RESEARCH ARTICLE

Genetic variability of *CYP2C19* in a Mexican population: contribution to the knowledge of the inheritance pattern of *CYP2C19*17* to develop the ultrarapid metabolizer phenotype

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Abstract

CYP2C19 is a polymorphic enzyme that metabolizes a wide variety of therapeutic drugs that has been associated with altered enzymatic activity and adverse drug reactions. Differences in allele frequencies of the *CYP2C19* gene have been detected in populations worldwide. Thus, we analysed the alleles *CYP2C19*2*, *CYP2C19*3*, *CYP2C19*4* and *CYP2C19*5* related to the poor metabolizer (PM) phenotype in a Mexican population sample ($n = 238$), as well as *CYP2C19*17*, unique allele related to ultrarapid metabolizer phenotype (UMs). Genotypes were determined using SNaPshot and TaqManqPCR assays. In addition to the wild-type *CYP2C19*1* allele (77.1%), we only found *CYP2C19*17* (14.3%) and *CYP2C19*2* (8.6%). Comparison with previous population reports demonstrated that these two SNPs are homogeneously distributed in Latin America ($P > 0.05$). Based on comparison with a previous pharmacokinetic study that determined the frequency of CYP2C19 phenotypes in the same population (western Mexican), we obtained the following findings: (i) based on the difference between the frequency of genotypes *CYP2C19*2/*2* (presumably PM) versus the observed prevalence of PM phenotypes (0.4 versus 6.3%; $\chi^2 = 9.58$, $P = 0.00196$), we inferred the plausible presence of novel *CYP2C19* alleles related to the PM phenotype; (ii) the prevalence of UMs was in disagreement with the dominant inheritance pattern suggested for *CYP2C19*17* (23.1 versus 4%; $P < 0.00001$); (iii) the apparent recessive inheritance pattern of *CYP2C19*17*, based on the agreement between homozygous *CYP2C19*17/*17* (presumably UMs) and the observed prevalence of UMs (2.1 versus 4%; ($\chi^2 = 1.048$; $P = 0.306$).

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Introduction

CYP2C19 is a polymorphic enzyme involved in the metabolism of around 15% of the drugs prescribed, including anxiolytics, antidepressants, antineoplastics, proton pump inhibitors and antiplatelet agents, among others (Rosemary and Adithan 2007). Differences for these drugs have been observed in pharmacologic responses and adverse drug reactions due to several factors, such as the presence of single-nucleotide polymorphisms (SNPs) in the *CYP2C19* gene that generate several alleles, influencing the enzymatic function (Li-Wan-Po *et al.* 2009). These alleles associated with the drug's metabolic ability define the *CYP2C19* genotype, which can be related to the following four metabolizer phenotypes: poor metabolizer (PM), homozygous or compound heterozygous genotypes for the loss-of-function (LOF) *CYP2C19* alleles; intermediate metabolizer (IM), heterozygous genotypes for the LOF *CYP2C19* alleles; extensive metabolizer (EM), carrying two functional or wild-type alleles; and ultrarapid metabolizer (UM), both heterozygous and homozygous for the *CYP2C19*17* that increase gene expression and thereby CYP2C19 activity (Sim *et al.* 2006; Santos *et al.* 2011). However, it has been claimed that only homozygous *CYP2C19*17/*17* displays the UM phenotype (Li-Wan-Po *et al*. 2009). In Latin America, population studies have shown differences in the genetic diversity of *CYP2C19*, mainly

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regarding LOF alleles related to the PM phenotype, such as *CYP2C19*2, CYP2C19*3, CYP2C19*4* and *CYP2C19*5*, principally (Bravo-Villalta *et al.* 2005; Luo *et al.* 2006; Isaza *et al.* 2007; Linden *et al.* 2009; Hoyo-Vadillo *et al.* 2010; Salazar-Flores *et al.* 2012). However, scarce population data exists in Latin American for *CYP2C19*17*, the unique allele associated to the UM phenotype. This allele shows relatively high frequencies in African American (21%), European (range 18–27%), and Brazilian populations (range 15.8–26.3%) (Santos *et al.* 2011); whereas low frequencies have been found in Hispanic American (12%) and Asian populations (0–4%) (Kearns *et al.* 2010).

