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ORIGINAL ARTICLE

Haplotype structure and allele frequencies of *CYP2B6* in Spaniards and Central Americans

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ABSTRACT

This study was aimed to investigate the potential differences in allele frequencies of the *CYP2B6* gene between Spaniards and Central Americans. Three single nucleotide polymorphisms of the *CYP2B6* gene 516 G>T, 785 A>G and 1459 C>T were assayed by a polymerase chain reaction in 180 Spaniards and 182 Central Americans. The allele frequencies for *CYP2B6*1*, *CYP2B6*4*, *CYP2B6*5*, *CYP2B6*6*, *CYP2B6*9* in Spaniards and Central Americans were 0.593 and 0.642, 0.062 and 0.073, 0.113 and 0.030, 0.215 and 0.230, 0.014 and 0.023, respectively. *CYP2B6*5* was less prevalent among Central Americans than in Spaniards ($P < 0.001$). In comparison to other previously studied populations, the *CYP2B6*5* allele frequency among Spaniards was similar to other Caucasian or African groups, and higher than that in Asian populations. The *CYP2B6*5* allele frequency in Central Americans was lower than that in Africans or Caucasian groups and higher than in Asians. The results indicate the presence of ethnic differences in *CYP2B6* genetic variants between Spaniards and Central Americans, and support the need for further investigations to explore whether these differences significantly alter the efficacy or toxicity of CYP2B6 substrate drugs.

INTRODUCTION

CYP2B6 belongs to the set of drug metabolizing P450 isoforms. CYP2B6 represents approximately 1–10% of the total CYP content in the liver and it is also present in other tissues, such as lung, brain, kidney, intestine, trachea, or oral mucosa [1–3].

CYP2B6 plays a significant role in the biotransformation of a number of endogenous and exogenous compounds including some procarcinogens [4,5]. The involvement of CYP2B6 in the metabolism of clinically used drugs is of a particular significance for bupropion [6,7], and efavirenz [8], artemisinin [11], and some others. CYP2B6 participates also in the biotransformation of testosterone, artemisin, ketamine, propofol, meperidine, sertoline, fluoxetine, tramadol, selegiline and methadone [9–11].

CYP2B6 expression levels and activity in human liver microsomes vary more than 100-fold among individuals [10]. Genetic polymorphism in the human *CYP2B6* is an important factor determining the wide interindividual variation in CYP2B6 expression and/or enzyme activity. To date, more than 48 variant alleles have been described at the *CYP2B6* gene [12]. Some of these variant alleles with significant impact on the CYP2B6 expression level and activity [3,13] are: *CYP2B6*4* (785 A>G), *CYP2B6*5* (1459 C>T), *CYP2B6*6* (516 G>T and 785 A>G), and *CYP2B6*9* (516 G>T). *CYP2B6*4* is

associated to higher enzyme activity both in vitro [14] and in vivo [15]. By contrast, a significant lower CYP2B6 activity in vitro has been observed to be associated with the *CYP2B6*5* allele [3,13]. The 516 G>T SNP, which is present in both *CYP2B6*6* and *CYP2B6*9* allele significantly reduces CYP2B6 protein expression and enzyme activity [16], with important repercussions on both pharmacokinetics and therapeutics outcomes of some substrate drugs, such as methadone [17], bupropion [18], and efavirenz [19]. The increase in CYP2B6 activity by the additional 785 A>G mutation in *CYP2B6*6* haplotype appears not to overcome the decrease in CYP2B6 expression or activity produced by the 516 G>T SNP [20,21].

Among the most important migratory flows to Spain, that of Central America has been increasingly growing due to the absence of any language barrier. El Salvador and Nicaragua are located very close within the same geographical area in Central America and these two countries have been affected by identical natural catastrophes and migratory movements and hence, the population structure is very similar. Mestizos are the most representative and the largest population group in Central America. They are descendents of Spanish (Caucasian) and Amerindian people.

The frequency of functional SNPs varies widely between ethnic groups and, hence, ethnicity is an important variable contributing to interindividual variability in drug metabolism, response and toxicity [22]. Therefore, it is important to determine the genetic variants responsible for altered CYP2B6 expression and/or activity.

The aim of this study was to detect interethnic differences in *CYP2B6* genetic polymorphisms between North Spaniards and Central American mestizo people.

