CLINICAL SCIENCE

Frequency of genetic polymorphisms of *PXR* gene in the Brazilian population

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INTRODUCTION: PXR polymorphisms have been implicated in modulating *CYP3A4* and PXR expression, potentially accounting for interindividual differences in drug metabolism. The prevalence of PXR polymorphisms varies among ethnic groups and data on the allelic distribution in the highly mixed Brazilian population is lacking. The aim of this study was to analyze genetic variations in the PXR gene in Brazilians and to compare the results to other ethnic groups.

METHODS: DNA samples from 117 healthy Brazilians underwent PCR amplification and sequencing.

RESULTS: Eleven polymorphisms were identified, 3 of which are highly associated with differences in *CYP3A4* expression. We also identified 1 new synonymous variant in 1.3% of the alleles. Among the functional polymorphisms, –25913 C>T and –6994T>C occurred at a higher frequency compared to the African alleles ($p < 0.05$) but at a lower frequency compared to Caucasian alleles. The 8055 C>T allele was found at a similar frequency to those described in Caucasians and Africans ($p > 0.05$).

CONCLUSION: We observed that functional variants of the PXR were frequent in our sample of the Brazilian population. Our results suggest that PXR gene variants may be of interest in pharmacogenetic studies involving Brazilians.

KEYWORDS: Pharmacogenetics; Allelic distribution; Brazilian population; Genetic variation.

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INTRODUCTION

The interindividual variability in human drug metabolism may be genetically determined by allelic variance in genes encoding P450 proteins and nuclear receptors.[1-4] Cytochrome P450s metabolize approximately 80% of clinical drugs and polymorphisms in the *CYP3A4* gene have been previously investigated due to its involvement in the metabolism of more than 50% of drugs. Despite the large interindividual differences in *CYP3A4* expression, polymorphisms that impair enzyme activity seem to be rare.[5-7]

CYP3A4 expression is mainly induced by the pregnane X receptor (PXR),[8-10] a member of the orphan nuclear receptor subfamily and the main transcriptional regulator of cytochrome P4503A enzymes.[8,9] The activation of PXR initiates in the cytoplasm. After exposure to ligands, PXR dimerizes with the retinoic acid receptor (RXR) and binds to nuclear receptor response elements in the upstream regulatory regions of target genes. These genes encode drug-metabolizing enzymes such as *CYP3A4* and drug transporters,[11] which are the main regulators in the uptake and transformation of many prescribed medicines.

Several *PXR* (also known as *NR1I2*) polymorphisms have been identified that influence the level of *PXR* expression and, therefore, indirectly regulate its target genes.[4,12] These polymorphisms account for the wide variability of CYP3A4 levels in the population.[4,13] The functional effects of these polymorphisms include aberrant DNA binding and alterations in the transactivation and expression of downstream target genes.[4,12]

While the allelic distribution of the *PXR* gene varies widely among different ethnic groups,[13-17] it has yet to be studied in the highly mixed Brazilian population. Here we analyze the frequency of *PXR* variants in a sample of healthy Brazilian subjects. We also compare the frequencies of *PXR* polymorphisms to those described in Asians, Caucasians, and Africans in order to determine whether *PXR* variants should be evaluated in pharmacogenetic studies involving Brazilian subjects.

Subjects

The study protocol was approved by the Ethical Committee of São Paulo University. Written consent was

obtained from all subjects. Healthy volunteers consisted of 76 females and 41 males with a mean age 25.8 ± 11.8 years. The self-reported ethnicity of this cohort was 87.2% Caucasian and 12.8% African. In addition, 88% were from the southeast, 8.5% from the northeast, and 3.5% from the southern region of Brazil.

METHODS

DNA samples from all subjects were obtained from peripheral blood leukocytes using a salting out procedure. All coding regions of the *PXR* gene were analyzed in addition to three variants in non-coding regions that had a minor allele frequency of at least ≥ 0.10 in other ethnic groups. We also looked for three variants, which modified the PXR and/or CYP3A4 expression, previously described in non-coding regions with a minor allele frequency ≥ 0.10 in other ethnic groups.[4,17] The *PXR* gene was amplified as previously described.[4,18] The amplified products were sequenced using the Big Dye Terminator Sequencing Kit™ (Applied Biosystem, Inc., Foster City, CA, USA) and capillary electrophoresis was conducted on an ABI PRISM 3100 sequencer (Applied Biosystem, Inc.). Sequence traces were analyzed using Sequencher (version 4.5 build 1416) and assembled to the reference (NCBI accession number AF364606).

