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Characterization of the CYP2D6 drug metabolizing phenotypes of the Chilean mestizo population through polymorphism analyses

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ABSTRACT

We tested the influence of four polymorphisms and gene duplication in *CYP2D6* on *in vivo* enzyme activity in a Chilean mestizo population in order to identify the most relevant genetic profiles that account for observed phenotypes in this ethnic group.

*CYP2D6**2 (2850C>T), *3 (2549A>del), *4 (1846G>A), *17 (1023C>T) and gene duplication were determined by PCR-RFLP or PCRL in a group of 321 healthy volunteers. Individuals with different variant alleles were phenotyped by determining debrisoquine 4-hydroxylase activity as a metabolic ratio (MR) using a validated HPLC assay.

Minor allele frequencies were 0.41, 0.01, 0.12 and 0.00 for *CYP2D6**2, *3, *4 and *17 variants, respectively, and the duplication frequency was 0.003. Genotype analysis correlated with phenotypes in 18 of 23 subjects (78.3%). 11 subjects were extensive metabolizers (EM), 8 were intermediate metabolizers (IM), 2 were poor metabolizers (PM) and 2 were ultra-rapid metabolizers (UM) which is fairly coincident with expected phenotypes metabolic ratios ranged from 0.11 to 126.41. The influence of *CYP2D6**3 was particularly notable, although only heterozygote carriers were present in our population. Individuals homozygous for *4 were always PM. As expected, the only subject with gene duplication was UM.

In conclusion, there was a clear effect of genotype on observed CYP2D6 activity. Classification of EM, PM and UM through genotyping was useful to characterize CYP2D6 phenotype in the Chilean mestizo population.

1. Introduction

Although expressed at lower levels compared to other human P450, CYP2D6 is one of the most studied and important isoenzymes given the number of metabolic routes of drugs and xenobiotics that it catalyzes. It is estimated that between 20 and 25% of clinically used drugs, some of which have a narrow therapeutic range, are metabolized by this enzyme. *CYP2D6* polymorphisms were discovered in the 1970s [1]. Subsequently, Mahgoub et al. [2] and Eichelbaum et al. [3] independently discovered that debrisoquine and sparteine are metabolized by CYP2D6, demonstrating the polymorphic nature of the drugs. More than 100 drugs have been shown to be substrates of this enzyme [2,3], including antidepressants, neuroleptics, β-blockers and antiarríthmics [4].

The *CYP2D6* gene is located on chromosome 22q13.1 in a gene cluster spanning about 45 kb, including the CYP2D7 and CYP2D8 pseudogenes with 97% and 92% homogeneity, respectively. CYP2D6 includes nine exons and eight introns, with an open reading frame of 1383 bp encoding 461 amino acids. The *CYP2D6* gene locus is complex and highly polymorphic, and variants within this locus affect CYP2D6 activity resulting in a wide range of enzyme activity from absence of activity to ultrarapid metabolism [5,6]. To date, more than 105 CYP2D6 variants and more than 20 SNPs without determined haplotypes have been described [7].

Abbreviations: CYP, cytocrome P450; HPLC, high performance liquid chromatography; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism.

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Table 1
Anthropometric characteristics of the studied population. Values represent means ± SD for the number of subjects indicated. The significant differences between mean values (p-value < 0.05), assessed by Student's t test. BMI, body mass index.

Characteristics	Female $n = 188$	Male $n = 133$	Total $n = 321$[a]	p-Value
Age (years)	31 ± 12.3	30 ± 12.2	31 ± 12.0	0.472
Weight (kg)	61.8 ± 9.1	74.9 ± 10.1	66.8 ± 11.4	0.001
Height (m^2)	1.60 ± 0.10	1.73 ± 0.07	1.65 ± 0.09	0.001
BMI (kg/m^2)	24.4 ± 3.2	25 ± 2.9	24.4 ± 3.0	0.087

[a] Amerindian–Caucasian admixture = 18%.

One example of the clinical importance of the CYP2D6 polymorphisms is the tricyclic antidepressant nortriptyline which is cleared by this enzyme: poor metabolizers (PM) need 30–50 mg compared with ultrarapid metabolizers (UM) requiring 500 mg to achieve similar plasma levels. The standard dose of 150 mg is therefore not suitable for PM and UM [8].

The metabolic ratios for CYP2D6 are distributed in a bimodal manner in Caucasian populations, existing individuals with deficient activity (PM) and others with high enzyme activity (UM) [9–12].

CYP2D6 polymorphisms in human populations exhibit large differences in allele frequencies according to ethnicity [13–15]. In Caucasian populations, UMs may have a duplication/multiplication of the gene in about 7% of the population, while single base mutations (e.g. *4, *6, *8, *10, *17) of reduced function [16], partial deletions (*3) or complete deletion of the gene (*5) are nonfunctional alleles [17], all resulting in PM, are found in more than 10% of this population. In Latin America, the frequency of PMs is about 6.6% in Colombia, 3.2% in Mexico, among 2.2% and 4.4% and 3.6% in Panama and Nicaragua and about 10% in Chile [14,18–20].

