ALLELIC VARIANTS OF THE *CYP2D6: *4, *6 AND *10* IN A SAMPLE OF RESIDENT FROM THE ARAGUA STATE, VENEZUELA

Carlos Flores-Angulo[1,a], Cecilia Villegas[1,b], Yuselin Mora[1,b]; Jose Antonio Martinez[1,c], Teresa Oropeza[1,d], Nancy Moreno[1,e]

ABSTRACT

The aim of this study was to determine the *CYP2D6: * 4, * 6 and * 10* gene variants frequency and to predict the metabolizer phenotype in a sample of 145 unrelated apparently healthy individuals residing in the state of Aragua, Venezuela. Genotypes were determined by Polymerase chain reaction assays followed by restriction endonucleases digestion. The metabolizer phenotype prediction was made based on the activity score system. The frequencies of *CYP2D6 * 4, * 6 and * 10* allelic variants were 14.5%, 0.3% and 1%. A significant percentage of individuals were categorized as heterozygote-extensive/intermediate (23.5%) and poor metabolizers (4.1%), this information has potential clinical impact, because the *CYP2D6* protein is involved in the metabolism of drugs frequently prescribed as: carvedilol, captopril, chloroquine, codeine, fluoxetine, fluvastatin, haloperidol, idarubicin, indinavir, imatinib, loperamide, nifedipine, ondansetron and tamoxifen.

Key words: CYP2D6; Genotype; Phenotype; Pharmacogenetics, Venezuela (source: MeSH/NLM)

INTRODUCTION

Pharmacotherapy is a pillar of modern medicine, but the therapeutic response is not always homogeneous: a broad range of responses is often observed. This interindividual variability is due to various factors, including polymorphisms in genes that encode drug-metabolizing enzymes, such as the cytochrome P450 (CYP) superfamily [(1)]. CYP protein, family 2, subfamily D isoform 6 (*CYP2D6*) belongs to this group, which is predominantly expressed in the liver and is responsible for the metabolism of 25% of pharmaceutical drugs [(1)].

More than 100 alleles have been identified in the *CYP2D6* gene, which are classified into four categories depending on the effect on the enzymatic activity: those that yield a nonfunctional protein and those that result in a protein with decreased, normal, or increased activity (http://www.cypalleles.ki.se). *CYP2D6* variants *4 and *6 correspond to a truncated protein without activity and *CYP2D6**10 variant correspond to an enzyme with a decreased activity [(2)]. Identification of *CYP2D6* genotypes allows for prediction of the hydroxylation ability, and therefore, of the metabolizer phenotype [(3-5)].

Due to the existence of various types of metabolizers, in the prospectus on 137 pharmaceutical drugs, the Food and Drug Administration (FDA) stated that people with certain phenotypic characteristics, whether as a result of mutations (or variants) or via drug interactions, are at

[1] *Instituto de Investigaciones Biomédicas* "Dr. Francisco J. Triana Alonso" (BIOMED). Faculty of Health Sciences. *Universidad de Carabobo* headquarters Aragua. Maracay, Aragua state, Venezuela

[a] Physician; [b] licenciate in bioanalysis; [c] magíster scientiarum; [d] specialist in public health, doctorate in molecular biology

This paper incoporates part of the results included in the dissertation: *Determinación del fenotipo metabolizador de CYP2D6 y polimorfismo -582C>T del gen TC/RRAS2 en voluntarios sanos y pacientes con cancer de mama* presented by Carlos Flores-Angulo to obtain the grade of magister scientiarum in biomedical sciences. *Instituto de Investigaciones Biomédicas* "Dr. Francisco J. Alonso Triana" from the *Universidad de Carabobo* headquarters Aragua.
Received: 6/2/2015 Approved: 10/7/2015

Citatiton: Flores-Angulo C, Villegas C, Mora Y, Martinez JA, Oropeza T, Moreno N. Allelic variants of the *CYP2D6: *4, *6* and **10* in a sample of resident from the Aragua state, Venezuela. Rev Peru Med Exp Salud Publica. 2015;32(4):746-51.

risk of adverse effects and may or may not respond to a specific treatment (http://www.fda.gov).