MATERIALS AND METHODS

Subjects

The total study population consisted of 361 healthy, unrelated individuals. Among them, 180 were Spaniards (97 males and 83 females) 89 from El Salvador (41 males and 48 females) and 92 from Nicaragua (43 males and 49 females). Means and standard deviations of age in years were 25.1 ± 4.9 (range, 18–48), 24.4 ± 4.7 (range, 18–46), and 27.3 ± 7.4 (range, 19–51) for those individuals from El Salvador, Nicaragua and Spain. All participants from Central America were mestizos (Amerindian and European descendents). The Spaniard participants were white people from the same geographical area (Northern of Spain). Individuals were randomly selected among students and personnel of the Medical Schools from the following Universities: El Salvador (San Salvador, El Salvador), Nacional Autónoma of Nicaragua (León, Nicaragua), and Zaragoza (Zaragoza, Spain). Written informed consent was obtained from all participants in the study which was approved by the Ethics Committee of Aragón (Zaragoza, Spain) and was conducted in accordance with the Declaration of Helsinki and its subsequent revisions.

Genotyping

Genomic DNA was extracted from peripheral blood, blotted and dried on filter paper using the QIAamp DNA microkit (Izasa, Madrid, Spain). For the *CYP2B6* variant, genotyping of 516 G>T in exon 4 (*CYP2B6*6*, *CYP2B6*9*), 785 A>G in exon 5 (*CYP2B6*4*, *CYP2B6*6*) and 1459 C>T in exon 9 (*CYP2B6*5*, *CYP2B6*7*) was carried out following a method previously described [23], with minor modifications. The method consisted of a two-step polymerase chain reaction (PCR).We used negative and positive controls in all reactions. In the first PCR step we performed a multiplex reaction for amplification of three fragments of 679 bp, 1524 bp and 920 bp in exons 4, 5 and 9 respectively. The second PCR step was an allele-specific reaction for each polymorphism. The products obtained were electrophoresed on a 3% agarose gel and stained with ethidium bromide. The primers used in this study for the multiplex reaction were: for fragment in exon 4, mpr4F-5′-AACTGTACTCACTCCCAGAGT-3′ and mpr4R-5′-CTGATTCTTCACATGTCTGCG-3′; for fragment in exon 5, mpr5F-5′-GTAGTCCTAACATGTCAGCAG-3′ and mpr6R-5′-AGAGCCTACAGTGCTCCCA-3′; and for fragment in exon 9, mpr9F-5′-ACAGAAGCTAGAAACTCTCTTATAT-3′ and mpr9R-5′-GCACTCACTTGCAATGTGACC-3′. For allele-specific reaction, the primers were: for 516G>T, 516G-5′-CCCACCTTCCTCTTCCAG-3′; 516T-5′-ACCCCACCTTCCTCTTCCAT-3′ and 516rev-5′-ATAAGCTGC ATTTCTGAGCCC-3′); for 785A>G, 785A-5′-GTAGGTGTCGATGAGGTCCT-3′; 785G-5′-TAGGTGTCGATGAGGTCC C-3′ and 785rev-5′-GAGATATAGAGTCAGTGAG TGA-3′; for 1459C>T, 1459C-5′-AGCGGGGCAGGAAGCG-3′; 1459 T-5′-CAGCGGGGCAGGAAGCA-3′ and 1459rev-5′-GAACGTGGCACATCCACTCA-3′.

Statistical analysis

CYP2B6 allele data from this study was analysed by the population genetic data analytical program ARLEQUIN

version 3.11 based on an expectation-maximization (EM) algorithm [24] for the following procedures:
(a) the calculation of *CYP2B6* allele and genotype frequencies;
(b) the estimation of heterozygosity in each polymorphism in Hardy–Weinberg (H–W) proportion (estimation of *P* values by the Markov chain method);
(c) the estimation of maximum-likelihood haplotype frequency;
(d) the pair-wise linkage disequilibrium (LD) by χ^2 test. Lewontin's D' values were obtained to further characterize the extent of LD using the Excoffier–Laval–Balding (ELB) algorithm by ARLEQUIN software. Differences in allele and genotype frequencies between populations were measured by the Fisher's exact or χ^2 test (MEDCALC 9.5 statistical software for Windows). For all statistical analysis, a *P* value of <0.05 was considered to be significant.

RESULTS

The frequencies of SNPs, haplotypes and genotype combinations were extremely similar in the two populations of El Salvador and Nicaragua ($P > 0.05$ in all comparisons). Therefore, these results allowed us to integrate these two populations in a single Central American population to be compared with that of Spaniards and other populations.

