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FREQUENCIES OF ALLELIC VARIANTS OF THE CYP2D6: *4, *6 AND *10 IN QUILOMBOLA COMMUNITY, MARANHÃO, BRAZIL

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ABSTRACT
The formation of the Brazilian territory had as one of its fundamental characteristics, the miscegenation of several population matrixes. In this context, people from Africa played a fundamental role in shaping what is now Brazil. Brazil has the largest black population outside Africa and the second largest in the world, surpassed only by Nigeria. More than 40% of the Brazilian population corresponds to Afro-descendants. Genotyping of the CYP2D6 gene may serve as a predictive tool for the pharmacological effectiveness and / or toxicity of various drugs. However, due to the high cost of genetic testing, it is necessary to know the population frequencies in order to facilitate the development of clinical and laboratory strategies. Based on these, the present study aimed to verify the allelic and genotype frequencies of the *4, *6 and *10 variants of the CYP2D6 gene in a quilombola community, located in the municipality of Icatu, Maranhão, Brazil. The research was performed with 75 people, DNA samples were obtained and used for genotyping of polymorphisms, from April 2013 to April 2014. The results demonstrated the presence of genetic polymorphisms of CYP2D6 *4, *6 and *10 in the remaining population of quilombo. Detection of alleles that did not carry the studied mutations was observed in 65% of the individuals, followed by heterozygosis in 37% and homozygosis in 1%. The most prevalent polymorph was *6 with 81% of heterozygotes.

KEYWORDS: CYP2D6; Polymorphism; Genetic Variability; Drug Metabolism; Pharmacogenetics.

INTRODUCTION

The formation of the Brazilian territory had as one of its fundamental characteristics, the miscegenation of several population matrixes. In this context, people from Africa played a fundamental role in the configuration of what is now Brazil (Munanga, 2012). Brazil has the largest black population outside Africa and the second largest in the world, surpassed only by Nigeria. More than 40% of the Brazilian population corresponds to Afro-descendants (Amabis and Martho, 1996; Batista, 2009).

According to estimates by the Federal Government, there are, currently in the country, around 3,000 remaining quilombo areas, mainly concentrated in the states of São Paulo, Rio de Janeiro, Pará, Maranhão, Pernambuco, Mato Grosso, Mato Grosso do Sul, Bahia, Sergipe, Goiás and Amapá. Of these, more than 1,826 are certified by the Palmares Cultural Foundation (PCF), totaling around 2.2 million people. (Ibge, 2017). Regarding the State of Maranhão, there are more than 682 remaining communities of quilombos (Ibge, 2017).

In Brazil, the Brazilian black population has a genetic specificity that distinguishes it from other black populations in the world, including that of Africa itself (Brasil, 2001). Therefore, it is known that individuals with distinct ancestries are known to vary in response to the drug, it may be attributed to a consequence of multiple factors influencing in pharmacokinetic and / or pharmacodynamic processes such as: age, gender, body mass, renal and hepatic function, concomitant therapy, nature of the disease, ethnicity, environmental and genetic factors (Clayton, 2006).

The new gene pool contributed greatly to a high degree of variability, directly affecting most of the polymorphic genetic traits, such as the genes of the cytochrome P450 superfamily. Cytochromes P450 are hemeproteins that

catalyze the metabolism of a large number of xenobiotics and endobiotics, responsible for promote the clearance of therapeutic drugs, for example (Deenem et al., 2011). With more than 100 variants and alleles, the gene encoding the CYP2D6 enzyme is the most polymorphic compared to the genes of the other enzymes in the family (Zhou, 2009), having as characteristic a high interindividual variability in the enzymatic activity (Gregori et al., 2010; Hicks et al., 2013) Genotyping of the CYP2D6 gene may serve as a predictive tool for the pharmacological effectiveness and / or toxicity of various drugs (Bernarda et al. , 2005). However, due to the high cost of genetic testing, it is necessary to know the population frequencies in order to facilitate the development of clinical and laboratory strategies. Based on these, the present study aimed to verify the allelic and genotype frequencies of the *4, *6 and *10 variants of the CYP2D6 gene in a quilombola community, located in the municipality of Icatu, Maranhão, Brazil.

Considering the need to add strategies for prevention and health promotion for remnants of quilombo population, this study aimed to verify the frequency of the polymorphism of CYP gene (Cytochrome) 2D6 in a quilombola community of Maranhão, since the variation of the activity of the enzyme has important therapeutic consequences, having a significant role in the development of therapeutic insufficiency or adverse events in individuals with enzymatic polymorphism (Zhou, 2009).

MATERIAL AND METHODS

Study area: The research was carried out in the quilombo of Boca da Mata, this one has 10 hectares and approximately 60 residences (Prefeitura Municipal Icatu, 2017). The territory is located in the North Coast Zone of the Eastern Meso-region and in the Lower Maranhense Oriental Microregion. Its population is estimated at 25,147 inhabitants (Ibge, 2013). The city of Icatu is the second oldest municipality of Maranhão.

Sampling: In this study, 75 DNA samples were obtained and used for genotyping of polymorphisms during the period from April 2013 to April 2014. Inclusion criteria were individuals belonging to the remaining quilombo community of Boca da Mata, older than 18 years. Pregnant women and those with mental disorders were excluded (which made it impossible to read and understand the Term of Free and Informed Consent - TCLE), as well as immunosuppressed, bedridden and children.

Genotyping: DNA was extracted from peripheral venous blood samples using QIAmp¯ DNA Mini and Blood Mini kits (Qiagen, USA) according to the manufacturer's protocols. The extracted DNA was stored at Maranhão Tumor Bank at -80 °C. The presence of CYP2D6 *4, CYP2D6 *6 and CYP2D6 *10 polymorphisms (Table 1) were investigated by PCR and restriction enzyme digestion using 100 ng of DNA as template, as previously described by Schur et al. (2000).