Various population-wide studies have shown that the frequency of alleles in the *CYP2D6* gene varies considerably from one population to another [6]; in Venezuela, data on *CYP2D6* have been reported in studies on the population of the central west region and several native Amerindian populations [7]. Because the degree of mixing varies among different Venezuelan geographical zones, knowledge on the frequency of this gene in other parts of the country is needed in order to obtain the basic information that is important for pharmacogenetic studies. In the present study, we determined the frequencies of CYP2D6 variants *4, *6, and *10 with the aim of predicting the metabolizer phenotype; we used a sample of individuals residing in Aragua State, Venezuela.

THE STUDY

The sample consisted of 145 unrelated individuals residing in various locations in the Aragua State; there were 72.4% of women (n = 105) and 27.6% of men (n = 40), all apparently healthy and of legal age, at age 32.5 ± 10.6 years (mean ± SD). The Aragua State is located in the central region of the country (9º20′ to 10º29′N and 66º40′ to 67º45′W). All participants signed an informed consent form, which was approved by the BIOMED Bioethics Committee.X A sample (1 mL) of peripheral blood was collected from each participant. Genomic DNA was isolated from the samples using a modification of the salting-out method [8].

On the basis of single nucleotide polymorphisms (SNP) and in accordance with those described in the CYP Allele Nomenclature Database (http://www.cypalleles.ki.se), the presence of alleles *CYP2D6*4 (1846G>A* and *100C>T)*, *CYP2D6*4M (1846G>A)*, *CYP2D6*6 (1707delT)*, and *CYP2D6*10* (100C>T) was tested. These variants were selected because they have been studied in population samples from the central-western region and groups of Venezuelan Amerindians [7].

The sequences that contain the SNPs of interest were amplified by polymerase chain reaction (PCR) using the following oligonucleotide primers: a) polymorphism *1846G>A* (rs3892097): 5′-GCT TCG CCA ACC ACT CCG-3′ (direct) and 5′-AAA TCC TGC TCT TCC GAG GC-3′ (reverse) [9]; b) polymorphism *1707 del T* (rs5030655): 5′-CCT GGG CAA GAA GTC GCT GGA CCA G-3′ (direct) and 5′-GAG ACT CCT CGG TCT CTC G-3′ (reverse) [10]; and c) polymorphism *100C>T* (rs1065852): 5′-AAC GCT GGG CTG CAC GGT AC-3′ (direct) and 5′-TGA TGG TCC ATG TCG GTG AGC A-3′ (reverse) (EGT, San Diego, CA, USA). The latter two primers were designed in the Primer3 software, version 0.4.0 (Cambridge, MA, USA).

The SNPs were identified by the restriction fragment length polymorphism (RFLP) method (Table 1), and the incubation conditions for the digestion reactions were those recommended by the manufacturer of the restriction enzymes (Promega®, Madison, WI, USA). The genotypes of each polymorphism were determined according to the restriction fragment profile, as evidenced by polyacrylamide gel electrophoresis. Subjects missing the SNPs under study were classified as CYP2D6*1.

The metabolizer phenotype was inferred according to the genotypes by means of the Activity Score (AS) [3,4] model. The value of 0 was assigned to variants CYP2D6*4 and *6, the value of 0.5 to CYP2D6*10, and 1 to CYP2D6*1 [3,4]. Individuals with an AS of 0 who showed genotypes composed of two inactive alleles (*4 and *6) were classified as poor metabolizers (PM), those with an AS of 0.5 to 1 or with genotypes *4/*10, *6/*10, or *10/*10 were considered intermediate metabolizers (IM), subjects with an AS of 1 to 1.5 and heterozygous for the dominant allele (*1): *1/*4, *1/*6, or *1/*10 were labeled heterozygote-extensive metabolizers for the dominant allele (hetEM),

Table 1. Fragments obtained after restriction digestion of DNA sequences that contain the following polymorphisms: *CYP2D6*4, CYP2D6*6, and CYP2D6*10*