Table I shows the frequencies of the SNPs 516 G>T, 785 A>G and 1459 C>T in Spaniards, Central Americans and also in subjects from other populations as described in previous reports [3,23,25–28]. The frequencies of 516 G>T and 785 A>G were similar in Spaniards and Central Americans ($P < 0.05$) with no significant differences in the comparisons with Asian, African and other Caucasian groups ($P > 0.05$), with the only exception of the Japanese group in the case of 516 G>T SNP (differences with Spaniards, $P < 0.01$; differences with Central Americans, $P < 0.001$).

The frequency of the 1459 C>T SNP among Spaniards was not different to that previously found in other Caucasian or African American populations ($P > 0.05$ in all cases) but was found to be higher than that observed in Japanese or Chinese subjects ($P < 0.0001$ in the two comparisons). Nevertheless, 1459 C>T frequency in Central Americans was markedly lower in relation to that found in Spaniards, other Caucasian populations such as British, Germans or Swiss ($P < 0.0001$ in all cases), and African-Americans ($P < 0.05$). This frequency in Central Americans was higher than in the Japanese ($P < 0.05$) or in Chinese ($P < 0.01$).

CYP2B6 allele (or haplotype) and genotype (or diplotype) frequencies observed in both Spaniards and Central Americans corresponded to those predicted by the Hardy-Weinberg law ($P > 0.05$ in all cases).

The results of the haplotype analysis using an expectation-maximization (EM) algorithm revealed five types of maximum-likelihood haplotypes in the two populations of Spaniards and Central Americans. These haplotypes for the loci studied here were *CYP2B6*1*, *CYP2B6*4*, *CYP2B6*5*, *CYP2B6*6*, and *CYP2B6*9*. The haplotype frequencies in percentages are shown in *Table II*. Data comparisons between the two populations showed that most of the frequencies were similar ($P > 0.05$). However, *CYP2B6*5* allele frequency was more prevalent among Spaniards in relation to Central Americans ($P < 0.0001$) (*Table II*). As shown in *Table III*, the rank order for *CYP2B6*5* allele frequencies in several populations was the following: Han Chinese < Japanese < Central Americans < African Americans < white Caucasians, the last group includes Germans, Spaniards, Swiss and British. In addition, *Table III* shows the

Table I *CYP2B6* SNPs frequencies (%) observed in this study and comparison with other populations.

		SNPs			*P* value 1459C>T		*P* value 516G>T		
Population	Subjects (*n*)	516G>T	785A>G	1459C>T	Spaniard	Central American	Spaniard	Central American	Reference
Spaniard	180	23.0	27.0	11.0		<0.0001		n.s	This study
Central American	181	25.0	30.0	3.1	<0.0001		n.s		This study
Caucasian (German)	215	28.0	32.0	14.0	n.s	<0.0001	n.s	n.s	[3]
Caucasian (Swiss)	141	24.8	28.7	12.1	n.s	<0.0001	n.s	n.s	[25]
Caucasian (British)	135	28.1	30.4	12.2	n.s	<0.0001	n.s	n.s	[23]
Japanese	265	16.0	26.0	1.0	<0.0001	<0.05	<0.01	<0.01	[26]
Han Chinese	193	21.0	28.0	3.0	<0.0001	<0.01	n.s	n.s	[27]
African-American	45, 42, 39	27.8	29.8	9.0	n.s	<0.05	n.s	n.s	[28]

n.s: not significant; *P* values are from χ^2 test.

Table II *CYP2B6* haplotype frequencies.

Haplotype	Expected effect on enzyme activity	Spaniards (Subjects, *n* = 180)		Central Americans (Subjects, *n* = 181)		*P* value
		Haplotype frequencies (CI)	*n*	Haplotype frequencies (CI)	*n*	
*CYP2B6*1* (Wild type)		59.35% (54.29–64.38)	214	64.22% (59.29–69.11)	233	0.2
*CYP2B6*4* (785 A>G)	Increased [25]	6.20% (4.06–9.07)	22	7.32% (4.94–10.31)	26	0.65
*CYP2B6*5* (1459 C>T)	No significant effect [37,38] Decreased (in vitro) [3]	11.38% (8.549–15.07)	41	3.03% (1.69–5.34)	11	<0.0001
*CYP2B6*6* (516 G>T; 785 > G)	Decreased [25]	21.57% (17.71–26.20)	78	23.06% (20.17–28.98)	84	0.69
*CYP2B6*9* (516 G>T)	Increased (in vitro) [20]	1.47% (0.58–3.19)	5	2.34% (1.11–4.28)	8	0.56

CI, 95% confidence interval; *P* values are from χ^2 test.

