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Distribution of *CYP2D6* and *CYP2C19* Polymorphisms Associated with Poor Metabolizer Phenotype in Five Amerindian Groups and Western Mestizos from Mexico

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Background: The distribution of polymorphisms in the *CYP2D6* and *CYP2C19* genes allows inferring the potential risk for specific adverse drug reactions and lack of therapeutic effects in humans. This variability shows differences among human populations. The aim of this study was to analyze single-nucleotide polymorphisms related to a poor metabolizer (PM) phenotype in nonpreviously studied Amerindian groups and Mestizos (general admixed population) from Mexico. *Methods:* We detected by SNaPshot® different polymorphisms located in *CYP2D6* (**3, *4, *6, *7,* and **8)* and *CYP2C19* (*2, *3, *4 and *5) in western Mestizos ($n = 145$) and five Amerindian groups from Mexico: Tarahumaras from the North ($n = 88$); Purépechas from the Center ($n = 101$); and Tojolabales ($n = 68$), Tzotziles ($n = 88$), and Tzeltales ($n = 20$) from the Southeast. Genotypes were observed by capillary electrophoresis. The genetic relationships among these populations were estimated based on these genes. *Results and Discussion:* The wild-type allele *(*1)* of both genes was predominant in the Mexican populations studied. The most widely observed alleles were *CYP2C19*2* (range, 0%–31%) and *CYP2D6*4* (range, 1.2%–7.3%), whereas *CYP2D6*3* was exclusively detected in Mestizos. Conversely, *CYP2C19*4* and **5,* as well as *CYP2D6*3, *6, *7,* and **8*, were not observed in the majority of the Mexican populations. The Tarahumaras presented a high frequency of the allele *CYP2C19*2* (31%) and of homozygotes *2/*2 (10.7%), which represent a high frequency of potentially PM phenotypes in this Amerindian group. The genetic distances showed high differentiation of Tarahumaras (principally for *CYP2C19* gene). In general, a relative proximity was observed between most of the Amerindian, Mexican-Mestizo, and Latin-American populations. *Conclusion:* In general, the wild-type allele (*1) predominates in Mexican populations, outlining a relatively homogeneous distribution for *CYP2C19* and *CYP2D6*. The exception is the Tarahumara group that displays a potentially increased risk for adverse reactions to CYP2C19-metabolized drugs.

Introduction

The genes *CYP2D6* and *CYP2C19* are clinically important for the metabolism of ~40%–50% of drugs on the market (Van der Weide and Hinrichs, 2006). The gene *CYP2D6* codifies for the enzyme debrisoquine-4-hydroxilase, which metabolizes nearly 25% of commonly prescribed drugs. This is the most important polymorphic enzyme active in drug metabolism (Evans and Relling, 1999; Eichelbaum *et al.*, 2006). CYP2D6 substrates are antidepressants, antipsychotics, antiarrhythmics, antiemetics, β-blockers, opioids, etc. (Zhou, 2009). Although 82 alleles have been described (www.cypalleles.ki.se/cyp2d6.htm), this number is growing progressively. Functionally, these alleles can be classified into different groups: (1) increased activity, (2) decreased activity, (3) total loss of activity, and (4) normal activity (Zhou, 2009).

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The most important null alleles are *CYP2D6*4* and **5*, involving a splicing defect and total deletion of the gene, respectively. Other common alleles are *CYP2D6*10*, **17*, and **41* (Ingelman-Sundberg *et al.*, 2007).