Polymorphism	PCR product (bp)	Restriction enzyme	Size of the wild-type allele (bp)	Size of the polymorphic allele (bp)
1846G>A	354	*Bst*OI	105, 249	354
1707delT	353	*Bst*OI	163, 190	23, 139, 190
100C>T	540	*Kpn*I	20, 520	540

Table 2. Frequency of CYP2D6 genotypes in the Aragua population

Genotype	Active genes	*Activity score*	Phenotype	n	%	(95% CI)
**4/*4*§	0	0	PM	6	4.1	(0.9-7.4)
**6/*6*	0	0	PM	0	0	0
**4/*6*	0	0	PM	0	0	0
**4/*10*	1	0.5	hetEM/IM	0	0	0
**6/*10*	1	0.5	hetEM/IM	0	0	0
**1/*4*‡	1	1	hetEM/IM	30	20.7	14.0-27.4)
**1/*6*	1	1	hetEM/IM	1	0.7	(0.2-1.0)
**10/*10*	2	1	hetEM/IM	0	0	0
**1/*10*	2	1.5	hetEM/IM	3	2.1	(0-4.4)
**1/*1*	2	2	EM	105	72.4	(65.1-79.8)

§: Individuals of this group had a genotype composed of alleles *4 and *4M; ‡Includes four subjects with genotypes *1/*4M; PM: poor metabolizer, hetEM: heterozygote-extensive metabolizer for the dominant allele; IM: intermediate metabolizer; EM: extensive metabolizer

and individuals with an AS of 2 or homozygous for allele *1 were considered extensive metabolizers (EM) [5]. Phenotypes IM and hetEM were included in the same group as proposed by Saladores *et al*. [5].

The resulting allele frequency was compared with that of other populations using the chi squared test with the Yate correction. Differences with $p < 0.05$ were considered statistically significant. The confidence interval was calculated with 95% probability (95% CI) using the SPSS software, version 19 (IBM®, Armonk, NY, USA). For each polymorphism, the Hardy-Weinberg equilibrium was calculated in the POPGENE software, version 1.31 (Edmonton, Canada).

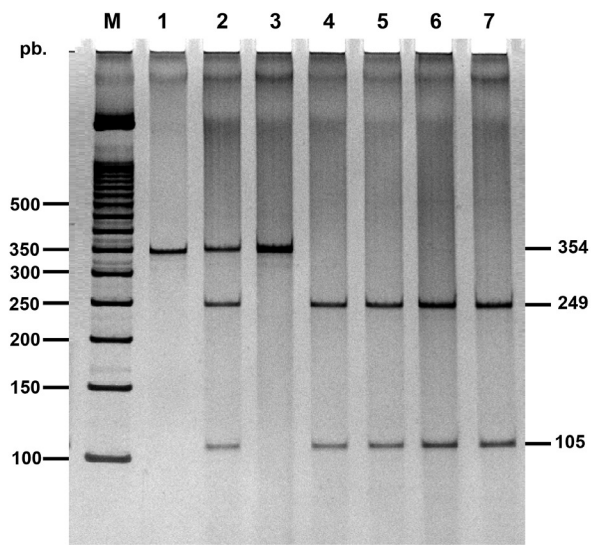

Figure 1. Analysis of the genotypes of polymorphism 1846G>A using polyacrylamide gel electrophoresis (9% gel). M: 50-bp DNA step ladder; Lane 1: PCR product of 354 bp undigested, Lane 2: a person of genotype *1/*4; Lane 3: an individual of genotype *4/*4; Lanes 4–7: subjects with genotype *1/*1

FINDINGS

Of the three polymorphisms analyzed in this work, the three expected genotypes were observed only in the case of *1846G>A* (Figure 1). SNP *1846G>A*, in the homozygous and heterozygous states, was detected at the frequency of 4.1% (95% CI: 0.9–7.4) and 20.7% (95% CI: 14.0–27.4), respectively. Polymorphisms *1707delT* and *100C>T* were detected only in the heterozygous form at the frequency of 0.7% (95% CI: 0–2.1) and 24.1% (95% CI: 17–31.2), respectively. The SNPs under study were found to be in the Hardy-Weinberg equilibrium ($p > 0.05$).