In Mexico, in addition to the wild-type allele *CYP2C19*1*, the *CYP2C19*2* allele has almost exclusively been detected in Mestizos (range 6.9–10.3%) and in Amerindian groups (range 3.6–31%) (Hoyo-Vadillo *et al.* 2010; Salazar-Flores *et al.* 2012). Interestingly, one previous pharmacokinetic study in western Mexico, the same population studied here, estimated the frequency of PM (6%), EM (90%), and UM (4%) phenotypes using Omeprazole as probe drug (González *et al.* 2003). Therefore, the primary purpose of this study was to detail the genetic variability of *CYP2C19* in western Mexicans, and to check its correspondence with: i) previous genetic population studies of *CYP2C19* throughout Latin America; and ii) the metabolizer phenotype frequency previously described in the same Mexican population.

Material and methods

Population sample

The total population sample consisted of 238 healthy unrelated Mexican Mestizos from the state of Jalisco (western Mexico). Mestizos are the result of admixture mostly between Spaniards, Amerindians and Africans after the European contact, and presently they represent most of the Mexican population (∼90%) (Salazar-Flores *et al.* 2012). All volunteers for the study signed a written informed consent form according to the ethical guidelines of the Helsinki Declaration. The protocol was approved by the Committee of Ethics and Research of the CUCiénega (UdeG).

Methods

Genomic DNA was extracted from peripheral blood using the standard phenol–chloroform method (Sambrook *et al.* 1989), and it was quantified with a Nanodrop 2000^{TM} instrument (Thermo Scientific, Wilmington, USA). To analyse the genetic variability of *CY2C19*, the alleles *CYP2C19*2, CYP2C19*3, CYP2C19*4* and *CYP2C19*5* were genotyped by SNaPshot^{TM} following the same conditions described in a previous report (Salazar-Flores *et al.* 2012). Detection of *CYP2C19*17* (rs12248560, c.−806 C_T) was performed by TaqManqPCR assay according to the manufacturer's instructions (Applied Biosystems, Foster City, USA); the Step One^{TM} Real-Time PCR system was employed for this purpose (Applied Biosystems). Moreover, a subset of samples was regenotyped for *CYP2C19*2* (rs4244285, c.19154 G_A) by TaqManqPCR assay to ensure accuracy. The presence of wild-type allele *CYP2C19*1* was deduced in each individual by the absence of the SNPs studied here. The phenotype was inferred theoretically from the genotype, as mentioned in the Introduction section of this article. Allele and genotype frequencies were determined by the gene counting method. For the SNPs detected, Hardy–Weinberg equilibrium (HWE) and pairwise comparisons with previous reports were carried out by chi-square tests (http://www.quantpsy.org/chisq/chisq.htm).

Results

We describe the genetic variation of *CY2C19* in Mexican-Mestizos. Although this issue has been addressed previously (Hoyo-Vadillo *et al.* 2010; Salazar-Flores *et al.* 2012), this report includes for the first time the analysis of *CYP2C19*17* in a Mexican population sample. We only detected the polymorphisms *CYP2C19*2* and *CYP2C19*17* with frequencies of 8.6% and 14.3%, respectively; thus, the wild-type allele *CYP2C19*1* exhibited the highest frequency with 77.1% (table 1). Genotype frequencies involving alleles *CYP2C19*1*, *CYP2C19*2* and *CYP2C19*17* were distributed according to the HWE ($P = 1.000$). Dual genotyping of *CYP2C19*2* (TaqManqPCR versus SNaPshot)

Table 1. Genetic variation, predicted and observed phenotypes for *CYP2C19* in a western Mexican Mestizo population.

CYP2C19 allele	$2n = 476$ (%)	*CYP2C19* genotype	$n = 238$ (%)	Predicted phenotype	Observed phenotype[a] *CYP2C19*	$n = 127$ (%)
**1*	367 (77.10)	**1/*1*	143 (60.08)	EM	UM	5 (4.0)
**2*	41 (8.61)	**1/*2*	31 (13.03)	IM	EM	114 (89.7)
**17*	68 (14.29)	**1/*17*	50 (21.01)	EM/UM[b]	PM	8 (6.3)
**3*4*5*	0 (0)	**2/*2*	1	PM		
		**2/*17*	8	Unknown		
		**17/*17*	5	UM		

EM, extensive metabolizer; UM, ultrarapid metabolizer; IM, intermediate metabolizer.
[a]Based on the pharmacokinetic analysis of González *et al.* (2003) in western Mexicans (showed for comparison purposes).
[b]Please see Discussion.

Table 2. Allelic frequency distribution of *CYP2C19*2*, *CYP2C19*3* and *CYP2C19*17* in Latin and Native American populations.