The gene *CYP2C19* codifies for the enzyme mephenytoin 4-prime-hydroxylase, which is of clinical importance for the metabolism of the anticonvulsant mephenytoin, antiulcer drugs (such as omeprazole), antidepressants, the antimalarial drug proguanil, the muscle relaxant carisoprodol, the antiplatelet clopidrogel, cyclophosphamide, and thalidomide, among others (Takada *et al.*, 2004; Hulot *et al.*, 2006; Li *et al.*, 2007). Twenty eight allelic variants have been described for *CYP2C19*. The alleles *CYP2C19*2* through *CYP2C19*8* have been reported to be without enzyme activity and *CYP2C19*9* through *CYP2C19*11* showed decreased activity *in vitro*. Finally, *CYP2C19*12* exhibits unstable activity *in vitro,* whereas *CYP2C19*17* demonstrates increased transcriptional activity (Sibbing *et al.*, 2010). The remaining alleles also exhibit the absence of enzyme activity (www.cypalleles.ki.se/cyp2c19.htm). Metabolizer phenotypes generated due to the variable enzyme activity of CYP2D6 and CYP2C19 include Ultrarapid (UM), Extensive (EM), Intermediate (IM), and Poor metabolizers (PM). The allele frequencies of these genes show differences among populations. For example, alleles *CYP2D6*3*, **4*, **5*, and **6* are responsible for 97% of PM in Caucasians, with *CYP2D6*3* and **4* the most common alleles (Sachse *et al.*, 1997), whereas in China, these alleles are rare and explain only ~1% of PM phenotypes (Wang *et al.*, 1993). For *CYP2C19*, *CYP2C19*2* and **3* are the most common alleles and are those mainly responsible for the PM phenotype in Caucasian and Asian populations, respectively (Xie *et al.*, 2001; Mizutani, 2003). PM frequency is 5% in Caucasian and African populations and ~20% in Asians.

In Mexico, there are two main populations: Native groups (Amerindians) and Mestizos the result of post-Columbian admixture. Mexican Amerindians constitute >68 Native groups, >85 languages and variant dialects, and are geographically located mainly in the Center and Southeast of the country (Martinez-Cortes *et al.*, 2010). Conversely, Mexican-Mestizos are widely distributed throughout the country, comprise ~93% of the total population, and arose during and after European contact with the New World by admixture among Spaniards, Amerindians, and, to a lesser degree, with African slaves (Rubi-Castellanos *et al.*, 2009). Presently, the distribution of alleles related with the PM phenotype for *CYP2D6* and *CYP2C19* is poorly known in both Mexican-Mestizos and Amerindians. *CYP2D6* alleles *CYP2D6*2, *3, *4, *5, *10,* and **17* in Mestizos from Mexico City display a frequency of 19.3%, 1.4%, 11.2%, 2.6%, 12.4%, and 1.6%, respectively (Lopez *et al.*, 2005). In Mestizos from Durango (Northwest), alleles *CYP2D6*2, *3, *4, *5,* and **10* were reported, with frequencies of 10.7%, 0.9%, 13.1%, 1.3%, and 2.3%, respectively, in addition to 6.8% of PM phenotypes, similar to those reported in Caucasians (10%) (Sosa-Macias *et al.*, 2006; Sosa-Macias *et al.*, 2010). In Mexican-Amerindian groups, this information is more limited; during the search for *CYP2D6*3, *4, *6*, and **10* in Tepehuanos from Durango (Northwest, Mexico), only *CYP2D6*4* was found at a low frequency (0.6%), suggesting that the PM phenotype is absent in this Amerindian group (Sosa-Macias *et al.*, 2006). In addition, *CYP2D6*4* (0.21%) and *CYP2D6*10* (0.05%) were also detected in Mennonites of Caucasian origin residing in Durango, Mexico, whereas *CYP2D6*6* was not found (Alanis-Bañuelos *et al.*, 2007). In Mexican-Americans, frequencies of alleles *CYP2D6*4* and **6* were 10 and 0.4%, respectively (Luo *et al.*, 2005). Recently, *CYP2D6* resequencing data from two Mexican-Mestizo populations allowed detection of 14 novel variants, including allele *CYP2D6*82* that was hypothesized as Amerindian because of its identification in three Mexican-Amerindian groups (Contreras *et al.*, 2011). In this study, the frequencies of *CYP2D6*1, *2, *4, *5, *10, *29, *53, *82*, and its duplications were 50.0, 25.5, 14.1, 2.0, 2.6, 1.0, 0.5, 2.1, and 3.6%, respectively.