Table 2 shows frequencies of the genotypes of *CYP2D6* variants and a prediction of the metabolizer phenotype. Genotypes identified as *1/*1 correspond to the wild type for each polymorphism analyzed here. Nevertheless, in this group of individuals, the poor, intermediate, and ultrarapid metabolizers are included. These will be discussed when other *CYP2D6* polymorphisms are analyzed.

Table 3 shows comparison of frequencies of allelic variants *CYP2D6*4*, **6*, *and *10* (according to this study) with those reported in Venezuelan populations and frequencies in other countries. Among all the analyzed chromosomes (n = 290), we found that the most frequent allele is *CYP2D6*1* (84.2%; 95% CI: 79.9–88.4). The frequency of allele *4 is 14.5% (95% CI: 10.4–18.6), with subtype *4M found within this group at the frequency of 3.5% (95% CI: 1.3–5.6). Alleles *6 and *10 showed the following frequencies: 0.3% (95% CI: 0–1.0) and 1% (95% CI: 0–2.2), respectively.

Table 3. Frequencies of the allele variants of CYP2D6 in Aragua and other populations

Population	# of evaluated chromosomes	Allele frequency of *CYP2D6* (%)			Reference
		**4*	**6*	**10*	
Aragua population	290	14.5	0.3	1.0	Present study
African Americans	544	3.9	0.6	2.9	3
Bari‡	80	42.5	0	6.3	7
Brazilians	2040	9.4	NE	2.1	11
American Caucasians	694	19.7	1.0	2.2	3
Central-western Venezuelans§	298	13.4	1.3	4.0	7
Colombians	242	19.4	0	NE	14
Costa Ricans	770	15.8	0.3	0.9	15
White Cubans	260	14.6	0.8	0.4	4
Cuban mestizos	252	14.3	1.2	0.8	4
Ecuadorians	236	10.6	0	1.3	16
Spaniards	898	16.5	1.2	2.2	17
Mexican mestizos	250	5.6	0	NE	12
Nicaraguans	196	14.3	0	3.1	4
Panare‡	92	5.4	0	3.3	7
Pemon‡	80	2.5	0	1.3	7
Warao‡	58	1.7	0	1.7	7

NE: Not evaluated; ‡Venezuelan Amerindians residing near peripheral areas of the country; §A sample from the city of Barquisimeto, the capital of the Lara State (9º20' to 10º48'N and 69º9' to 70º24'W).

DISCUSSION

The distribution of *CYP2D6* alleles has great variability according to the population. Our work shows that the study population contains significant differences ($p < 0.05$) in the frequencies of allele *4 with population samples of Brazilians (9.4%) [11], Mexican mestizos (5.6%) [12], and Venezuelan Amerindians: Panare (5.4%) [7], Pemon (2.5%) [7], Warao (1.7%) [7], and Bari (42.5%) [7] (Table 3). The differences in the distribution of *CYP2D6* alleles can be explained by the evolutionary traits of the populations, which are currently revealed by the variability in metabolism and the biological effects of multiple xenobiotics [13].

On the other hand, the frequency of allele *4 found in the Aragua population sample (14.5%) is not statistically different ($p > 0.05$) from that reported in individuals of the central-western region of Venezuela (13.4%) [7] and in populations of American Caucasians (19.7%) [3], Colombians (19.4%) [14], Costa Ricans (15.8%) [15], white Cubans (14.6%) [4], Cuban mestizos (14.3%) [4], Ecuadorans (10.6%) [16], Spaniards (16.5%) [17], and Nicaraguans (14.3%) [4] (Table 3).