Statistical Analysis

When appropriate, identified allele frequencies were compared to the expected normal distribution in a population (Hardy-Weinberg equilibrium) by using a goodness-of-fit x^2 test.

Differences in allele frequencies between ethnic groups were analyzed using the x^2 test. Values of $p < 0.05$ were considered to be significant.

RESULTS

The allelic frequencies of *PXR* polymorphisms that we identified in this study are shown in Table 1 along with previously published data from Asian, Caucasian, and African populations. The genotypic frequencies that we found are listed in Table 2. All polymorphisms were in Hardy-Weinberg equilibrium. We identified 11 polymorphisms (Tables 1 and 2), one of which is a new synonymous variant located in exon 4 (4306 C>G) that was present in 1.3 % of Brazilian alleles. Eight polymorphisms were identified in the coding regions of the *PXR* gene and 3 polymorphisms in non-coding regions. Of the variants identified, 8055 C>T, -25913 C>T, and -6994 C>T are known to be associated with functionality.

The 8055 C>T (rs2276707) variant was found in 12.5% of alleles, which is similar to its frequencies in Caucasian and African alleles of 15% and 18% ($p > 0.05$),[12] respectively, but lower than its frequency in Chinese alleles of 45% ($p < 0.05$).[19] The -25913 C>T (rs1523130) variant occurred in 50% of the Brazilian alleles, which is less frequent than in Caucasians (70%, p = 0.003, 95% CI: 0.66-0.76) but more frequent than in Africans (28%, $p < 0.001$, 95% CI: 0.49-0.61). The -6994 C>T (rs2472677) polymorphism was found in 46% of the Brazilian alleles, which is a higher frequency than in Africans (38.1%, p = 0.03, 95% CI: 0.80-0.85) but a lower frequency than in Caucasians (62%, p = 0.01, 95% CI: 0.70-0.79).

The 79 C>T (rs12727613) polymorphism was found in 2.1% of Brazilian alleles. This variant is significantly more frequent in African alleles (20%, $p < 0.001$) and not present in Caucasians or Asians. The 106 G>A (rs12721607) polymorphism, which is absent in Asians, was found in 1.3% of alleles in this population, similar to its frequencies in Caucasians and Africans of 5% and 4%, respectively ($p < 0.05$). The 4321 G>A (rs12721608) polymorphism is absent in Africans but present in Brazilians at a similarly low frequency as that found in Caucasians ($p < 0.05$). The other five polymorphisms were detected at a frequency lower than 1% [4447 T>C (rs12721611), 4499 C>T (rs12721600), 4773 G>A (rs12721612), and 8528 G>A (rs59152710)].

DISCUSSION

PXR polymorphisms have been implicated in modulating *CYP3A4* and *PXR* expression, potentially accounting for interindividual differences in drug metabolism.[20] These variants impact drug response and some types of drug interactions by affecting serum levels of the medicines and endogenous compounds, which can include PXR ligands and substrates of enzymes and drug transporters.[13] The distribution of allelic variants in the *PXR* gene varies widely among different ethnic groups,[12,14] which helps to explain variations in the pharmacokinetics and pharmacodynamics of drugs that are PXR ligands.

Table 1 - Allelic frequency of polymorphisms in the *PXR* gene in Brazilians compared to Asians, Caucasians, and Africans.

Location	dbSNP	*Position in AF364606	Amino Acid Change	Asians		Caucasians		Africans[a]		This study	
				n°	Frequency	n°	Frequency	n°	Frequency	n°	Frequency
5′UTR	rs1523130	-25913 C>T	-	80[1]	0.67	80[1]	0.70	80[1]	0.28	234	0.50
Intron 1	rs2472677	-6994 C>T	-			92[4]	0.62	714[#]	0.381	234	0.457
Exon 2	rs12727613	79 C>T	Pro27Ser	80[1]	0	300[2]	0	66[3]	0.20	234	0.021
Exon 2	rs12721607	106 G>A	Gly36Arg	80[1]	0	80[1]	0.05	80[1]	0.04	234	0.013
Exon 4	-	*4306 C>G*	*Arg156Arg*							234	0.013
Exon 4	rs12721608	4321 G>A	Gln122Arg			144[3]	0.01	714[#]	0	234	0.012
Exon 4	rs12721611	4447 T>C	Thr164Thr	80[1]	0	80[1]	0	80[1]	0.03	234	0.008
Exon 5	rs12721600	4499 C>T	Gly220Gly							234	0.008
Exon 6	rs12721612	4773 G>A	Gly317Gly							234	0.004
Intron 6	rs2276707	8055 C>T	-	80[1]	0.51	150[5]	0.15	22[5]	0.18	234	0.125
Exon 8	rs59152710	8528 G>A	Ala370Thr			312[2]	0	64[2]	0.016	234	0.004