For *CYP2C19,* the first pharmacokinetic study was conducted in western Mexican-Mestizos, obtaining a frequency of 6% and 4% for PM and UM, respectively (González *et al.*, 2003). Afterward, in a case–control study carried out in the state of Nuevo León (Northeast Mexico), estimated PM frequency of *CYP2C19* was 8% in the general population and 11.9% in cases (Garza-González *et al.*, 2007). Recently, in one gene-disease association study, the estimated frequency of *CYP2C19*1* and **2* was 91.6% and 8.4% in healthy volunteers from Mexico City, respectively, whereas *CYP2C19*3* was not found (Hoyo-Vadillo *et al.*, 2010). To our knowledge, in the unique study of *CYP2C19* in Amerindian groups, PM individuals were not found in the Cuna Amerindian group from Panama (Inaba *et al.*, 1988). These studies in Native Americans are interesting, taking into account their particular lifestyle, customs, and geographic location, because these are related with the clinical impact of these alleles in terms of conferring risk for or susceptibility to adverse drug reactions. Because of the relationship between a genotype and a phenotype, at least to some extent, these analyses help to define the prevalence of different drug phenotype metabolizers in populations (Goldstein, 2001; Desta *et al.*, 2002). The aim of this work was to define *CYP2D6* and *CYP2C19* allele frequency in five Native groups from different regions (North, West, and Southwest) of Mexico, in addition to Mestizos from western Mexico. We found a high frequency of the wild-type allele in the Mexican populations, but an elevated frequency of homozygous *CYP2C19 *2/*2* in the Tarahumara group (North-Central Mexico) that could represent a higher risk for adverse drug reactions for CYP2C19-metabolized drugs.

Materials and Methods

DNA samples

A total of 416 and 506 unrelated individuals from five Amerindian groups and western Mestizos from Mexico were analyzed for *CYP2D6* and *CYP2C19*, respectively. Difference in population sample sizes was due to the scarce quantity and bad quality (degradation) of some DNA samples. DNA was extracted from peripheral blood samples by the salting-out method (Miller *et al.*, 1988) or with the Qiagen® FlexiGene DNA kit. The characteristics of the population samples analyzed here and those utilized for comparison purposes are described in Table 1. Written informed consent was obtained from all volunteers. The experimental protocol was approved by the Committee of Ethics in Research of the Centro Universitario de la Ciénega, Universidad de Guadalajara (CUCI-UdeG).

Genotyping

Five *CYP2D6* alleles (*CYP2D6*3*, **4*, **6*, **7*, and **8*) and four *CYP2C19* alleles (*CYP2C19*2*, **3*, **4*, and **5*) were analyzed by

Table 1. Origin, Region, Abbreviation, Sample size (*n*), and Reference of the Populations Analyzed in This Study and Those Used for Comparison Purposes with *CYP2D6* and *CYP2C19*

				Sample size (n)		
Mexican populations	*State*	*Region*	*Abbr.*	CYP2D6	CYP2C19	*Reference*
Tarahumaras	Chihuahua	North, Mex	Tar	88	84	This study
Purepechas	Michoacán	West, Mex	Pur	85	101	This study
Tojolabales	Chiapas	Southeast, Mex	Toj	43	68	This study
Tzotziles	Chiapas	Southeast, Mex	Tzo	56	88	This study
Tzeltales	Chiapas	Southeast, Mex	Tze	19	20	This study
Tepehuanos	Durango	Northwest, Mex	TepDur	85	——	Sosa-Macias *et al.* (2006)
Mestizos	Jalisco	West, Mex	MxJal	125	145	This study
Mestizos	Mexico	Center, Mex	MxDF	243	342	Lopez *et al.* (2005); Hoyo-Vadillo *et al.* (2010)
Mestizos	Durango	Northwest, Mex	MxDur	110	——	Sosa-Macias *et al.* (2006)
Menonitas	Durango	Northwest, Mex	MenDur	21		Alanis-Bañuelos *et al.* (2007)
Reference populations						
Mexican-Americans		United States	MxAmer	——	346	Luo *et al.* (2006)
Colombia		South America	Col	121	189	Isaza *et al.* (2000); Isaza *et al.* (2007)
Bolivia		South America	Bol	——	778	Bravo-Villalta *et al.* (2005)
Venezuela		South America	Ven	100	——	Grimán *et al.* (2009)
Brazil		South America	Bra	——	38	Linden *et al.* (2009)
Spaniards		Europe	Spa1 and Spa2	290,105	32	Crescenti *et al.* (2007); Menoyo *et al.* (2006); Pachkoria *et al.* (2007)
Chinese		North Asia	Chi	223	280	Ji *et al.* (2002); Yang *et al.* (2010)
Indians		South Asia	Ind	26		Naveen *et al.* (2006)

