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ORIGINAL ARTICLE

Differences between Spaniards and Ecuadorians in *CYP2A6* allele frequencies: comparison with other populations

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ABSTRACT

This study was designed to investigate the potential differences between Spaniards and Ecuadorian Mestizo people regarding *CYP2A6*1A*, *CYP2A6*1B1*, *CYP2A6*1x2A*, *CYP2A6*9A*, and *CYP2A6*4A* variant alleles at the *CYP2A6* gene and also to compare the observed frequencies with those previously reported in different ethnic groups. DNA from 234 Spaniard and 300 Ecuadorian subjects were analyzed by either PCR or PCR-restriction fragment length polymorphism. Differences between Spaniards and Mestizo Ecuadorians were detected in relation to the frequencies of the alleles linked to either absent enzyme activity, *CYP2A6*4A* (4 and 7.1%, respectively), or reduced CYP2A6 enzyme activity, *CYP2A6*9A* (6.4 and 10.3%, respectively). *CYP2A6*4A* and *CYP2A6*9A* frequencies in Ecuadorians were higher than those in Africans or Caucasian groups and lower than those in Asians. This study provides, for the first time, the result of the analysis of *CYP2A6* allele frequency in a South American population and demonstrates the presence of ethnic differences in *CYP2A6* genetic variants between Spaniards and Mestizo Ecuadorians, which should be considered in allele–disease association studies and, in particular, in those involving *CYP2A6* genetic polymorphisms and tobacco-related cancer.

INTRODUCTION

Human CYP2A6 catalyzes the metabolism of a number of pharmaceuticals such as coumarin, tegafur, losigamona, and valproic acid. CYP2A6 is the major enzyme involved in the metabolism of nicotine [1] and also in the bioactivation of some procarcinogens such as aflatoxin B_1 and the tobacco-related N-nitrosamines, 4-(methylnitrosamine)-1-(3-pyridyl)-butanone (NNK) and N-nitrosonornicotine (NNN) [2].

CYP2A6 is predominantly expressed in the liver with a wide interindividual variation of more than 100-fold in both CYP2A6 mRNA and protein levels [3,4]. Genetic variation accounts for a part of this variability. To date, more than seventy different alleles have been characterized (http://www.imm.ki.se/CYPalleles/CYP2A6.htm), including those with gene duplication (*CYP2A6*1x2A*) [5], gene conversion (*CYP2A6*1B1*) [6,7], gene deletion (*CYP2A6*4A*) [8,9], and single nucleotide polymorphisms (SNP) in coding and regulatory regions (*CYP2A6*9A*) [10]. *CYP2A6*9A* and *CYP2A6*4A* are associated, respectively, to decreased [11] or absent CYP2A6 enzyme activity [12–14]. By contrast, *CYP2A6*1x2A* and *CYP2A6*1B1* have been observed to be linked to higher CYP2A6 activity [5,14,15]. These genetic polymorphisms may be of relevance to cancer risk associated with tobacco consumption by changing the rate of NNN and NNK activation [16]. In fact,

defective alleles *CYP2A6*9A* and, especially, *CYP2A6*4A*, appear to protect against lung [17,18], head and neck [19], upper aerodigestive tract [20,21], and bladder cancers [22] and, conversely, *CYP2A6*1x2A*, which is linked to higher CYP2A6 activity [5] has been found to be overrepresented in larynx and lung cancer patients [23]. In addition, impaired metabolism has been proposed to reduce cigarette smoking in slow metabolizers of nicotine [24] which may indirectly decrease the risk of developing tobacco-related tumors.

Large interethnic differences in *CYP2A6* allele frequency have been extensively reported with a higher frequency of the defective alleles among Asians in relation to Caucasian and African populations [6,14,25]. Previous reports indicate that Spaniard Caucasians show different frequencies for some polymorphisms in P450 (CYP) genes than those of other European populations [26,27]. However, the information about the frequency of common *CYP2A6* allelic variants in Spaniards is scarce and restricted to the *CYP2A6*2* [28] and *CYP2A6*4A* alleles [8,29], and the frequencies in South Americans from Ecuador have not been documented so far.

