

Lower frequency of *CYP2C9*2* in Mexican-Americans compared to Spaniards

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ABSTRACT

Interethnic differences in cytochrome *P450* polymorphism might be responsible, at least in part, for the variations in drug disposition between ethnic groups. Of the various *CYP2C9* alleles, *CYP2C9*2* and *CYP2C9*3* have been reported to have altered catalytic activities compared to the wild-type *CYP2C9*1*. The present study is aimed at analysing the *CYP2C9* polymorphism in a Mexican-American compared with a Spanish population. Differences between the two populations of healthy volunteers, Mexican-Americans ($n=98$ subjects) and Spaniards ($n=102$ subjects), regarding the *CYP2C9* allele frequencies have been found. *CYP2C9* genotypes among the studied Mexican-American population are in equilibrium. The 95% CI were, respectively, 0.81–0.90 for *CYP2C9*1* ($n=169$), 0.05–0.13 for *CYP2C9*2* ($n=16$) and 0.031–0.10 for *CYP2C9*3* ($n=11$). *CYP2C9*4*, *5 and *6 were found in none of the studied subjects. The frequency of *CYP2C9*2* was lower among Mexican-Americans compared to Spaniards ($P<0.05$). The obtained frequency of *CYP2C9* alleles is compatible with the genomic assembly of the constitutive potential ethnic origin of this population, and supports the need of pharmacogenetic studies for optimizing the recommended drug dosages to Mexican-Americans.

The Pharmacogenomics Journal (2004) 4, 403–406. doi:10.1038/sj.tpj.6500278
Published online 28 September 2004

Keywords: *CYP2C9*; Mexican-Americans; Hispanics; Spaniards; pharmacogenetics

INTRODUCTION

The genetic polymorphism of the cytochrome *P450* enzymes is one of the major determinants of the interindividual variability of pharmacokinetics and drug response. Human cytochrome *P450* (CYP) is a group of haemoproteins catalysing the oxidative phase I metabolism of many exogenous and endogenous substrates.¹ Among them, the CYP2 family, especially the CYP2C subfamily, is large and complex.² *CYP2C9* constitutes approximately 20% of the total human liver microsome CYP protein content, and metabolizes approximately 10% of therapeutically important drugs.^{3–5} The *CYP2C9* isoenzyme is primarily responsible for the oxidative metabolism of several clinically important compounds, including the narrow therapeutic index drugs warfarin and phenytoin, and other routinely prescribed compounds such as losartan, tolbutamide and numerous nonsteroidal anti-inflammatory drugs such as ibuprofen, piroxicam, tenoxicam and diclofenac.⁵

CYP2C9 exhibits marked interindividual variability in its expression and catalytic activity due to functionally significant genetic variations. This can result in either drug toxicity (eg, warfarin-induced bleeding complications) or

Received: 15 July 2004
Revised: 28 July 2004
Accepted: 29 July 2004
Published online 28 September 2004

therapeutic failure in some patients who take standard doses of CYP2C9 substrate drugs.^{6,7}

The human gene coding for the CYP2C9 protein has been mapped to chromosome 10q24.2 and is greater than 55 kb in length.⁴ At present, at least there are six CYP2C9 alleles studied in humans. The most common allele is designated as CYP2C9*1, and is considered the wild-type allele. A cytosine-to-thymine transversion at nucleotide 430 encodes for a cysteine substitution at amino-acid residue 144, producing the CYP2C9*2 variant allele. An adenine-to-cytosine transversion at nucleotide 1075 encodes for a leucine substitution at amino-acid residue 359, producing the CYP2C9*3 variant allele. Recently, a substitution at base pair 1076 has been detected, coding for an Ile₃₅₉Thr amino-acid change, producing the CYP2C9*4 variant allele. A cytosine-to-guanine polymorphism at base pair 1080, encoding for an Asp₃₆₀Glu amino-acid substitution (CYP2C9*5), has also recently been identified. Finally, CYP2C9*6 variant allele has been identified; this is a new null polymorphism containing an adenine base pair deletion at nucleotide 818, which results in a premature stop codon and a truncated inactive protein.^{8,9}

The genotypic features of the CYP2C9 gene have been studied in several ethnic groups (Table 1); very low frequency of alleles CYP2C9*2 and *3 has been found among Oriental populations. We have previously shown the genetic polymorphism of CYP2C9 among Spaniards.¹⁰ Despite the fact that Hispanics constitute one of the largest groups in the world, including USA, so far there is a paucity of pharmacogenetic studies in this group. Hispanics from Mexican origin is one of the largest ethnic groups in California; the ethnical background of this group includes an admixture of ancient Amerindians living in Mexico and Europeans from Spain.