Regarding allele *6, no significant differences were observed ($p > 0.05$) in comparison with other populations [3,4,7,15,17] (Table 3); similarly, the same is true for *CYP2D6*10*, whose frequency (1%) is not significantly different ($p > 0.05$) from that previously reported for American Caucasians, Spaniards, and Latin American populations [3,4,11,15-17] (Table 3). Nevertheless, there are significant differences ($p < 0.05$) with two samples of Venezuelan populations: the central-western population (4%) and Bari Amerindians (6.3%) [7]. The statistically significant differences observed after comparison of the frequency in the Aragua population with the one reported in Venezuelan Amerindian populations [7]are consistent with historical data, namely: during the first 150 years of Spanish conquest, the population of Amerindians was reduced by 95%. Therefore, the current traits of the Venezuelan mestizo population serve as evidence of a smaller genetic contribution from these groups relative to the Spanish contribution [18]. It is reported that in the central region where the Aragua State is located, the Spanish contribution is much greater (0.604 ± 18) than the Amerindian (0.235 ± 5) and African (0.161 ± 22) [19] contribution. This fact, along with migration from Europe to America [18], has caused the Venezuelan population to have a considerable frequency of allele *CYP2D6*4*, which is characteristic of the Caucasian population; these data are statistically significantly similar to the data on populations of American Caucasians ($p = 0.063$) and Spaniards ($p = 0.475$; Table 3).

Regarding the frequency of the PM phenotype, we found it to be 4.1%: twice that reported in samples from the population of Venezuelan mestizos in the city of Barquisimeto [7]. This frequency is also greater than frequencies observed in several Latin American mestizo populations [11,12,15,16], in contrast to the Bari Amerindian group, in which 25% frequency [7] was reported. This information has possible applications in clinical practice because it is probable that the PM group represents adverse effects or a weak response to certain common prescription drugs that are metabolized by enzyme CYP2D6, for example, amiodarone, amitriptyline, bisoprolol, carvedilol, captopril, chloroquine, codeine, domperidone, fluoxetine, fluvastatin, haloperidol, indinavir, imatinib, loperamide, nifedipine, ondansetron, tamoxifen, and venlafaxine. For several of these pharmaceutical drugs, there are dosage guidelines based on the genetic traits of patients. This information is available in the PharmGKB® database.

Accordingly, the dose guidelines for the pharmaceutical drug metoprolol, commonly prescribed for hypertension and cardiac arrhythmias, recommend reducing the standard dose by 75% in subjects with the PM genotypes [20] because these people are at risk of adverse effects characteristic of intoxication, such as blurred vision, chest pain, dizziness, syncope, weakness, oliguria, and dyspnea. This example highlights the importance of

continuing research on gene *CYP2D6*, in particular the studies with a greater sample size, both in the central and other regions of the country. The above example also points to the need for analysis of other alleles that have been detected in Venezuela and Latin American populations.

There are few studies in Venezuela on *CYP2D6* and other genes involved in the variability of responses to pharmaceutical drugs; therefore, it is not yet possible to integrate pharmacogenetic knowledge into clinical practice. The results of this research are important for the design of clinical studies because knowledge of the allele frequency is necessary to properly calculate the sample size in case-control, cohort, and survival studies and thus to obtain knowledge that will help to adjust the dose of a particular pharmaceutical drug in accordance with the traits of each individual. This individualized approach may reduce the incidence of adverse effects and increase the desired therapeutic effect in patients with diseases common in the Venezuelan population.

Acknowledgements: the authors thank the volunteers that participated in this study. We are grateful to doctors Flor Herrera and Heriberto Correia of BIOMED-UC for allowing us to perform experiments in their labs and for providing some reagents for the research.

Author contributions: NM and CFA participated in the conception and design of the study. CFA, CV, YM, and TO participated in collection and analysis of data. NM, CFA, CV, and JM participated in the analysis and interpretation of data. CFA participated in writing of the manuscript. NM and JM participated in critical review of the manuscript. NM, CFA, CV, YM, JM, and TO approved the final version. CFA, CV, and TO participated in the recruitment of patients and collection of study material. NM obtained the funding.

Funding sources: funding was received from the National Fund for Science, Technology, and Innovation (FONACIT) through projects 2012001248 and 2012002328. Some of the equipment was funded by Mission Science project No. 2008000911-1.

Conflicts of interest: the authors declare that the research was conducted in the absence of any commercial relationship or funding that could result in a possible conflict of interest.

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Correspondence: *Nancy Moreno.*
Address: BIOMED Final de la calle Cecilio Acosta Las Delicias - Maracay Estado Aragua Venezuela
Phone number: 58-243-2420577 y 58-243-2425822.
E-mail: nanmorja@hotmail.com