The absence of any language barrier has caused an important migratory influx of Ecuadorians to Spain over the last years. Mestizos are the most representative and the largest group in Ecuador. They are descendents of Spaniards (Caucasian) and Amerindian people originating from Asia. This study was designed to detect interethnic differences in *CYP2A6*1A*, *CYP2A6*1B1*, *CYP2A6*1x2A*, *CYP2A6*9A*, and *CYP2A6*4A* variant alleles between a Spaniard and an Ecuadorian Mestizo population.

MATERIAL AND METHODS

Subjects

The total study population consisted of 534 healthy, unrelated individuals. From these, 234 were white European subjects from Spain (111 men and 123 women) and 300 (148 men and 152 women) were subjects from Ecuador. Age ranges in years of the participants were 18–48 and 18–52 for Spaniards and Ecuadorians, respectively. All participants from Ecuador were 'Mestizo' (Amerindian and European descent), as assessed by the morphological traits, skin color, self-identification, and genotyping with a panel of ancestry-informative markers, AIM (Power PlexR® 16 System kit, Promega Co, Madison, WI, USA). Individuals were randomly selected among students and personnel of the Medical School of the University of Zaragoza (Zaragoza, Spain) and 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.

Genotyping

Genomic DNA was extracted from peripheral blood, blotted and dried on filter paper using the QIAamp DNA Micro Kit (Izasa, Madrid, Spain). The method used to determine the three alleles *CYP2A6*1A*, *CYP2A6*1B1* y *CYP2A6*4A* was performed as previously described [30]. The primers used were the 8S (5′-CACCGAAGTGTACC CTATGCTG-3′) and R2 (5′-AAAATGGGCATGAACGCCC-3′). After the amplification, the polymerase chain reaction product was double-digested with the *Eco81 I* and the *Acc II* restriction enzyme. Then, the digests were electrophoresed on a 2% agarose gel and stained with ethidium bromide. The *CYP2A6*1A* yielded 800-, 434-, and 104-bp fragments, the *CYP2A6*1B1* yielded 800-, 285-, 149-, and 104-bp fragments, and the *CYP2A6*4A* yielded 759-, 285-, 149-, 104-, and 41-bp fragments.

For the detection of the duplication variant *CYP2A6*1x2A*, we used a one-step genotyping assay, as has been previously described [5]. This PCR used a CYP2A7-specific (5′-CATTTCCTGGATGAC-3′) or CYP2A6-specific (5′-CATTTCCTGAATGAG-3′) forward primer with the CYP2A7 (5′-GCACTTATGTTTTGTGAGACAT-CAGATAGAG-3′) reverse primer for the detection of the wild-type *CYP2A7* and duplicated *CYP2A6* variants, respectively. The products (1258 bp) were separated by gel electrophoresis on a 1.2% agarose gel stained with ethidium bromide.

For genotyping of *CYP2A6*9A*, an allele-specific PCR method previously reported was used [11]. The primers used were 2A6*9S (5′-GATTCCTCTCCCCTGGAAC-3′) and 2A6*9AS-wild type (5′-GGCTGGGGTGGTTTGCCTT TA-3′) or 2A6*9AS-mutant type (5′-GGCTGGGGTGGTT TGCCTTTC-3′). The expected size of the PCR product was 368 bp, and it was analyzed by electrophoresis on a 2% agarose gel.

Statistical analysis

The differences in allele frequencies between different populations were determined using the chi-square 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 chi-square statistic. Probability values of <0.05 were regarded as statistically significant.

RESULTS

The *CYP2A6* genotype frequencies among both Spaniards and Ecuadorians correspond to those predicted by the Hardy–Weinberg law ($P > 0.05$).