As the CYP2D6 genotype has been associated with interindividual variability in the activity of the enzyme, there are several *in vivo* assays to evaluate this activity on the basis of the urinary metabolic ratio (MR: drug/urinary metabolite) for sparteine, metoprolol, dextromethorphan and debrisoquine, debrisoquine being the most widely used substrate probe due to its ability to discriminate EM, IM and UM phenotypes. This drug is an antihypertensive agent no longer in clinical use, metabolized by CYP2D6 to 4-hydroxydebrisoquine [1].

Variation in CYP2D6 metabolic activity is one of the most important factors giving rise to inter-individual variation in response to several drugs [21]. Determining CYP2D6 phenotypes, or metabolic capability, promises to help identify individuals for whom a change in drug or drug dosage is appropriate. To achieve this goal, analysis of genotypes is the preferred method when genotypes translate well to observed phenotype in metabolism; however, this is not confirmed in all populations. There can be substantial differences in test interpretation as there is no standardized process of translating CYP2D6 genotype to purported phenotype.

We studied the presence of *CYP2D6**2 (2850C>T, rs1135840, R296C), *3 (2549A>del, rs35742686, frameshift), *4 (1846G>A, rs3892097, splicing defect), *17 (1023C>T, rs28371706, T107I) and the duplication of the gene, with respect to enzyme activity in a Chilean mestizo population in order to identify the most relevant genetic profiles that account for observed CYP2D6 catalytic activity.

2. Materials and methods

2.1. Study population

321 people healthy volunteers (both sexes, 31 ± 12 years old) were recruited from the general population in Santiago, Chile (Table 1). All subjects provided informed consent. The protocol was approved by the Ethics Committee for Human Research at the Faculty of Medicine, University of Chile. The Amerindian–Caucasian admixture was determined to be 18%using ABO blood group distribution (BioClone®, ABO Pharmaceuticals, EE.UU) the Hardy–Weinberg and Bernstein equation [22–24].

A sub-group of 25 individuals, selected according to genotype, were asked to participate in an *in vivo* determination of CYP2D6 enzyme activity, were informed accordingly and consented to participate. Inclusion criteria were: healthy subjects, nonsmokers and without a history of alcohol or drug use. All individuals were screened for suitability by an extensive review of their medical history, physical examination such as blood pressure and pulse, and interpretation of standard biochemical analyses (e.g. glucose, blood urea nitrogen, creatinine, total protein, albumin, total bilirubin, alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase, drugs of abuse). Exclusion criteria included: HIV, hepatitis B or hepatitis C infection; consumption of any prescribed or over-the-counter medication 60 days prior to participation; involvement in a clinical trial 90 days prior to participation. For female subjects exclusion criteria included pregnancy, planning pregnancy and breast-feeding.

2.2. DNA extraction and genotyping

Extraction of genomic DNA from whole blood was performed using a commercial kit (High Pure PCR Template Preparation Kit, Roche Diagnostics®). DNA samples were quantified and stored at −20 °C until further analysis. Genomic DNA was amplified by PCR using primers for the specific variants studied (Table 2). PCR-RFLP was used to determine *CYP2D6**2, *3, *4, and *17. Amplicons were digested using appropriate restriction enzymes (Table 2). Electrophoresis was performed according to previously reported methods, with minor modifications, in agarose 2% or polyacrylamide16% gels [25]. *CYP2D6* gene duplication was detected using a modified long PCR method (PCRL), described previously [26,27]. The method is based on the detection of a homologous 3.6 kb sequence downstream of *CYP2D6* and *CYP2D7*, which identifies the presence of the duplication. Representative PCR analyses are shown in Fig. 1.

2.3. Evaluation of CYP2D6 enzyme activity

Phenotyping was performed in 25 previously genotyped subjects (26 ± 9.2 years old, body mass index: 22.8 ± 2.4 kg/m^2), according to inclusion and exclusion criteria as describe in Section 2.1. Volunteers were instructed about the procedure for collecting urine at home and were provided with appropriate receptacles and a single tablet of Declinax®, containing 22.5 mg debrisoquine sulfate, equivalent to 20 mg debrisoquine. Flyers were provided instructing not to eat the previous day from 19:00 pm until 00:00 am, and avoid coffee, alcohol and strenuous exercise. Instructions indicated to take a urine sample at 23:00 pm, designated as "control" and proceed to completely empty the bladder, and then ingest the tablet with 250 mL of water. Following 8 h, a subsequent urine sample was to collected, designated as "debrisoquine sample". Samples were processed up to 2 h after completion of the collection of urine samples, and were kept refrigerated (2–6 °C) throughout the process of collection and transportation to the laboratory. 10 mL aliquots of urine samples were stored at −20 °C for subsequent HPLC analyses.

2.4. Substrate determination

Following liquid–liquid urine extraction, chromatographic analysis was performed using a validated method adapted [28,29]. HPLC (Shimadzu LC-20 AT) separation employed to ODS-3 C18 Inertsil column followed by detection with a fluorescence detector