SNapShot™ with previously described primers and conditions (Bender, 2005). However, the poor reproducibility of the Long PCR protocol to amplify the *CYP2D6* fragment (5.1 kb) compelled us to employ an alternative PCR multiplex assay with the following primers sets previously reported (Gersdorff, 2005): (1) forward 5′-GGCTGGCAAGGTCCTACGC-3′ and reverse 5′-CATTCCTCCTGGGACGCTCA-3′ for *CYP2D6*3* (196 bp); (2) forward 5′-GCCTGGGCAAGAAGTCGCT-3′ and reverse 5′-AGGGAGGCGATCACGTTGCT-3′ for *CYP2D6*4/*6/*8* (227 bp); and 3) forward 5′-CGTTCTGTCCCGAGTATGCT-3′ and reverse 5′-GCCCTATCACGTCGTCGAT-3′ for *CYP2D6*7* (420 bp). To validate SNaPshot assays, genotypes in 10 individuals were confirmed with commercially available qPCR assays using TaqMan probes for the most common variant allele *CYP2D6*4* (Applied Biosystems).

PCR multiplexes were performed with the Qiagen Multiplex PCR kit (Qiagen) and with 10 ng of genomic DNA in a 10 μL of total volume. PCR products were observed by 6% (29:1) polyacrylamide gel electrophoresis followed by silver staining. After amplification, 0.2 μL of PCR product was purified with 1 μL of ExoSAP-IT (Amersham, Biosciences). The mix was incubated at 37°C for 15 min followed by 15 min at 80°C to inactive the enzyme. The minisequencing reaction was conducted in a total volume of 5 μL with 1 μL of purified PCR product, 2.5 μL of SNaPshot reaction mix (Applied Biosystems), and 0.7 μL of single base extension (SBE) primer mix. SBE primers and cycling conditions were those specified by Bender (2005). Five μL of the SBE product were treated with 1 μL of the SAP enzyme (Amersham Biosciences) for 60 min at 37°C, followed by 15 min at 80°C for enzyme inactivation. SNaPshot products were observed in the ABI Prism 3130® Genetic Analyzer (Applied Biosystems). One microliter of SBE product was mixed with 9 μL of HiDi™ formamide and 0.3 μL of size standard LIZ-120 (Applied Biosystems); these were injected for 16 s at 1.2 kV and 5 μA at 60°C in a 36-cm-long capillary filled with POP7®. *CYP2D6* and *CYP2C19* genotypes were interpreted with GeneMapper® v3.2 (Applied Biosystems) software. The presence of the wild-type allele (*CYP2D6*1* and *CYP2C19*1*) was inferred when derived states were absent in all single-nucleotide polymorphisms (SNP) detected.

Statistical analysis

Allele and genotype frequencies were estimated by the gene counting method. Genotype distribution agreement with Hardy–Weinberg expectations was checked by exact tests. Genetic relatedness between populations based on *CYP2D6* and *CYP2C19* was evaluated by pairwise comparisons (exact tests) and genetic distances (F_{ST}), which additionally were represented in a multidimensional scaling plot (MDS) with SPSS 19.0.0 for Windows software. The Arlequin 2000 program was employed for genetic data analyses (Excoffier *et al.*, 2005).

Results and Discussion

To our knowledge, this is the first report of *CYP2C19* and *CYP2D6* SNPs related to PM phenotypes in the Mexican populations here analyzed. Although a low allele diversity was observed in the majority of populations, some limitations must be considered regarding these findings: (1) European or Asian prevalence of the alleles identified, which means that alleles of Amerindian origin are not represented, such as the variant *CYP2D6*82* recently described

Table 2. Allele and Genotype Frequencies (%) for *CYP2D6* and *CYP2C19* Genes in Amerindian Groups and Mestizos from Mexico

		Frequencies (%) CYP2D6								*Frequencies (%)* CYP2C19					
		Alleles				*Genotypes*				*Alleles*			*Genotypes*		
Population	n	**1*	**3*	**4*	**6,*7,*8*	**1/*1*	**1/*3*	**1/*4*	n	**1*	**2*	**3,*4,*5*	**1/*1*	**1/*2*	**2/*2*
Mestizos	125	93.2	1.2	5.6	0	86.3	2.5	11.2	145	93.1	6.9	0	87.6	11	1.4
Tarahumaras	88	92.7	0	7.3	0	85.3	0	14.7	84	69	31	0	48.8	40.5	10.7
Purépechas	85	97.1	0	2.9	0	94.1	0	5.9	101	94.6	5.4	0	89.1	10.9	0
Tojolabales	43	98.8	0	1.2	0	97.6	0	2.4	68	96.3	3.6	0	93.3	6.6	0
Tzotziles	56	97.3	0	2.7	0	94.3	0	5.7	88	94.3	5.6	0	88.6	11.3	0
Tzeltales	19	94.7	0	5.3	0	89.5	0	10.5	20	100	0	0	100	0	0