The allele frequencies in Spaniards were not different ($P > 0.05$) to those previously found in other Caucasian populations (*Table I*), with the only exception being that of the *CYP2A6*4A* allele associated with abolished enzyme activity. In this regard, although the *CYP2A6*4A* prevalence in Spaniards (4%) was the same than that previously reported in a French Caucasian population [15], it was higher than that previously found among white Canadian people (1.2%) ($P < 0.001$) [25]. In addition, the comparison of the *CYP2A6*4A* frequencies between Spaniards and a Finnish population (1%) [8] shows a borderline significance level ($P = 0.07$). On the other hand, *CYP2A6*4A* frequency in this work was similar ($P > 0.05$) to that observed in our previous study in 100 Spaniards [8].

As shown in *Table I*, the frequency of the *CYP2A6*1A* allele among Spaniards and other Caucasian groups was between those reported in Orientals [8,17,32] and those in Africans [33] with highly significant differences in the comparisons ($P < 0.001$ in all cases). On the contrary, the opposite rank order was observed when the *CYP2A6*1B1* allele frequencies were compared: Africans < Spaniards and other Caucasian groups < Orientals ($P < 0.001$ in all comparisons) (*Table I*).

The frequencies of the *CYP2A6*4A* and *CYP2A6*9A* alleles were similar in Spaniards and Africans ($P > 0.05$). By contrast, as expected, the *CYP2A6*4A* and *CYP2A6*9A* alleles were more prevalent in Orientals than in Spaniards and in other Caucasian groups ($P < 0.001$) (*Table I*). No differences between ethnic groups have been found when the *CYP2A6*1x2A* allele frequencies were compared ($P > 0.05$ in all comparisons).

Spaniards and Ecuadorians show similar frequencies of *CYP2A6*1A*, *CYP2A6*1B1*, and *CYP2A6*1x2A* (*Table I*) without significant differences in the comparisons ($P > 0.05$ in all cases). By contrast, differences between these two populations were found when the frequencies of the alleles linked to null or reduced CYP2A6 enzyme activity (*CYP2A6*4A* and *CYP2A6*9A*, respectively) were compared. Thus, the frequency of the deletion of the whole *CYP2A6* gene (*CYP2A6*4A)* was 4% in Spaniards and 7.1% in Ecuadorians ($P < 0.05$), and that of the *CYP2A6*9A* allele, which has a point mutation in the TATA box (−48 T > G), was 6.4 and 10.3% in Spaniards and Ecuadorians, respectively ($P < 0.05$) (*Table I*). The *CYP2A6*4A* and *CYP2A6*9A* allele frequencies in Ecuadorians were also higher than those reported earlier in a Caucasian population [25] ($P < 0.01$ and $P < 0.05$, respectively); these frequencies in Ecuadorians being between those found earlier in Orientals [8,25] (either Chinese, $P < 0.05$ or Japanese, $P < 0.001$) and those previously reported in Africans [33] ($P < 0.01$) (*Table I*).

DISCUSSION

Understanding the ethnic differences in allele frequencies offers the possibility to explain some observed ethnic variability in drug response and disease prevalence as

Table I Frequencies of *CYP2A6* alleles (%) observed in this study compared with those found in other populations

	Population					
CYP2A6 allele	Spaniard (this study)	Ecuadorian (this study)	Caucasian	Chinese	Japanese	African from Ghana
*CYP2A6*1A*	64.9 (468)	61.7 (600)	67.0 (926) [15]	27.2 (192) [8]	16.4 (2444) [17]–20.3 (368) [32]	80.5 (420) [33]
*CYP2A6*1B1*	30.9 (468)	31.2 (600)	33.5 (1416) [25]	34.5 (192) [8]–51.3 (226) [25]	27.0 (368) [32] –48.4 (128) [25]	11.9 (420) [33]
*CYP2A6*4A*	4.0 (468)	7.1 (600)	1 (200) [8]–1.2 (2336) [25] –4.0 (926) [15]	6.7 (224) [25]–15.1 (192) [8]	24.2 (128) [25]	1.9 (420) [33]
*CYP2A6*9A*	6.4 (468)	10.3 (600)	7.1 (1856) [25]	15.6 (224) [25]	20.3 (128) [25]	5.7 (420) [33]
*CYP2A6*1x2A*	1.2 (468)	0.5 (600)	0.7 (2296) [25]–1.7 (592) [5]	0.4 (226) [25]	0.0 (124) [25]	n.d.

n.d., not determined.

