

*CYP3A5*3*, *CYP3A4*1B* and *MDR1* C3435T genotype distributions in Ecuadorians

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Abstract. Polymorphisms in *CYP3A* genes, such as *CYP3A5* and *CYP3A4*, as well as in the *MDR1* gene, which encodes for P-glycoprotein, have been implicated as genetic markers in several disorders. Differences in the frequency distribution of the allelic variants *CYP3A5*3*, *CYP3A4*1B*, and *MDR1* 3435T have been demonstrated between distinct ethnic groups. In this study we examined the frequency of these allelic variants in 317 healthy Mestizo individuals from Ecuador and made comparisons with results reported in the literature. The genotypes were determined by PCR-RFLP. Allele and genotype differences were studied by chi-square test. The *MDR1* T allele frequency was similar to that of Spaniards or Asian populations, which is consistent with the ethnic origin of Ecuadorian Mestizo individuals (Amerindian and Spaniard Caucasians). By contrast, the *CYP3A5*3* allele frequency was significantly lower in Ecuadorians than in Spaniards and other white populations and higher than in Central Americans, Asians and blacks. *CYP3A4*1B* was more common in Ecuadorians than in Caucasian or Asian populations but less present than in blacks. The differences in the polymorphism found in this work should be considered in allele-disease association studies.

Keywords: *CYP3A5*3*, *CYP3A4*1B*, *MDR1* C3435T, Ecuadorians, disease markers

1. Introduction

The CYP3A subfamily of the human cytochrome P450 is involved in the metabolism of more than 50% of clinically used drugs, as well as in the hydroxylation of endogenous steroids and bioactivation of some genotoxicants [1]. The human *CYP3A* locus contains the genes encoding for *CYP3A4*, *CYP3A5*, *CYP3A7* and *CYP3A43*. Of these, *CYP3A4* and *CYP3A5* are the major enzymes responsible for drug metabolizing in adults; *CYP3A7* is the major fetal enzyme and

CYP3A43 has shown lower catalytic activity toward *CYP3A4* substrates [2].

CYP3A5 is polymorphically expressed. The major defective allele is *CYP3A5*3*. This mutant allele (A6986G) in the intron 3 of *CYP3A5* leads to alternative splicing and protein truncation, which results in absence of enzyme activity. In individuals carrying at least one *CYP3A5*1*, which encodes a normally spliced *CYP3A5*, the *CYP3A5* isoform contributes up to 50% of hepatic CYP3A protein [3].

The *CYP3A4* expression is highly variable. It exhibits a common variant in the 5'-flanking region designated *CYP3A4*1B*. It has been shown that *CYP3A4*1B* polymorphism is associated to disease risk [4–6], and some in vitro studies indicate an association between the presence of the *CYP3A4*1B* allele and higher expression or activity. However, data about the function-

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al effect on drug disposition in vivo are controversial, suggesting the possibility of linkage disequilibrium between *CYP3A4*1B* and *CYP3A5*1* [3].

P-glycoprotein (P-gp), an ATP-dependent membrane efflux transporter, is the product of the multidrug resistance gene (*MDR1*). Expression of the P-gp is influenced by *MDR1* C3435T genetic polymorphism of the human *MDR1* gene [7]. The common C3435T polymorphism in exon 26 has been reported to be associated with lower P-gp expression and drug uptake; individuals with the CC genotype have approximately two-fold higher oral bioavailability of digoxin than individuals homozygous for the T allele variant [7]. C3435T polymorphism of the *MDR1* gene has also been linked to an increased risk of development of several diseases [8–14].

Population differences in genetic polymorphism of enzymes and transporters involved in drug disposition can result in phenotype exhibiting poor, extensive, or even multiextensive metabolism. Therefore, genetic differences in the metabolism of drugs, xenobiotics and some endogenous compounds can lead to severe toxicity, risk of disease or therapeutic failure. Understanding the ethnic differences in allele and genotype frequencies has the potential to explain some of the observed ethnic variability in drug response and disease prevalence as well as to improve clinical practice and to optimize the clinical evaluation of the therapeutic efficacy and safety of drugs for patients throughout the world.