Table III CYP2B6 allele frequencies (%): comparisons between Central Americans and other populations including Spaniards.

Population (*n*)	*CYP2B6*1*	*CYP2B6*4*	*CYP2B6*5*	*CYP2B6*6*	*CYP2B6*9*	References
Han Chinese (386)	67.1 (n.s.)	9.1 (n.s.)	0.3[c]	18.4 (n.s.)	1.8 (n.s.)	[27]
Japanese (530)	68.5 (n.s.)	9.3 (n.s.)	1.1[a]	16.4[b]	NA	[26]
Central American (362)	64.2	7.3	3.0	23.1	2.3	This study
African-American (70)	44.3[c]	0.0[b]	8.6[a]	32.8 (n.s.)	0.0	[28]
German (430)	50.7[d]	4.0[a]	10.9[c]	25.6 (n.s.)	NA	[3]
Spaniard (360)	59.3 (n.s.)	6.2 (n.s.)	11.4[d]	21.6 (n.s.)	1.4 (n.s.)	This study
Swiss (282)	55.6[b]	3.9 (n.s.)	12.1[d]	24.8 (n.s.)	NA	[25]
British (270)	53.7[c]	2.2[c]	12.2[d]	28.1 (n.s.)	NA	[23]

NA: data not available; n.s.: non significant differences; *P* values are from χ^2 test: a: borderline significance ($P = 0.06$); b: $P < 0.05$; c: $P < 0.01$; d: $P < 0.001$.

CYP2B6 allele frequencies in these latter populations, as well as a comparative analysis between Central Americans and the other groups.

Linkage disequilibrium (LD) analysis among pairs of SNPs in each population was performed to further analyse the haplotype data. ARLEQUIN pair-wise LD between SNPs showed that LD was significantly present (χ^2 test based in EM algorithm) in Spaniards (between 516 G>T and 785 A>G, $P < 0.0001$; between 516 G>T and 1459 C>T, $P = 0.0003$;and between 785 A>G and 1459 C>T, $P = 0.0001$) and also in Central Americans (between 516 G>T and 785 A>G, $P = 0.0001$; between 516 G>T and 1459 C>T, $P = 0.045$; and between 785 A>G and 1459 C>T, $P = 0.0192$). *Table IV* shows the extent of LD, measured by Lewontin's D' values, which are independent on the SNP frequency. LD was similar in the two populations with a strong to complete LD between locus pairs as reflected by the high D' values in Spaniards and Central Americans.

The most frequent genotype among Spaniards was *CYP2B6*1/*1* (35.5%), followed by *CYP2B6*1/*6* (25%), *CYP2B6*1/*5* (15%), *CYP2B6*1/*4* (6.1%), and *CYP2B6*6/*6* (5.5%), and the most frequent genotypes among Central Americans was *CYP2B6*1/*1* (43.6%), followed by *CYP2B6*1/*6* (24.3%), *CYP2B6*1/*4* (9.4%), *CYP2B6*6/*6* (7.1%), *CYP2B6*1/*5* (4.9%), and *CYP2B6*4/*6* (4.9%).

Table IV Lewontins D' values in several populations.

Population	*n*	SNP pairs			References
		516-785	516-1459	785-1459	
Asian American	61	1,000	1,000	1,000	[40]
Caucasian American	60	0,838	0,980	0,974	[40]
Hispanic American	77	0,875	0,987	0,997	[40]
African American	93	0,975	0,999	0,998	[40]
Spaniard	180	0,899	1,000	1,000	This study
Central American	181	0,875	1,000	1,000	This study

DISCUSSION

In the present study, we determined the allele frequencies for three SNPs at the *CYP2B6* gene in a Spaniard population (*Table I*), since only limited information was available regarding *CYP2B6* polymorphism variations in this population; the information being restricted to

516 G>T polymorphism [29]. In addition, although the frequencies of *CYP2B6* polymorphisms in other Caucasian populations is well documented [3,23,25] a geographical gradient according to latitude has been observed for some polymorphisms in Europe [30,31]. According to the frequency distribution of the variants 516 G>T, 785 A>G and 1459 C>T, we have found that Spaniards present a similar profile than that reported for other Caucasian groups. Therefore, like in other Caucasian populations, the frequency of the allele variant 785 A>G was similar in Spaniards, Asians, and African-Americans. This same result was obtained in relation to the 516 G>T SNP with the exception of a higher frequency in Spaniards than in Japanese. In this regard, it should be noted that differences between Japanese and Chinese have been reported in relation to 516 G>T frequency [32]. With regard to the 1459 C>T SNP, a higher prevalence in Spaniards and other Caucasians than in Asian or African populations has been detected, which is in agreement with previous reports on Caucasian groups [3,23,25].