(Contreras *et al.*, 2011). (2) Deletions related with the PM phenotype were not included, particularly *CYP2D6*5*, which has been described with a low frequency (0.5%) in Tepehuanos, one Amerindian group from Northwest, Mexico (Sosa-Macias *et al.*, 2010). (3) Alleles related to the UM phenotype were not included in this study, such as *CYP2C19*17* (Sim *et al.*, 2006) and *CYP2D6* duplications. (4) The small population sample of some Amerindian groups, such as Tzeltales ($n = 19$), which is explained by the difficulty to obtain samples from these isolated communities. These limitations should be considered to design additional studies in Mexican populations, principally Amerindian groups, in the near future.

Allele frequencies

In general, low allele diversity was observed due to the predominance of the wild-type allele for both genes (>92%) (Table 2). The exception was the Tarahumara group that exhibited a lower *CYP2C19*1* frequency (69%). The alleles observed in the majority of Mexican populations were *CYP2C19*2* (range, 0%–6.9%) and *CYP2D6*4* (range, 1.2%–7.3%). Conversely, alleles *CYP2D6*6, *7*, and **8*, as well as *CYP2C19*3, *4*, and **5*, were not observed in the majority of Mexican population samples. It was particularly noteworthy that *CYP2D6*3* was observed exclusively in Mestizos, and that *CYP2C19*2* had an elevated frequency in Tarahumaras (31%), but was absent in Tzeltales, who solely presented the wild-type allele *CYP2C19*1* (Table 2).

These frequencies in Mexican populations are similar to those reported in European populations in which *CYP2C19*2* and *CYP2D6*4* are the modal alleles (Sachse *et al.*, 1997; Mizutani, 2003). In particular, *CYP2C19*2* frequency in Mestizos (6.9%) is similar to that reported in Mexico City (8.4%) (Hoyo-Vadillo *et al.*, 2010) and Mexican-Americans (9.7%) (Luo *et al.*, 2006). Regarding the high frequency of *CYP2C19*2* in Tarahumaras, this could be related with the high differentiation by genetic drift described for this group, in addition to their proximity to non-Amerindian groups, such as the NaDene groups located in North America (Rangel-Villalobos *et al.*, 2000; Rangel-Villalobos *et al.*, 2008). With respect to the allele *CYP2D6*4* frequency in Mestizos (5.6%), although this was lower than those reported in Mexico City (11.2%) and Durango (13.1%) (17–18), this difference was not significant ($p > 0.1268$). Finally, all of the SNPs analyzed in these Mexican populations were in Hardy–Weinberg equilibrium ($p > 0.05$) (data not shown).

CYP2C19 *genotype frequencies*

The scarce presence of a sole additional allele (*CYP2C19*2*) in the majority of Mexican populations explains the high frequency of wild-type homozygous **1/*1* (range, 48.8%–100%). Therefore, presence of heterozygous **1/*2* (range, 0%–11.3%) and homozygous **2/*2* (range, 0%–1.4%) also was low, except in Tarahumaras (40.5% and 10.7%, respectively). This suggests, in general, limited frequency of PM phenotypes in Mexican populations. Compared with previous reports, the frequency of homozygous **2/*2* in Mexican-Mestizos (1.4%) is similar to those observed in Mestizos of Colombia and Bolivia (Bravo-Villalta *et al.*, 2005; Isaza *et al.*, 2007). However, a previous study also carried out in western Mexican Mestizos, but based on the metabolic ratio (MR log) omeprazole/hydroxy-omeprazole, estimated a frequency of 6% for PM (González *et al.*, 2003). This constitutes a difference with respect to the PM frequency predicted by the genotypes **2/*2* detected in our study (6% vs. 1.4%) ($p = 0.047 \pm 0.0019$). Although this difference could be explained by the limited number of SNPs detected here, and to additional factors that influence the CYP2C19 pharmacokinetic phenotype (Shah, 2005; Jacob *et al.*, 2009), the answer to this finding would require further research.