Socio-economic reasons as well as the absence of any language barrier are causing an important influx of Ecuadorians to Spain. In the last few years Spain has become one of the most significant destinations for Ecuadorian migrants. Mestizos are the most representative and the largest group in Ecuador. They are descendants of Spanish (Caucasian) and Amerindian people.

The aim of this study was to detect differences in the frequency of *CYP3A* and *MDR1* C3435T polymorphisms in the Ecuadorian Mestizo population.

2. Material and methods

2.1. Subjects and study protocol

The total study population consisted of 317 unrelated healthy individuals (150 males and 167 females) from Ecuador. Means \pm standard deviations of age were 26.5 ± 6.7 (range, 18–52). All participants from

Ecuador were “Mestizo” (Amerindian and European descent). Individuals were randomly selected among students and personnel of the Metropolitan Hospital of Quito (Ecuador).

All individuals were healthy as assessed by medical history and physical examination. All subjects gave their written informed consent to participate in this study, which was approved by the Human Research Ethics Committee of Aragón (Zaragoza, Spain), and was conducted in accordance with the Declaration of Helsinki and its subsequent revisions.

2.1.1. Genotyping

Genomic DNA was extracted from peripheral blood, blotted and dried on filter paper using the QIAamp DNA Micro Kit (Izasa, Madrid, Spain).

CYP3A4

The method used to determine the genotype was performed as previously described [5]. This method consisted of a two-step PCR-based restriction fragment length polymorphism assay to determine the genotype of a *CYP3A4*1A/B* single nucleotide polymorphism at the promoter region (–290) on the nifedipine-specific response element (A to G). We use a negative control in all reactions. The first PCR reaction generated a 319-bp fragment, covering the nifedipine-specific response element. The PCR products were diluted 1:500 and one μ l of this dilution was used for a secondary PCR reaction to amplify a 168-bp fragment. This PCR was done using a set of primers: 5'-GGACAGCCATAGAGACAAGGCCA-3' (forward) and 5'-CACTCACTGACCTCCTTTGAGT TCA-3' (reverse). Ten μ l of the second PCR products were digested at 37°C for 2 h. Then, the digests were electrophoresed on a 3% agarose gel and stained with ethidium bromide. *CYP3A4*1A* homozygotes exhibited a 168-bp fragment and *CYP3A4*1B* homozygotes showed 146-bp and 22-bp fragments.

CYP3A5

For the detection of *CYP3A5*1* and *CYP3A5*3*, we used an allele-specific PCR, as has been previously described [15]. *CYP3A5* gene-specific primers specifically amplified a 750-bp fragment of the region of interest. This PCR product was diluted 10 times and served as template for the subsequent allele-specific PCR reaction. The forward primer of this PCR was 5'-CATGACTTAGTAGACAGATGA-3' and it was used together with either 5'-CAGGGAAGAGATAC-3' for identification of the *CYP3A5*3* allele or 5'-

Table 1
Allele and genotype frequencies of the *CYP3A5* variants observed in this study, compared with those found in other populations

Population	N° alleles	Allele frequency		Genotype frequency (%)			Ref.
		*1	*3	*1/*1	*1/*3	*3/*3	
African American	76	0.66**	0.34	45.0	42.0	13.0	4
African American	40	0.45**	0.47	25.0	25.0	35.0	3
South Asian							
Indian	180	0.40**	0.60	12.2	56.7	31.1	35
Malay	196	0.39**	0.61	10.2	57.1	32.7	35
East Asian							
Chinese	216	0.25*	0.75	8.3	32.4	59.3	35
Central American	464	0.24**	0.76	3.0	41.4	55.6	34
Ecuadorian	634	0.12	0.88	2.2	20.2	77.6	This study
North American	874	0.10	0.90	0.7	17.0	82.0	4
Spaniard							
South Spaniard	354	0.09	0.91	2.8	12.4	84.7	36
North Spaniard	408	0.09*	0.91	1.0	15.2	83.8	34
German	856	0.06*	0.94	0.2	11.9	87.9	33

* $P < 0.05$; ** $P < 0.001$.

