Allele frequency distribution of CYP2C9*2 and CYP2C9*3 polymorphisms in six Mexican populations

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Abstract
Allele frequency differences of functional CYP2C9 polymorphisms are responsible for some of the variation in drug response observed in human populations. The most relevant CYP2C9 functional variants are CYP2C9*2 (rs1799853) and CYP2C9*3 (rs1057910). These polymorphisms show variation in allele frequencies among different population groups. The present study aimed to analyze these polymorphisms in 947 Mexican-Mestizo from Mexico City and 483 individuals from five indigenous Mexican populations: Nahua, Teenek, Tarahumara, Purepecha and Huichol. The CYP2C9*2 allele frequencies in the Mestizo, Nahua and Teenek populations were 0.051, 0.007 and 0.005, respectively. As for CYP2C9*3, the allelic frequencies in the Mestizo, Nahua and Teenek populations were 0.04, 0.005 and 0.005, respectively. The CYP2C9*2 and CYP2C9*3 alleles were not observed in the Tarahumara, Purepecha and Huichol populations. These findings are in agreement with previous studies reporting very low allele frequencies for these polymorphisms in American Indigenous populations.

1. Introduction
Several studies have reported that some interethnic differences in drug response have their origin in the presence of functional polymorphisms with varying allele frequencies in genes related to the absorption, distribution, metabolism and excretion of drugs, as well as their therapeutic targets (Evans and McLeod, 2003; Ingelman-Sundberg, 2001; Zhou et al., 2008). It has been estimated that genetics account for approximately 20% of the total human CYP protein content in adult liver (Hitchen, 2006). Genetic polymorphisms of cytochrome P450 (CYP) enzymes are major determinants of inter-individual variability in therapeutic responses and adverse events (Rendic and Di Carlo, 1997). A recent study focused on the CYP superfamily has indicated that many of the genes in this superfamily show substantial genetic differentiation in human populations (Polimanti et al., 2012). Within the CYP superfamily, the CYP2C subfamily plays a key role on the metabolism of many drugs commonly prescribed world-wide. CYP2C9 is a CYP enzyme that accounts for approximately 20% of the total human CYP protein content in adult liver (Xie et al., 2002). CYP2C9 is responsible for the metabolism of approximately 16% of clinically used drugs cleared by oxidative pathways (Williams et al., 2004). Several of these drugs have narrow therapeutic indices, such as the anticoagulant warfarin and the anticonvulsant phenytoin (Lee et al., 2002; Ross et al., 2010). Other therapeutically important drugs metabolized by CYP2C9 include the anti-diabetic drugs tolbutamide, glipizide and glibenclamide, the diuretic torsemide, inflammatory drugs, including ibuprofen, diclofenac (Miners and Birkett, 1998; Zanger et al., 2008).

The human CYP2C9 gene has been mapped to chromosome 10q24 (Nelson et al., 1996; Zanger et al., 2008). The most common allele, considered as the wild type, is denoted CYP2C9*1. Many polymorphisms within the CYP2C9 gene have been reported, with at least eight known as nonfunctional alleles found in different ethnic groups (De Lozier et al., 2005). These codify enzymes with either decreased activity or with a truncated inactive protein. Among these functional variants, CYP2C9*2 (rs1799853) and CYP2C9*3 (rs1057910) have been extensively studied.
The CYP2C9*2 allele encodes a moderately defective protein whereas the CYP2C9*3 allele has lower affinity and markedly lower intrinsic clearance for numerous drugs both in vitro and in vivo (Lee et al., 2002). The CYP2C9*2 allele is primarily restricted to European, Middle Eastern and Central/South Asian populations, and is absent or found at very low frequencies in other geographic regions (Africa, East Asia, Oceania and the Americas). The CYP2C9*3 allele has a broader geographic distribution, but the highest allele frequencies are also found in European and Central/South Asian populations (Ross et al., 2010).