As previously described, the main finding in this work regarding *CYP2C19* comprises the high frequency in Tarahumaras of heterozygous **1/*2* (40.5%) and homozygous **2/*2* (10.7%). This result deserves additional pharmacokinetic studies to confirm the possible increment of PM phenotypes in this Native American group that—presumably—would increase the risk for adverse drug reactions. In psychiatry, for instance, the CYP2C19 phenotype affects the pharmacokinetics of antidepressants, including monoamine oxidase inhibitor moclobemide, tricyclic antidepressants amitriptyline and clomipramine, and selective serotonin reuptake inhibitors (SSRI) sertraline and citalopram (Ingelman-Sundberg *et al.*, 2007); in addition to anxiolytics, such as diazepam and clonazepam (Desta *et al.*, 2002) and antiepileptics, such as clobazam (Seo *et al.*, 2008). Another potential effect of the high frequency of PM for CYP2C19 would be a lesser clinical response to prodrugs, such as clopidogrel, thalidomide, and cyclophosmamide (Takada *et al.*, 2004; Hulot *et al.*, 2006; Li *et al.*, 2007). However, the final impact of *CYP2C19*-based adverse drug reactions in Tarahumaras would be limited because of the rare application of these drugs in this Native group. This is due to the Tarahumaras' natural lifestyle and high physical activity in the mountains and canyons of the

Sierra Madre of Chihuahua (North, Mexico), which additionally complicates the presence of public health services (Pintado-Cortina, 2004). Conversely, modification of the Tarahumara lifestyle by their incorporation into urban areas—and into social health services—probably will increase their exposure to environmental factors and to CYP2C19-metabolized drugs (Yampey, 1981; Torres *et al.*, 2003). This should be considered to avoid adverse drug reactions in Tarahumaras currently incorporated into urban centers. In addition, *CYP2C19* has been described as a possible risk factor for lung (odds ratio [OR] = 3.23), esophageal (OR = 3.18), and stomach cancer (OR = 2.86) (Tsuneoka *et al.*, 1996; Shi and Chen, 2004). Therefore, research on *CYP2C19* genotypes and phenotypes and prevalence of the previously mentioned cancers in Tarahumaras will probably offer interesting results on this topic. This is of particular note considering additional studies that report weak or no association between *CYP2C19* alleles and cancer (Brockmoller *et al.*, 1996; Wadelius *et al.*, 1999). On the other hand, *CYP2C19* allele variants do not always represent a risk factor. For example, proton pump inhibitors (PPI) in PM achieve better suppression of gastric acid and efficient eradication of *Helicobacter pylori* (Furuta *et al.*, 2007; Chaudhry *et al.*, 2008). Based on the generally accepted idea that therapeutic efficiency of omeprazole is influenced by the *CYP2C19* genotype, some authors have suggested that this also affects the effectiveness of different PPIs. However, the less clear influence of the CYP2C19 genotype over further PPIs, support that this relationship actually depends on the kind of PPI (Chang *et al.*, 1995; Sapone *et al.*, 2003).

CYP2D6 *genotype frequencies*

The frequency of the wild-type homozygous *1/*1 was even greater for *CYP2D6* (range, 85.3%–97.6%) than for *CYP2C19.* This was a result of the limited presence of additional alleles *CYP2D6*4* and *CYP2D6*3*; the latter allele only observed in Mestizos. Therefore, the low frequency of heterozygous **1/*4* (range, 2.4%–14.7%) and **1/*3* (2.5% in Mestizos) and the absence of compounds heterozygous **3/*4* or homozygous for these alleles suggest a low frequency of PM for CYP2D6 in Mexican populations. Previous reports of *CYP2D6* in Mestizos from Mexico City and Durango (Lopez *et al.*, 2005; Sosa-Macias *et al.*, 2006) showed a similar allele distribution to that of the western Mestizos studied here ($p = 0.1564$ and $p = 0.5268$, respectively). This is a result of the relatively similar frequency of the heterozygous **1/*3* (0.41 and 1.13 vs. 2.5%, respectively) and heterozygous **1/*4* (10.7 and 15.9 vs. 11.2%, respectively). The impact of the heterozygotes is difficult to establish because of wide interindividual variation in enzyme activity for these genotypes (Carcillo *et al.*, 2003). Conversely, the unique antecedent of *CYP2D6* in Mexican-Amerindians derives from Tepehuanos (Northwest) (Sosa-Macias *et al.*, 2006), who exhibited a similar allele frequency to that of all five Native groups and the western Mestizos studied here ($p > 0.1229$). However, it was particularly noteworthy that the frequency of genotype **1/*4* (1.2%) in Tepehuanos was the lowest with respect to the five Amerindian groups (range, 2.4%–14.7%).

