results of studies in other populations (Fiegenbaum et al., 2005; Becker et al., 2010; Jeovanio-Silva et al., 2012).

Brazil presents a large miscegenation of Native American Indians, European Caucasians, and African blacks originating from various countries. Because of the geographical enormity, topographic diversity, and colonization history of Brazil, different regions display a singular prevalence of ancestry that is reflected in the population subtypes (Alves-Silva et al., 2000). This justifies the large variation in the results of studies in Brazilian population genetics, underscoring the need for studies focusing on different regions of Brazil and different population subtypes.

In conclusion, the variant frequency of *CYP3A4* polymorphisms among different ethnic groups may significantly contribute to the drug efficacy and toxicity. Based on these results, it is necessary to specifically examine the genetic frequencies of the *CYP3A4* -392A>G polymorphism in different regions in Brazil, in order to promote greater understanding of their role in pharmacological response.

Conflicts of interest

The authors declare no conflict of interest.

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