This study was approved by the Research Ethics Committee of the Presidente Dutra University Hospital of the Federal University of Maranhão on 04/30/2012 (CEP 007/2012) and informed consent was obtained from all registered patients.

Statistical Analysis: Statistical data were analyzed using SPSS Statistics 20.0. Genotypic frequencies were tested for Hardy-Weinberg Equilibrium (HWE). The chi-squared test was also used to evaluate the deviations of the allelic frequencies of the Hardy-Weinberg Equilibrium.

RESULTS AND DISCUSSION

Among the 75 individuals analyzed, the age ranged from 18 to 85 years, with a mean of 39,0 years (standard deviation ± 19,1 years), of which 27 (36,0%) were men and 48 (64,0%) were women. According to the results obtained between the polymorphisms CYP2D6 *4, *6 and *10, in a total of 225 analyzes performed in the volunteers, was verified that 141 (65%) of the individuals did not present any of the polymorphisms studied, 74 (34%) were heterozygous for one of the polymorphisms studied and 10 (1%) of the individuals surveyed were homozygous for one of the three polymorphisms studied.

Analysis of the results demonstrated the detection of CYP2D6*4 polymorphism in heterozygosity in 17 (23%) individuals and 1% homozygous, resulting in a frequency, of this allele, of 24%.

A frequency of 12% for homozygous for the CYP2D6*10 allele was found. It was verified that 66 (76%) individuals of the study population presented the polymorphic *6 in heterozygosis, making this the most frequent polymorphism in the sample.

In relation to the genotype frequencies of the CYP2D6 polymorphism, * 4 were 44 (21.9%) as AG and 6 (3.0%) as GG.

The allele frequency of the -392A allele was 86.1% and the -392G allele was 13.9%. Genotypic frequencies were in line with the Hardy-Weinberg Equilibrium (HWE) ($p > 0.05$). With the exception of CYP2D6*4, all polymorphisms analyzed were in HWE in the Brazilian population. Homozygous variants of CYP2D6 * 10 were not found in the population, with the -238AA genotype found only once (0.4%) and the -308GA genotype.

The study showed that the population is in equilibrium, for variants *4 and *10, that is, there is no statistic difference between observed and expected frequencies. However, for the variant *6 ($X2 = 28,17$) the population is not in equilibrium, because the results show a difference between observed and expected frequencies.

Evidencing that this population will probably come into equilibrium after several generations.

The study of this polymorphism is of great importance for the metabolism and effects of many drugs, besides being of interest to Brazil due to the vast miscegenation of the population.

It is important to note that polymorphisms in CYP2D6, as well as most polymorphisms, exhibit different frequencies according to ethnicity. Therefore, different populations may express the same polymorphisms or not, or even if they express patterns of similar polymorphisms, they may be distributed at different frequencies between these populations (Sistonen, 2007; Hicks, et al., 2013).

Table. 1. Single Nucleotide Polymorphisms (SNP) primer sequences.

Polymorphism	Primers	ºC	Enzyme
CYP2D6*4	F: 5' GCCTTCGCCAACCACTCCG 3'	60	BstNI
rs3892097	R: 5' AAATCCTGCTCTTCCGAGGC 3'		
CYP2D6*6	1: 5' TCCCAGCTGGAATCCGGTGTCG 3'	63 / 53	–
rs5030655	2: 5' GGAGCTCGCCCTGCAGAGACTCCT 3'	(10x / 30x)	
	11: 5' TCCTCGGTCACCCA 3'		
	Tmut: 5' GTCGCTGGAGCAGG 3'		
CYP2D6*10	F: 5' CCATTTGGTAGTGAGGCAGGTAT 3'	58	HpHI
rs1065852	R: 5' CACCATCCATGTTTGCTTCTGGT 3'		

Table. 2. Genotypic and allelic distribution.

Polymorphisms	Frequencies		Hard-Weinberg equilibrium	
	No.	(%)	x^2	p value[a]
CYP2D6*4				
*1/*1	57	(76.0)	0.045	0.832
*1/*4	17	(22.7)		
*4/*4	1	(1.3)		
Wild-type allele		(87.3)		
Variant allele	–	(12.7)		
CYP2D6*6				
*1/*1	18	(24.0)	28.174	< 0.001
*1/*6	57	(76.0)		
*6/*6	0	(0.0)		
Wild-type allele		(62.0)		
Variant allele	–	(38.0)		
CYP2D6*10				
*1/*1	66	(88.0)	0.940	0.580
*1/*10	9	(12.0)		
*10/*10	0	(0.0)		
Wild-type allele		(94.0)		
Variant allele	–	(6.0)		
Total	**75**	**(100.0)**		

x^2 - Chi-square.

[a] Equilibrium was assumed when $p > 0.05$.

CONCLUSION

The results demonstrated the presence of genetic polymorphisms of CYP2D6 *4, *6 and *10 in the population of remaining of quilombos. Detection of alleles that did not carry the mutations studied was observed in 65% of the individuals, followed by heterozygosis in 37% and homozygous in 1% of the individuals surveyed. The most prevalent polymorph was 6 with 81% of heterozygotes.

COMPLIANCE WITH ETHICAL STANDARDS

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All of the procedures of this study which involved human beings were approved by the Committee on Ethics in Research of Maranhão's Federal University's (UFMA) Presidente Dutra University Hospital, in accordance with the National Health Council's (Brazil) Resolution number 466/2012 and the Declaration of Helsinki (1964) and its posterior alterations or comparable to the standards of ethics in health. This

article does not contain any procedures involving animals.

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