Genetic relationship

We attempted to describe genetic relationships among Mexican populations based on these two genes, adding certain populations to the analysis (Table 1). For *CYP2C19*, the similarity was evident among Mestizos, Latin-American, and Amerindian populations (Fig. 1A). The exception was the Tarahumara group that was similar to populations from Brazil and China (Mongolia). These populations formed a cluster ($p > 0.01$) based on their high *CYP2C19*2* frequency. As previously mentioned, separation of Tarahumaras could be explained by gene flow received from nearby Native North Americans, such as the NaDene groups, with a particular gene pool regarding Amerindians located geographically from Mesoamerica to South America (Schurr, 2004), for example, the haplogroup C* of the Y-chromosome, which is restricted to Native populations from North America (Zegura *et al.*, 2004). This hypothesis is in agreement with the genetic differentiation described for Tarahumaras by means of autosomal short tandem repeats and Y-chromosome markers (Rangel-Villalobos *et al.*, 2000; Rangel-Villalobos *et al.*, 2008). For *CYP2D6*, Mexican-Mestizos presented similarity ($p > 0.01$) (data not shown) with the majority of populations due to their intermediate position between Amerindian groups and Latin-American/Spanish populations (Fig. 1B). Interestingly, Amerindian groups were closer to China, but only China displayed a consistent differentiation with Latin-American and Spanish populations for *CYP2D6* ($p > 0.01$) (data not shown).

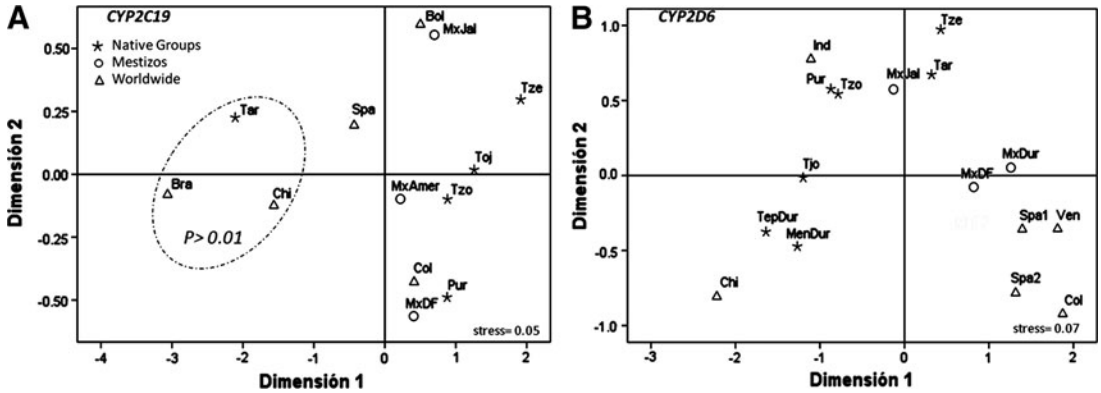

FIG. 1. Multidimensional scaling plot that shows genetic distances between Mexican and reference populations based on: **(A)** *CYP2C19* and **(B)** *CYP2D6*.

Conclusion

The wild-type allele for PM phenotypes in *CYP2D6* and *CYP2C19* genes was prevalent in western Mestizos and five Amerindian groups from Mexico. *CYP2C19*2* and *CYP2D6*4* were widely observed, but *CYP2D6*3* was only observed in Mestizos. Tarahumaras were distinguished by their high frequency of *CYP2C19*2*, which potentially represents a risk factor for this Amerindian group. In general, based on these SNPs, a relative homogeneity was observed among these Mexican populations.

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Author Disclosure Statement

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