CAGGGAAGATAT-3' for identification of the *CYP3A5*1* allele, using specific reverse primers for each allele and the same forward primer for both alleles. The products were electrophoresed on a 3% agarose gel and stained with ethidium bromide.

C3435T

The genotype was determined using the method previously used in our laboratory [16] with some modifications. Briefly, this method consists of a two-step PCR-based restriction fragment length polymorphism assay to determine the C3435T polymorphism. Primers were designed from known sequences of exon 26 (Genbank accession nos: AY910577 and M29445). The first PCR reaction generates an 802 bp fragment. The PCR products were diluted 1:10 and one μ l of this dilution was used for a secondary PCR reaction to amplify a 244 bp fragment. This PCR was done using a set of primers: 5'-GAT CTG TGA ACT CTT GTT TTC A-3' (forward) and 5'-GAA GAG AGA CTT ACA TTA GGC-3' (reverse). Ten μ l of the second PCR products were digested at 37°C for 2 h by the *Sau3AI* enzyme. Then, the digests were electrophoresed on a 3% agarose gel and stained with ethidium bromide. The fragments obtained were of 244 bp to TT genotype, 170 bp and 70 bp fragments to CC genotype, and 244 bp, 170 bp, and 70 bp to CT genotype.

2.2. Statistical analysis

The differences in genotype frequencies between different populations and genotypic groups were determined using the χ^2 test of goodness of fit with one

degree of freedom. Hardy-Weinberg equilibrium was assessed by comparing the genotype frequencies with the expected values using a contingency table χ^2 statistic. Partial linkage between alleles was determined by the χ^2 test. Probability values of less than 0.05 were regarded as statistically significant.

3. Results

The *CYP3A5*, *CYP3A4* and *MDR1 C3435T* genotype frequencies among Ecuadorians correspond to those predicted by the Hardy-Weinberg law ($p > 0.05$ in all cases).

Table 1 shows the *CYP3A5* allele and genotype frequencies in Ecuadorians compared to those previously found in other ethnic groups. The frequency of *CYP3A5*3* allele among Ecuadorians (88%) was significantly lower as compared with North Spaniards (91%) ($p = 0.04$) and another European population (94%) ($p = 0.003$). However, the frequency of *CYP3A5*3* allele in Ecuadorians resulted to be significantly higher than that observed in Central Americans (76%) ($p < 0.001$) and also in other ethnic groups such as Asian populations ($p = 0.003$) or African American people ($p < 0.001$).

As shown in Table 2, *CYP3A4*1B* allele frequency in Ecuadorians (8%) was similar to that in Central Americans (13%) and higher than in North Spaniards (4%) ($p = 0.04$) or Asian groups ($p < 0.001$). This frequency was lower in comparison with African American or Guinean populations ($p < 0.001$ in both cases).

The presence of the haplotype *CYP3A4*1B/CYP3A5*1* in Ecuadorians was significantly higher

Table 2

Allele and genotype frequencies of the *CYP3A4* variants observed in this study, compared with those found in other populations

Population	N° alleles	Allele frequency		Genotype frequency (%)			Ref.
		*1A	*1B	*1A/*1A	*1A/*1B	*1B/*1B	
Guinean	100	0.28**	0.72	10.0	36.0	54.0	37
African American	76	0.46**	0.54	21.0	42.0	37.0	4
Central American	464	0.87	0.13	75.9	23.3	0.8	34
Hispanic American	242	0.89	0.11	N.A	N.A	N.A	38
Ecuadorian	634	0.92	0.08	83.6	15.8	0.6	This study
Spaniard							
South Spaniard	354	0.96	0.04	91.5	8.5	0.0	36
North Spaniard	408	0.96*	0.04	91.7	8.3	0.0	34
German	856	0.97	0.03	94.4	5.6	0.0	33
South Asian							
Malay	208	1**	0	N.A	N.A	N.A	39
Indian	202	1**	0	N.A	N.A	N.A	39
East Asian							
Chinese	220	1**	0	N.A	N.A	N.A	39
Japanese	254	1**	0	N.A	N.A	N.A	39

N.A: data not available; * $P < 0.05$; ** $P < 0.001$.

than expected for independent genes ($\chi^2 = 29.93$, $p < 0.001$) suggesting a certain linkage disequilibrium.