The present study was aimed at analysing the CYP2C9 genetic polymorphism in a Mexican-American population compared with a Caucasian Spanish population.

RESULTS

The CYP2C9 genotype frequencies among the Mexican-American subjects studied (Table 2) corresponded to those predicted by the Hardy-Weinberg law ($\chi^2=2.18$, $df=5$, $P=0.82$). Among these Mexican-American healthy volunteers, only one individual (1%) was found to carry two

Table 1 Allele and genotype frequencies for CYP2C9 in different populations

Populations	(% CYP2C9 alleles)			n	(% Genotype)						Ref.
	*1	*2	*3		*1/*1	*1/*2	*1/*3	*2/*2	*2/*3	*3/*3	
<i>Europeans</i>											
British	79	12.5	8.5	100	62	19	15	3	0	1	18
Italian	79.6	11.2	9.2	157	65	16.8	14	2.5	1.9	1.3	19
Spanish	74.5	15.6	9.8	102	57.8	19.6	13.7	3.9	3.9	1.0	10
Swedish	81.9	10.7	7.4	430	66.7	18.6	11.6	0.5	1.9	0.7	20
<i>Americans</i>											
White-American	82.5	13.2	4.3	140	67.1	22.1	8.6	2.1	0	0	21
White-American	86	8	6	100	72	16	12	0	0	0	22
White-American	78	15	7	325	60.3	22.8	12.6	2.8	1.6	0	23
Afro-American	98.5	1	0.5	100	97	2	1	0	0	0	22
Afro-American	94.6	2.5	1.3	120	89.2	5	2.5	0	0	0	21
Canadian Indian	91	3	6	114	82.5	6.1	11.4	0	0	0	23
Inuit	100	0	0	151	100	0	0	0	0	0	23
<i>Asians Africans and others</i>											
Egyptians	82	12	6	247	66.4	19	11.7	2.4	0	0.4	24
Iranian	79.3	11	9.7	160	64.4	17.5	13.7	2.5	0	1.9	25
Turkish	79.4	10.6	10	499	61.7	18	17.2	1	1.1	0.8	26
Ethiopian	93.3	4.3	2.3	150	87	8.7	4.3	0	0	0	19
<i>Oriental</i>											
Chinese	98.3	0	1.7	115	96.5	0	3.5	0	0	0	27
Chinese	95.1	0	4.9	102	89.2	0	10.8	0	0	0	23
Japanese	98.2	0	1.8	140	96.4	0	3.6	0	0	0	28
Japanese	97.9	0	2.1	218	95.9	0	4.1	0	0	0	29
Korean	98.9	0	1.1	574	97.7	0	2.3	0	0	0	30
Taiwanese	97.4	0	2.6	98	91.8	0	8.2	0	0	0	22

n = number of subjects.

Table 2 CYP2C9 genotypes among Mexican-Americans (n=98)

Genotype	n	Observed frequency	95% CI	Expected frequency ^a
CYP2C9*1/*1	72	0.74	0.64–0.81	0.74
CYP2C9*1/*2	15	0.15	0.095–0.24	0.14
CYP2C9*1/*3	10	0.10	0.055–0.18	0.10
CYP2C9*2/*2	0	—	—	0.007
CYP2C9*2/*3	1	0.01	0.00–0.061	0.009
CYP2C9*3/*3	0	—	—	0.003

^aCalculated by Hardy–Weinberg law.

mutated alleles (CYP2C9*2/*3). No subject homozygous for CYP2C9*2 or CYP2C9*3 alleles was detected in this population.

The frequencies among Spaniards and Mexican-American volunteers on the CYP2C9 allele are shown in Table 3. The frequency of CYP2C9*2 was lower ($P < 0.05$) among Mexican-Americans compared to Spaniards. The CYP2C9*4, *5 and *6 allelic variants were not found in any of the subjects.