Figures in parenthesis represent number of alleles tested.

well as to improve and optimize the clinical practice and the evaluation of the efficacy and safety of drugs for patients throughout the world.

First, in this study, we determined the frequencies for five allelic variants at the *CYP2A6* gene in a Spaniard population, because the information regarding *CYP2A6* polymorphisms in Spaniards is limited and restricted to *CYP2A6*2* [28] and *CYP2A6*4A* [8] polymorphisms. In addition, although the frequencies of *CYP2A6* polymorphisms in other Caucasian populations is well documented [8,15,25], a geographic gradient according to latitude has been observed for some polymorphisms in Europe [26,27].

In this regard, no differences between Spaniards and other Caucasian populations in the frequencies distributions of the *CYP2A6*1A*, *CYP2A6*1B1*, *CYP2A6*9A*, and *CYP2A6*1x2A* have been found in this work (*Table I*).

Even though the *CYP2A6*4A* allele frequency distribution in Spaniards has been detected to be similar to that previously found in a French Caucasian population [15], it was higher than that reported in a wide cohort of Caucasian–Canadians [25]. Moreover, differences in *CYP2A6*4A* frequencies between Spaniards in this study and Finnish in a previous report [8] were of borderline significance ($P = 0.07$). In this regard, differences between Spaniards and other European Caucasian populations have been reported for other polymorphisms with a North–South gradient [26,27], probably because of a higher intermingling of gene pools between northern African people and Spaniards; the geographic proximity leading to a gene flow across the coast since ancient times [34].

Our study is the first to describe the frequency distribution of *CYP2A6* alleles in a South American population. Whereas no differences between Ecuadorians and Spaniards or other Caucasian groups in the frequencies of the alleles linked to either normal (*CYP2A6*1A*) or increased (*CYP2A6*1B1* and *CYP2A6*1x2A*) enzyme activity have been found, the alleles associated with either null (*CYP2A6*4A*) or reduced (*CYP2A6*9A*) enzyme activity have been detected to be more prevalent among Ecuadorians than in Spaniards or other Caucasian populations. The pattern of *CYP2A6*4A* or *CYP2A6*9A* in Ecuadorians found here could be explained by the biracial admixture of populations in Mestizo people, with a gene pool derived from Native Amerindian groups (originating from Asia) with other Caucasians coming from Spain and other European countries. In fact, as shown in *Table I*, with the magnitude of these frequencies in Ecuadorians being located in an intermediate position between Caucasians (including Spaniards) and Asians.

A number of studies have demonstrated that *CYP2A6* genetic variations leading to decreased or absent CYP2A6 activity are associated with reduced risk of smoking, lower amount smoked, less smoking intensity, and increased quitting [10,12,24,35–37]. On the other hand, several reports dealing with *CYP2A6* genetic variants and cancer risk indicate that *CYP2A6* alleles linked to lower or null enzyme activity are less prevalent among cancer patients [17–22]. Taken together, these data and the results of the present study could suggest that on a population basis, Ecuadorians are relatively more protected from developing tobacco-related cancer, through a direct (lower bioactivation of procarcinogens in tobacco) and indirect (more moderated smoking behavior) effect.

In summary, the frequencies of the *CYP2A6*1A*, *CYP2A6*1B1*, and *CYP2A6*1x2A* have been detected to be similar in Spaniards and Mestizo Ecuadorians. By contrast, the frequency of the *CYP2A6*4A* and *CYP2A6*9A* was higher in Ecuadorians compared to Spaniards, other Caucasian groups, and Africans, but lower than Asians. These data clearly provide further evidence of ethnic heterogeneity in *CYP2A6* polymorphism and also reinforce the notion about the biracial origin of the Mestizo population from Ecuador.

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