MDR1 C3435T allele and genotype frequencies in Ecuadorians compared to previous results obtained in populations of different ethnicities are shown in Table 3. Among Ecuadorians the C and T allele frequencies were found to be 52% and 48%, respectively. C allele frequency in Ecuadorians was similar to that in Caucasian groups and Central Americans ($p > 0.05$ and $p = 0.92$, respectively) and significantly lower than in the African American population ($p < 0.001$). However, this C allele frequency was found to be significantly higher than in Indian (38%) ($p = 0.014$) and South-west Asian populations (34%) ($p = 0.002$).

4. Discussion

The *MDR1* C3435T genetic polymorphism has been shown to be associated to both disease risk and pharmacokinetic variability. In fact, an association between *MDR1* C3435T polymorphism and susceptibility to risk of development of either early-onset Parkinson's disease [8], Balkan endemic nephropathy [9] or ulcerative colitis (UC) [10] has been reported. *MDR1* genotype might also be important as a potential target for therapy in patients with refractory Crohn's disease and UC [17]. There are some reports dealing with the relationship between *MDR1* C3435T and susceptibility to cancer, such as the association between T allele presence and risk of either epithelial tumour [11], childhood ALL [12], colon cancer [13] or acute myeloid leukemia [14]. On the other hand, some studies are

showing growing evidence that *MDR1*/P-gp modulates *CYP3A* activity; this modulation being associated to the *MDR1* polymorphism [18].

In this work we have observed a similar frequency distribution of *MDR1* CC, CT, and TT genotypes in Ecuadorians relating to that previously found in Spaniard and East Asian groups (Table 3). This could be explained by the ethnohistory of the Mestizo people. Indeed, Mestizos are characterized by a biracial mixture with a gene pool derived from Native Amerindian groups (originating from Asia) with Caucasians coming from Spain and other European countries. This would also be consistent with the lack of differences in *MDR1* C3435T genotype between Ecuadorians and Central Americans, since in both cases the study population was restricted to Mestizo people. Only African populations have been shown to have a very high frequency distribution of the C allele which has been suggested to be a consequence of selective advantage against gastrointestinal infections [19].

The *CYP3A5*3* allele frequency in Ecuadorians observed in this study was significantly lower than in Spaniards ($p = 0.04$) and much higher than that reported in Asian populations ($p = 0.003$) (Table 1); this reflecting the Ecuadorian genetic admixture between Spaniards and American Indians with a major contribution from European parental populations, as determined for other genetic features [20]. By contrast, the relative genetic contribution of Spaniards and Amerindians to the admixture in Central Americans appears to be higher for Amerindian groups as observed for other genetic traits [21,22]. This could explain the differences between Ecuadorians and Central Americans, the

Table 3
Allele and genotype frequencies of *MDR1 C3435T* variants observed in this study, compared with those found in other populations