DISCUSSION

The Mexican-American population is a complex admixture of races, combining the genetic background of several Native American Indian groups derived originally from a single migration of Asians to Beringia, with White Europeans coming from Spain. The present results agree with the resulting ethnical background for this population, as CYP2C9*2 allele has been found to be absent in Orientals (Table 1). Moreover, the frequency of CYP2C9*3 allele found among Mexican-Americans is similar to that described among White Europeans (Table 1) and Hispanics,¹¹ which is in agreement with the presence of migrations of Spaniards to Mexico and California. The CYP2C9*2 allele frequency has been shown to be similar to the one found in a previously studied Western Mexican population.¹² Several studies have shown the clinical implications for patients carrying CYP2C9*2 and CYP2C9*3 alleles.⁷ Some CYP2C9 substrates have a narrow therapeutic index (warfarin, acenocoumarol, glipizide and phenytoin), and thus the CYP2C9 poor metabolizers (PMs), mostly *3/*3 carriers, may experience severe drug toxicity, such as a risk of haemor-

rhage described during treatment with warfarin.^{6,7} Conversely, these PMs may have inadequate drug response and therapeutic failure when taking a pro-drug that requires CYP2C9-mediated bio-activation to produce its drug effects, such as losartan or cyclophosphamide.^{13,14} Thus, genotyping for CYP2C9 variants might allow optimization of the treatment with CYP2C9 substrate drugs by dose individualization.

A significant difference on the frequency of the CYP2C9*2 allele was found in Mexican-Americans compared to Spaniards, while the CYP2C9*3 allele frequency was similar to the previously reported among White Europeans and Americans and different from African-Americans (Table 1). To the best of our knowledge, this is the first study analysing CYP2C9*1, *2, *3, *4, *5 and *6 allelic variants among Hispanics, and one of the first in USA. The allelic distribution of the CYP2C9 gene in this Mexican-American population is compatible with the genomic assembly of the constitutive ethnic origin of this Hispanic group, an admixture of ancient Indians living in Central and North America and White Europeans coming mostly from Spain. Thus, there is a need of pharmacogenetic studies in this population to optimize the recommended drug dosages, especially for drugs with therapeutic narrow index.

MATERIALS AND METHODS

Subjects and Study Protocol

A group of 98 unrelated healthy Mexican-American individuals (48 males and 50 females) living in Los Angeles, CA, were studied. The mean (\pm SD) age was 35.3 ± 10.7 . They were defined as people with at least three grandparents born in México. A group of 102 White Europeans living in Spain were analysed for comparison.¹⁰

All subjects were informed about the aims of the study and gave their consent to participate. The study was approved by the UCLA Human Subjects Protection Committee. The purpose of this study was to obtain samples for a national repository to serve as a resource for genetic studies. Samples were anonymized and deposited at the Coriell Institute for Medical Research; they are available to scientists with approved research protocols.

CYP2C9 Genotyping

Venous blood (10 ml) was obtained from each volunteer. Blood samples were collected in plasma EDTA tubes, and

Table 3 Allele frequencies for CYP2C9 in Mexican-American and Spanish (10) populations

Alleles	n		Frequency (95% CI)	
	Mexican-Americans	Spaniards	Mexican-Americans	Spaniards
CYP2C9*1	169	152	0.86 (0.81–0.90)	0.74 (0.68–0.80)
CYP2C9*2 ^a	16	32	0.08 (0.05–0.13)	0.16 (0.11–0.21)
CYP2C9*3	11	20	0.06 (0.03–0.10)	0.10 (0.06–0.15)

^a $P < 0.05$, difference between Mexican-Americans and Spaniards, using Fisher's exact test; n = number of subjects.

genomic DNA was isolated using Gentrapuregen kit (Indianapolis, IN, USA). *CYP2C9*2* and *CYP2C9*3* allelic variants were detected as reported previously.¹⁰ *CYP2C9*4*, **5* and **6* alleles were studied using primers and restriction enzymes described elsewhere.^{15–17}

Statistics

The *CYP2C9* allele frequencies were compared by using the Fisher's exact test. Hardy–Weinberg equilibrium was determined by comparing the genotype frequencies with the expected values using a contingency table χ^2 statistic with Yates's correction. A *P*-value less than 0.05 was regarded as statistically significant in all cases. Statistical analyses were performed using GraphPad QuickCalcs (GraphPad Software, Inc.).

ACKNOWLEDGEMENTS

The present study was supported by the US National Institutes of Health (Grants GM61394, RR017365, RR000865, RR16996, RR017611 and DK063240) and by the Spanish Ministry of Science and Technology (Grant SAF2003-05716), and by an award from the Dana Foundation. The collaboration of MC Cáceres (University of Extremadura) is acknowledged.

DUALITY OF INTEREST

None of the authors has a conflict of interest to declare.

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