Latin American population	2*n*	*CYP2C19* allele frequency **2* *n*	**2* %	**3* *n*	**3* %	**17* *n*	**17* %	Comparison χ^2; *P* value	Reference
Western Mexicans Mestizos	476	41	8.61	–	0	68	14.29	–	This study
Mexican-Americans	692	67	9.7	1	0.1		NA	**2*=0.384; 0.5354	Luo *et al.* (2006)
Mexicans (control)	704	59	8.4	–	0		NA	**2*=0.02; 0.8875	Hoyo-Vadillo *et al.* (2010)
Western Mexicans Mestizos	290	20	6.9	–	0		NA	**2*=0.725; 0.3945	Salazar-Flores *et al.* (2012)
Bolivian	1556	121	7.8	2	0.1		NA	**2*=0.348; 0.5552	Bravo-Villalta *et al.* (2005)
Colombians	378	33	8.7	–	0		NA	**2*=0.004; 0.9495	Isaza *et al.* (2007)
Caucasian-Brazilians	76	19	25.0	–	0		NA	**2*=18.16; 0.00002	Linden *et al.* (2009)
Amerindian-Brazilians	366	38	10.4	–	0	58	15.8	**2*=0.762; 0.383 **17* = 0.396; 0.091	Santos *et al.* (2011)
Hispanic Americans	216	NA		NA		26	12.0	**17* = 0.64; 0.4237	Kearns *et al.* (2010)
Native Americans	100	11	11	–	0	9	9	**2*=0.573; 0.449 **17* = 1.994; 0.158	Oestreich *et al.* (2014)

Comparison of our results for *CYP2C19*2* and/or *CYP2C19*17* regarding different Latin American populations, respectively; NA, not analysed; –, not observed.

allowed confirmation of dubious SNaPshot results, specifically for some heterozygous *CYP2C19*1/*2,* which could be confused with homozygous *CYP2C19*1/*1* due to the low signal for the *CYP2C19*2* allele. Finally, all *CYP2C19* genotypes were established without doubt.

We compared the allele frequencies estimated here for *CYP2C19*2* and *CYP2C19*17* with respect to previously studied Latin American populations (table 2), which are characterized by the absence or negligible frequency (≤0.1%) of *CYP2C19*3*, *CYP2C19*4* and *CYP2C19*5* alleles. The allele frequency estimated here for *CYP2C19*2* was similar to previous reports from Latin American and Native American populations ($\chi^2 = 8.071$; $P = 0.4265$), except for Caucasian Brazilians ($P < 0.00002$), probably explained by higher European ancestry than the rest of the populations. Similarly, the *CYP2C19*17* allele frequency of western Mexicans was comparable with Hispanic Americans and Native Americans (table 2). Finally, the global differentiation test (excluding Caucasian Brazilians) demonstrated homogeneity among these populations for *CYP2C19*2* and *CYP2C19*17* ($P > 0.05$).

Discussion

Among alleles related to the PM phenotype, the nearly exclusive presence of *CYP2C19*2* (8.6%) in the studied population is in agreement with previous reports on Mexican, Latin American and Native American populations (table 2). As could be expected by the allele frequencies estimated in Mexican Mestizos, the main genotype is the wild-type *CYP2C19*1/*1* (60.1%), followed by genotypes related to the UM phenotype *CYP2C19*1/*17* (21%), and *CYP2C19*17/*17* (2.1%). Conversely, unique genotypes related to IM and PM phenotypes observed here were *CYP2C19*1/*2* (13%) and *CYP2C19*2/*2* (0.4%), respectively (table 1).

The prevalence of homozygous *CYP2C19*2/*2* estimated in this work (0.4%), and those previously estimated in Mexico (≤1.4%), suggest a low frequency of the PM phenotype in this country (Hoyo-Vadillo *et al.* 2010; Salazar-Flores *et al.* 2012). However, the predicted PM frequency based on the genotype *CYP2C19*2/*2* was in disagreement with the PMs frequency observed in the pharmacokinetic study of González *et al.* (2003) (0.4 versus 6.3%; $\chi^2 = 9.58$, $P = 0.00196$), who used omeprazole as probe-drug to estimate the prevalence of CYP2C19 phenotypes in Mexican population (table 1). Although both the expression of the gene *CYP3A4* and environmental factors might influence the PM phenotype for CYP2C19, this finding could also imply additional *CYP2C19* alleles (Andersson *et al.* 1994; González *et al.* 2003; Linden *et al.* 2009; Kearns *et al.* 2010), similar to the Amerindian mutation *CYP2D6*82* recently described in Mexican population (Contreras *et al.* 2011). The plausible presence of novel alleles is consistent with the elevated genetic variability described for *CYP2C19*, which includes at least 28 polymorphic alleles and encourages deeper studies to explore new functional mutations (Rosemary and Adithan 2007). Therefore, to confirm this hypothesis for *CYP2C19*, DNA sequencing would be required in Mexican individuals with PM phenotype but discordant *CYP2C19* genotype (e.g. *CYP2C19*1/*1)*, which by now is out of the purposes of this study.