Population	N° alleles	Allele frequency		Genotype frequency (%)			Reference
		C	T	CC	CT	TT	
African American	176	0.84**	0.16	68.0	31.0	1.0	[40]
Ghanaian	412	0.83**	0.17	67.0	34.0	0.0	[40]
Kenyan	160	0.83**	0.17	70.0	26.0	4.0	[40]
Sudanese	102	0.73*	0.27	52.0	43.0	6.0	[40]
Japanese	228	0.61	0.39	35.0	53.0	12.0	[41]
Chinese Kazakh	216	0.60	0.40	38.0	44.4	17.6	[42]
Filipino	120	0.59	0.41	38.0	42.0	20.0	[40]
Chinese	530	0.56	0.44	32.0	48.0	20.0	[43]
Saudi	192	0.55	0.45	37.0	38.0	26.0	[40]
Central American	464	0.53	0.47	29.2	46.7	24.1	[34]
Ecuadorian	634	0.52	0.48	24.9	53.0	22.1	This study
North Spaniard	408	0.52	0.48	27.0	51.0	22.0	[34]
German	376	0.52	0.48	27.0	48.0	24.0	[7]
South Spaniard	177	0.50	0.50	25.0	50.0	25.0	[36]
Caucasian (UK)	380	0.48	0.52	24.0	48.0	28.0	[40]
Malay	198	0.48	0.52	25.0	46.0	28.0	[10]
Chinese Uygur	322	0.47	0.53	24.8	44.7	30.4	[42]
German	922	0.46	0.54	21.0	50.0	29.0	[44]
Russian	580	0.46	0.54	21.4	48.6	30.0	[45]
Portuguese	200	0.43	0.57	22.0	42.0	36.0	[40]
Indian	186	0.38*	0.62	18.0	39.0	43.0	[10]
South-west Asian	178	0.34*	0.66	15.0	38.0	47.0	[40]

* $P < 0.05$; ** $P < 0.001$.

latter showing a significantly lower *CYP3A5*3* allele frequency.

The *CYP3A4*1B* allele frequency in Ecuadorians has shown to be significantly higher ($p = 0.04$) than in Spaniards and notably higher than in Asian populations ($p < 0.001$) (Table 2). As a result of the genetic background of Ecuadorians which combines that of white Europeans with Native American groups, we would have expected to find a lower *CYP3A4*1B* frequency. The existence of a selection factor against this allele in non-African populations has been recently suggested; because *CYP3A4* is involved in the vitamin D metabolism, rickets may have been the underlying selection factor [23]. We can only presume that the presence of the negative selection factor has been less present in Central and South America. In addition to differential selection, a founder effect and genetic drift can not be excluded. Indeed, the colonization process of the New World, carried new diseases and violent deaths with population loss.

The higher frequency of the *CYP3A5*1* allele in the Ecuadorian population compared with Spaniards and other white populations may be clinically relevant since subjects carrying the *CYP3A5*1* allele appear to require higher doses of *CYP3A5* substrates such as tacrolimus [24] or midazolam [25].

The association between *CYP3A5* polymorphism and high blood pressure or hypertension risk is con-

troversial [26,27]. By contrast, the antihypertensive response to verapamil appears to be better in carriers of two functional *CYP3A5* alleles [28]. On the other hand, lipid-lowering response to the statins metabolized by *CYP3A5* may be reduced in *CYP3A5*1* carriers [29] but these individuals may benefit from a reduced risk of statins-induced rhabdomyolysis [30]. In addition, *CYP3A5* polymorphism has been associated with Balkan endemic nephropathy [31], oesophageal cancer [32] and also with prostate cancer, similar to that detected for *CYP3A4*1B* [4].

Several studies have implicated *CYP3A4*1B* as a candidate allele in several disorders including prostate cancer [4], increased susceptibility to lung cancer [6], and early puberty [5]. As *CYP3A4*1B* and *CYP3A5*1* have been found to be in linkage disequilibrium in both Europeans [33] and blacks [4] it is probable, as suggested by Kuehl et al. [3] that the presence of *CYP3A5*1* might be responsible for the high expression phenotype towards endogenous or exogenous substrates [3]. In this regard, the *CYP3A5*1/CYP3A4*1B* genotype combination in Ecuadorians (7.5%) has been more common than that previously reported for Spaniards (5.4%) [34] and Germans (4%) [33], but lower than in Central Americans (16.9%) [34] and African Americans (53%) [4]. Hence, these latter results seem to reinforce the notion about a higher *CYP3A* activity in Ecuadorians.

In summary, the findings of this study and, especially the differences in CYP3A4*1B and CYP3A5*3 allele frequencies could contribute to better clinical care of Mestizo population from Ecuador and should be considered in allele disease association studies.

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