The majority of the Mexican population is comprised of Mestizos, the result of an admixture process between the original Mexican indigenous groups and Europeans who arrived to Mexico early in the 16th century, and, to a smaller extent, Africans brought to the country as slaves. Some studies have indicated that admixture proportions show regional variation, with an increased European background in the north and a relatively larger indigenous contribution in the south (Bonilla et al., 2005; R.M. Cerda-Flores et al., 2002; R. Cerda-Flores et al., 2002b; Lisker et al., 1986; Silva-Zolezzi et al., 2009). In addition to the Mestizo population, Mexico currently has 62 indigenous groups representing 9.54% of the total population (Navarrete-Linares, 2008). Variation in allele frequencies has been described for the CYP2C9*2 and CYP2C9*3 polymorphisms in Mexico. These alleles are absent or have very low frequencies in the limited number of Mexican indigenous groups that have been studied and Mexican Mestizo samples are characterized by intermediate frequencies between European and indigenous groups (Aguilar et al., 2008; Dorado et al., 2011; Llerena et al., 2004; Ross et al., 2010). The aim of this study was to characterize the frequency of the CYP2C9*2 and CYP2C9*3 variants in five Mexican indigenous populations: Nahua, Teenek, Tarahumara, Purepecha and Huichol, and a Mestizo population from Mexico City, in order to increase the information available for these polymorphisms of pharmacogenomic relevance in the Mexican population.

2. Material and methods

2.1. Subjects and study protocol

Four hundred eighty-three unrelated healthy Mexicans from five indigenous groups (Nahua, Teenek, Tarahumara, Purepecha and Huichol), living in different states of Mexico, were included in the present study. The Tarahumara, Purepecha and Huichol population have been previously described elsewhere (García-Ortiz et al., 2004; Nuño-Arana et al., 2005; Villalobos-Arámbula et al., 2000). The 212 Nahua individuals came from 6 municipalities: Agua Blanca, Metepec, Tenango de Doria, Tututepec, Acaxochitlán and San Bartolo, which are located in central Mexico in the area between the Tulancingo valley and the Huasteca Hidalguense region. The 98 Teenek individuals were from the Huasteca Potosina region within the Panuco River basin, east of the state of San Luis Potosí. The 48 Purepecha individuals were from the municipalities of Erongericuaro, Cutzamala de Pinzón, Uruapan, Mapízaro, Patzcuaro and Cueneo in Michoacán. The 52 Tarahumara participants from the northern region of Chihuahua came from 6 different municipalities: Bocoyna, Guachochi, Urique, Guazapares, Temosachi, and Uruachi. Finally, the 73 Huichol individuals came from Nayarit in the following municipalities: Mezquítica, Nayar, Jesús María, Guasave, La Yesca, Tepic and Rosa Morada. To be considered as Mexican indigenous, subjects’ families must have lived at least three generations in the same community, and speak their native indigenous language. In addition, we analyzed 947 unrelated Mestizo individuals who were recruited at the blood bank of the Mexican Institute of Social Security (IMSS) located in the southern area of Mexico City. To be considered as Mexican Mestizo, their families must have lived in Mexico for at least three generations. Written consent was obtained from all participants, if participants do not speak Spanish a translator explained the purpose of the study and signed the consent form.

<table>
<thead>
<tr>
<th>CYP2C9 genotype frequencies among Mexico City Mestizo and five Native Mexican populations.</th>
<th>CYP2C9 genotype</th>
<th>Mexico City-Mestizo n = 947</th>
<th>Nahua n = 212</th>
<th>Teenek n = 98</th>
<th>Tarahumara n = 947</th>
<th>Purepecha n = 48</th>
<th>Huichol n = 73</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C9*1</td>
<td>Expected frequency</td>
<td>0.81–0.85</td>
<td>0.014</td>
<td>0.49</td>
<td>0.15</td>
<td>0.12</td>
<td>0.27</td>
</tr>
<tr>
<td>CYP2C9*2</td>
<td>Expected frequency</td>
<td>0.08</td>
<td>0.063–0.08</td>
<td>0.005–0.01</td>
<td>0.0009</td>
<td>0.0004</td>
<td>0.0001</td>
</tr>
<tr>
<td>CYP2C9*3</td>
<td>Expected frequency</td>
<td>0.02</td>
<td>0.006–0.03</td>
<td>0.000–0.01</td>
<td>0.0002</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
</tbody>
</table>
| a Expected frequency calculated by Hardy-Weinberg law.
The percentages of women in Mexico City Mestizos, Nahua, Teenek, Tarahumara, Purepecha and Huichol groups were 67.5%, 80%, 66%, 61.5%, 71.4% and 32%, respectively. The average ages in Mexico City Mestizos, Nahua, Teenek, Tarahumara, Purepecha and Huichol groups were 58, 35, 31, 37 and 28 years, respectively.