[a]African-American population. n°: number of alleles. Data from Asians, Caucasians, and Africans was obtained from [1]King et al. 2007, [2]Hustert et al. 2001, [3]Wang et al. 2008, [4]Lamba et al. 2008, and [5]Zhang et al. 2008. [#]Data from sub-Saharan Africans (Svard et al. 2010). Italics: new variant. *Position in relation to translation start site of PXR (NR1I2): GenBank Accession AF364606.

Table 2 - Genotypic frequency of polymorphisms in the *PXR* gene in Brazilians.

Location	dbSNP	*Position in AF364606		Frequency
5'UTR	rs1523130	-25913 C>T	CC	0.325
			CT	0.35
			TT	0.325
Intron 1	rs2472677	-6994 C>T	CC	0.368
			CT	0.352
			TT	0.282
Exon 2	rs12727613	79 C>T	CC	0.957
			CT	0.043
			TT	0
Exon 2	rs12721607	106 G>A	GG	0.974
			GA	0.026
			AA	0
Exon 4	-	*4306 C>G*	*CC*	*0.974*
			CG	*0.026*
			GG	*0*
Exon 4	rs12721608	4321 G>A	GG	0.974
			GA	0.026
			AA	0
Exon 4	rs12721611	4447 T>C	TT	0.983
			TC	0.017
			CC	0
Exon 5	rs12721600	4499 C>T	CC	0.983
			CT	0.017
			TT	0
Exon 6	rs12721612	4773 G>A	GG	0.992
			GA	0.08
			AA	0
Intron 6	rs2276707	8055 C>T	CC	0.769
			CT	0.214
			TT	0.017
Exon 8	rs59152710	8528 G>A	GG	0.992
			GA	0.08
			AA	0

Italics: new variant, *Position in relation to translation start site of PXR (NR1I2): GenBank Accession AF364606.

This study was carried out in order to determine the frequency of variants of the *PXR* gene in a sample of the Brazilian population. We found 11 polymorphisms in the *PXR* gene, including the 4306 C>G substitution in exon 4 that has not been previously described. This variant is not predicted to alter the splice site in exon 4 of the *PXR* gene and is unlikely to alter protein function.

The Brazilian population is highly heterogeneous, resulting from centuries of interbreeding among peoples from three continents: European colonizers (mostly Portuguese), African slaves, and native Indians.[21] Although Brazilians contain approximately 28% of African alleles, we found that the variant C79T (rs12727613), which occurs in 20% of alleles Africans, occurred at a much lower than expected frequency in our sample (2.1%). On the other hand, the –25913 C>T (rs1523130) polymorphism that is present in 28% of African alleles was present at a significantly higher frequency in our population (50%, $p = 0.003$). We also found that the -6994T>C (rs2472677), which is present in 38% of African alleles, was significantly more frequent in the Brazilian alleles (46%, $p = 0.03$). Finally, the –25913 C>T and –6994T>C polymorphisms were not as frequent in the Brazilian alleles as in Caucasian alleles ($p < 0.05$).

Our results are distinctly clustered. This could represent the unique admixture of the regional subpopulation from the southeast region of Brazil, which has similar ancestral contributions from African (34%), European (31%), and Native American (33%) alleles, in contrast to the higher proportion of African lineages observed in the northeast region.[21-23]

Our study emphasizes the population-dependent frequency of *PXR* polymorphisms. The polymorphism frequencies found in our sample differ greatly from those previously described in Caucasians and Africans. Our results suggest that variants in the *PXR* gene should be considered in pharmacogenetic studies involving Brazilians from the southeast region, since the miscegenation varies from region to region.

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