Our study is the first to describe the distribution of *CYP2B6* SNPs (*Table I*), haplotypes (*Table II*) and genotypes in a Central American population. With regard to this population, a similar frequency profile of 516 G>T and 785 A>G SNPs has been found in relation to Spaniards. By contrast, the 1459 C>T SNP in Central Americans was less frequent than that in Spaniards ($P < 0.001$), other Caucasian groups ($P < 0.001$) and African-Americans ($P < 0.05$), and higher than that previously found in either Japanese ($P < 0.01$) or Chinese ($P < 0.001$). The biracial admixture of population in mestizo people with a gene pool derived from Native Amerindian groups (originating from Asia) with Caucasians coming from Spain and other European countries could explain the pattern of 1459 C>T frequency found here in Central Americans. In this regard, we have observed a similar result for *CYP3A5*1* allele with a frequency distribution between that of Spaniards and that of Asians [33]. The 1459 C>T frequency profile in Central Americans would be in consonance with the fact that rare variants, such as 1459 C>T appear to be recently derived and also sensitive indicators of recent migration and relation between various populations [34].

The lower frequency of the haplotype *CYP2B6*5* in Central Americans in relation to Spaniards (*Table III*) is something expected since *CYP2B6*5* contains the 1459 C>T SNP which has resulted to be less prevalent among Central Americans. Thus, the *CYP2B6*5* allele frequency in Central Americans was between that of Spaniards and Asian groups (*Table III*).

Linkage disequilibrium (LD) analysis has shown complete LD ($D' = 1$) between the pairs of loci sharing the rare 1459 C>T allele. Nevertheless, a D' value below 1 has been obtained for LD between the common alleles 516 G>T and 785 A>G in both Spaniards and Central Americans, the latter probably giving some evidence of historical recombination. Indeed, common alleles are generally older than rare alleles, there has been more historical opportunities for recombination to break down ancestral haplotypes, and show less LD than rare, younger or more geographically localized variants [35,36].

In this study, three non-synonymous mutant alleles of the *CYP2B6* gene, 516 G>T, 785 A>G and 1459 C>T have been genotyped. We inferred that the samples in which these mutant alleles were not detected had the wild-type *CYP2B6*1*. This could be a limitation of the present study. However, the frequencies of the variant alleles not analysed in this work are extremely low (*CYP2B6*2*) or even absent (CYP2B6*17 and *CYP2B6*18)* in Caucasian and in Oriental populations [39], and therefore the present study design is able to detect over 95% of *CYP2B6* variant alleles in the populations analysed in this study. Another potential limitation could rise from the absence of genetic markers of the mestizo population included in this study. However, subjects were classified as mestizo people by the morphological traits, skin colour and self-identification and these are the data that are available for clinicians in the current clinical practice.

Although lower enzyme activity and protein expression has been clearly found to be linked to *CYP2B6*5* haplotype in 'in vitro' studies [3,13], lack of significant phenotype/genotype association in 'in vivo' studies using efavirenz has been reported [37,38]. The discrepancy between in vitro and in vivo data has been explained by an increased specific activity of *CYP2B6*5* variant towards efavirenz which may thus compensate an inherent low expression [39]. Hence, it remains to be investigated for which substrates and under which conditions a lower frequency of *CYP2B6*5* could be clinically important.

In summary, the frequencies of the *CYP2B6*1*, *CYP2B6*4*, *CYP2B6*6* and *CYP2B6*9* have been detected to be similar in Spaniards and Central Americans. By contrast, the frequency of *CYP2B6*5* allele was lower in Central Americans compared to Spaniards and other Caucasian groups, but higher than in Asian

populations. These data reflect the biracial origin of mestizo population of Central America and provide further evidence of ethnic heterogeneity in *CYP2B6* polymorphism.

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