The protocol was approved by the Institutional Review Board of the National Ethical Committee of the IMSS and is in compliance with the Ethical principles for medical research involving human subjects of the Helsinki Declaration.

2.2. Genotyping

Genomic DNA was extracted from a peripheral blood sample using a QIAamp (Qiagen, Germany) kit, and DNA concentration was determined using a VICTOR3 1420 spectrophotometer (Perkin-Elmer, Germany). The SNP analyses were made using real-time PCR (RT-PCR) using TaqMan allelic discrimination assay C_25625805_10 for CYP2C9*2 and C_27104892_10 for CYP2C9*3 (7900HT Applied Biosystems, Foster City, CA, USA), following standard protocols. The plate was run at 95 °C for 10 min, 92 °C for 15 s and then 60 °C for 1 min for 40 cycles. To verify genotyping quality, 714 samples were genotyped as blind duplicated, and the concordance rate between the samples and duplicates was 100%.

2.3. Statistical analysis

Deviations of the CYP2C9 genotype frequencies from Hardy–Weinberg proportions were observed using the tests available at http://ihg2.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl, (Strom and Wienberg, 2009). Allele frequency differences between populations were evaluated using the Fisher’s exact test. A P value < 0.05 was regarded as statistically significant in all cases. The confidence intervals of the allele frequencies were estimated with GraphPad QuickCalc (GraphPad Software, Inc., San Diego, CA).

3. Results

The CYP2C9 genotype frequencies for the six Mexican populations studied are shown in Table 1. No significant deviations from Hardy–Weinberg proportions were observed in populations where variants are present. No CYP2C9*2 or CYP2C9*3 alleles were present in the Tarahumara, Purepecha and Huichol samples. The allele frequencies of CYP2C9*2 in the Mexico City Mestizo, Nahua and Teenek samples were 0.051, 0.007 and 0.005, respectively (Table 2). As for the CYP2C9*3 allele, the frequencies observed in the Mexico City Mestizo, Nahua and Teenek were 0.039, 0.004 and 0.005, respectively (Table 2). Four CYP2C9*2/2 and two CYP2C9*3/3 homozygous individuals were observed in the Mexican Mestizo from Mexico City. This is the first study to find homozygous states for these variants in the Mexican population. Additionally, seven Mestizo individuals were heterozygotes carrying both the CYP2C9*2 and CYP2C9*3 alleles (Table 1). Table 2 shows the results of the Fisher’s exact tests for all possible pairwise comparisons. There were significant differences between the frequencies observed in the Mexico City sample and the five Mexican indigenous groups. In contrast, no significant differences were observed between any of the five indigenous groups, in which alleles CYP2C9*2 or CYP2C9*3 are absent or found in extremely low frequencies (Table 3).

4. Discussion

This study examined the allele frequencies of two polymorphisms known to have important clinical implications, CYP2C9*2 (rs1799853) and CYP2C9*3 (rs1057910), in five indigenous groups and a Mestizo sample from Mexico City. Several studies have indicated that the functional CYP2C9*2 and CYP2C9*3 variants are absent or present in extremely low frequencies in indigenous American populations (Dorado et al., 2011; Gaedigk et al., 2001; Ross et al., 2010; Sosa-Macias et al., 2013). Our results are in overall agreement with previous reports: the CYP2C9*2 and CYP2C9*3 alleles were not present in three of the five indigenous populations included in our analysis (Tarahumara, Purepecha and Huichol) and the frequency of both alleles is lower than 1% in the other two indigenous groups (Nahua and Teenek). The samples for the Tarahumara and Purepecha groups are relatively small (around 50), but even in these samples we would expect to identify variants with frequencies higher than 1%. In contrast, the frequencies of the CYP2C9*2 and CYP2C9*3 alleles in the Mexico City Mestizo sample were substantially