CYP2C19 genotype frequencies estimated in the Mexican population analysed are ostensibly correlated with the frequency of altered metabolizer phenotypes (table 1). In this case, UM would be the most prevalent altered phenotype based on the frequency of the *CYP2C19*17* allele. However, there are two criteria for predicting the prevalence of UMs: some authors describe *CYP2C19*1/*17* individuals as UMs (Wang *et al.* 2009), whereas others consider them as EMs, within the same group of the wild-type homozygous *CYP2C19*1/*1* (Sugimoto *et al.* 2008). A previous study based on a MEDLINE search shed light

to this topic: researchers concluded that only homozygous *CYP2C19*17/*17* could be considered UMs and that they are likely at an increased risk to suffer therapeutic ineffectiveness, principally for drugs with a very narrow therapeutic window, such as Clopidogrel (Li-Wan-Po *et al.* 2009). We were able to contribute to this topic by comparing our results with the previously mentioned pharmacokinetic study on CYP2C19 in Mexican population González *et al.* (2003) (table 1). Interestingly, the 4% prevalence for the UM phenotype is in agreement with the 2.1% prevalence of homozygous *CYP2C19*17/*17* observed here ($\chi^2 = 1.048$; $P = 0.306$), assuming that this would be the unique genotype resulting in UM (table 1). Therefore, our results support the hypothesis of the recessive inheritance of *CYP2C19*17* which by definition comprises the allele that causes the phenotype only in homozygous, and never in heterozygous. Eventually, this inheritance pattern must be confirmed by comparison between the *CYP2C19*17* genotype and the pharmacokinetic profile in a population sample.

To our knowledge, *CYP2C19*17* is the unique allele related to the UM phenotype (Li-Wan-Po *et al.* 2009; Kearns *et al.* 2010). Thus, under a dominant inheritance pattern of *CYP2C19*17*, the observed genotypes including this allele, such as *CYP2C19*17/*17* and *CYP2C19*1/*17*, should correspond to the prevalence of UMs. We tested this hypothesis, comparing the predicted frequency of UMs based on the genotypes *CYP2C19*17/*17* plus *CYP2C19*1/*17* with regard to the prevalence of UMs previously observed in the pharmacokinetic study (23.1 vs. 4%; $\chi^2 = 22.16$; $P = 0.0000025$) (González *et al.* 2003) (table 1). This difference allows discarding the dominant inheritance pattern of *CYP2C19*17* for expressing the UM phenotype at least for those obtained by the administration of omeprazol as the probe drug.

The importance of discarding the dominant inheritance pattern of *CYP2C19*17* is based on the significant reduction of the predicted frequency of UMs in Mexican population based on *CYP2C19*17* (dominant 23.1% vs. recessive 2.1%). This fact would impact the therapeutic activity of various clinically relevant drugs, such as voriconazole, tamoxifen, and proton pump inhibitors among others. The frequency of the *CYP2C19*17* allele in Mexico, and the knowledge of its inheritance pattern permit us to estimate a relatively low health-care impact based on the predicted prevalence of UMs (*CYP2C19*17/*17* = 2.1%), as well as PMs (*CYP2C19*2/*2* = 0.4%). Interestingly, these conclusions are probably valid for Native American and Latin American populations as suggested by the homogeneous distribution demonstrated for *CYP2C19* at the continental level.

Conclusion

In brief, we describe the genetic variability of *CYP2C19* in a Mexican population which was similar to Latin American and Native American populations. Comparison with a previous pharmacokinetic report on the same Mexican population allowed the following: (i) to suggest the probable presence of novel *CYP2C19* alleles related to the PM phenotype in our population; and (ii) to discard the dominant inheritance pattern of allele *CYP2C19*17* for resulting in the UM phenotype.

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