Table 2

| Ethnic group | N     | CYP2C9*1 Frequency | 95% CI | CYP2C9*2 Frequency | 95% CI | CYP2C9*3 Frequency | 95% CI |
|--------------|-------|------------------)   |       | (95% CI)           |       | (95% CI)           |       |
| Nahua        | 424   | 0.989            | 0.975–0.997 | 0.007            | 0.002–0.024 | 0.004            | 0.000–0.018 |
| Teenek       | 196   | 0.990            | 0.961–0.999 | 0.005            | 0.000–0.031 | 0.005            | 0.000–0.031 |
| Tarahumara   | 104   | 1.0              | –       | 0.0              | –       | 0.0              | –       |
| Purepecha    | 96    | 1.0              | –       | 0.0              | –       | 0.0              | –       |
| Huichol      | 146   | 1.0              | –       | 0.0              | –       | 0.0              | –       |
| Mexico City Mestizo | 1894 | 0.910        | 0.896–0.922 | 0.051          | 0.041–0.061 | 0.039          | 0.031–0.048 |

N represents the number of alleles.

* P < 0.05 compared with Mexican Mestizos from Mexico City using Fisher’s exact test.

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higher (5% and 3.9% respectively), and significantly different from the frequencies observed in the indigenous samples (Table 2). Fig. 1 depicts the frequencies for the CYP2C9*2 and CYP2C9*3 alleles in indigenous and Mestizo populations from throughout the Americas (Aguilar et al., 2008; Bravo-Villalta et al., 2005; Dorado et al., 2011; Duconge et al., 2009; Gaedigk et al., 2001; Isaza et al., 2010; Llerena et al., 2004; Ross et al., 2010; Sosa-Macías et al., 2013; Vianna-Jorge et al., 2004), in addition to selected European populations (Llerena et al., 2004; The International HapMap Consortium, 2013a,b). The frequencies of the CYP2C9*2 and CYP2C9*3 alleles in indigenous groups are lower than those observed in the Mestizo samples. Some variation in allele frequencies is evident both in the indigenous and Mestizo groups, probably reflecting differences in European genetic contributions. Supporting this view is the fact that in the Centre d’Etude du Polymorphisme Humain-Human Genome Diversity Project (CEPH-HGDP) Maya samples, which have shown evidence of European admixture in studies based on dense SNP panels (Bryc et al., 2010), the frequencies of the CYP2C9 functional variants are higher than in other indigenous populations. Similarly, the higher frequencies of the CYP2C9 functional variants in the Mexican American sample from LA, and the Mexican
Mestizo sample from Monterrey, with respect to the Mexico City Mestizo sample, are also in agreement with the higher levels of European admixture reported for these populations (Martínez-Fierro et al., 2009; Martínez-Margnicac et al., 2007; Parra et al., 2011).

The genetic variation of the CYP2C9 gene affects drug dosage, and it is clinically relevant. The well-known effect of CYP2C9 variants on warfarin-dosing is an excellent example. Warfarin is the most broadly used antiocoagulant worldwide and it has a narrow therapeutic index (Caraco et al., 2008; Higashi et al., 2002). Incorrect dosing is responsible for a high rate of adverse effects and warfarin is the leading cause of hospitalization in the emergency room due to adverse events in the elderly (Budnitz et al., 2011). Importantly, there is a substantial inter-individual variability in the required warfarin dose (International Warfarin Pharmacogenetics Consortium, 2009; Takahashi et al., 2006) and a large proportion of this variability is explained by polymorphisms in the VKORC1 and CYP2C9 genes (Carquist et al., 2006; Takeuchi et al., 2009; Wadelius et al., 2005). Many studies have demonstrated the usefulness of the inclusion of CYP2C9 genotypes in warfarin-dosing algorithms (Anderson et al., 2012; Gong et al., 2011; Marin-Leblanc et al., 2012; Pavani et al., 2012). Adding genetic information to conventional algorithms (Anderson et al., 2012; Gong et al., 2011; Marin-Leblanc et al., 2012) indicates that genetic information enhances the efficacy and safety of anticoagulation: a prospective randomized controlled study. Clin. Pharmacol. Ther. 83, 460–470.


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Martinez-Marignac et al., 2007; Parra et al., 2011).


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