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CYP2C8 polymorphism among the Portuguese

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Abstract

Cytochrome P450 2C8 (CYP2C8) is a polymorphic phase I drug-metabolising enzyme involved in the metabolism of a wide variety of xenobiotics, as well as a proposed player in the regulation of vascular tone. Polymorphisms in this gene may have an impact on the metabolism of therapeutic drugs such as paclitaxel and verapamil. In this report we have determined the frequencies of the main non-synonymous *CYP2C8* alleles, 805A>T (*CYP2C8*2*), 416G>A/1196A>G (*CYP2C8*3*) and 792C>G (*CYP2C8*4*) in a sample representative of Portuguese Caucasians. The allelic frequencies determined were 1.2%, 19.8%, and 6.4% for *CYP2C8*2*, *CYP2C8*3*, and *CYP2C*4*, respectively. The observed *CYP2C8*3* prevalence is significantly different from the frequencies previously reported in North European populations.

Keywords: allele; CYP2C8; cytochrome P450; polymorphism; Portugal.

Introduction

The human cytochrome P450 2C8 (CYP2C8) enzyme is part of the four-member CYP2C subfamily of CYP450 enzymes. This enzyme is mainly expressed in liver, as well as in various extra-hepatic tissues, such as the vascular smooth muscles (1, 2).

The *CYP2C8* gene is located in a cluster on chromosome 10 with the other *CYP2C* genes (3). As for all the other members of this subfamily, several single nucleotide polymorphisms (SNPs) have been described for *CYP2C8* (http://www.imm.ki.se/CYPalleles/CYP2C8.htm). Diversity in this gene is apparently lower than that observed for the other *CYP2C* genes, an idea which has been recently supported by analysis of the full *CYP2C8* open reading frame in a set of individuals of very diverse geographical origin (4).

The main *CYP2C8* polymorphisms known code for the amino acid changes I269F, R139K, K399R and I264M. These variants have been documented as being associated with altered enzyme activity in vitro towards the probe drug paclitaxel (5, 6). These SNPs define three main non-wild-type alleles, *CYP2C8*2* (harbouring I269F), *CYP2C8*3* (carrying both R139K and K399R) and *CYP2C8*4* (I264M).

The CYP2C8 enzyme plays an important role in the metabolism of several therapeutic drugs, including paclitaxel (7), amodiaquine (8), troglitazone (9), amiodarone and verapamil (10, 11). Furthermore, it has been suggested that CYP2C8 is involved in the activation of procarcinogenic compounds (12). CYP2C8 also plays a role in the biosynthesis of endogenous vasoregulating factors from arachidonic acid (5). The importance of its endogenous functions might be reflected in the relatively low number of non-synonymous SNPs documented for *CYP2C8*.

In this work we studied the aforementioned *CYP2C8* alleles among the Portuguese. In addition, due to its expected effect on the structure of the protein, we further screened our population for the presence of a recently described three-nucleotide deletion (–/TTG, 461delV) on exon 9 (http://www.ncbi.nlm.nih.gov/SNP/, ID: 3832694) (13).

Materials and methods

Blood samples were obtained from 164 unrelated, healthy Caucasian Portuguese from Southern Portugal (67 men and 97 women), recruited from local medical check-ups, upon informed consent. This study was approved by the Ethical Boards of the institutions involved, and followed the Declaration of Helsinki guidelines.

Genomic DNA was extracted from peripheral blood using established phenol-chloroform methods and analysed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) according to previously described methods to test for the presence of A805T (Ile269Phe), G416A (Arg139Lys), C792G (Ile264Met) and A1196G (Lys399Arg) (5, 6, 14). To detect the deletion in exon 9, the following specific primers were designed: forward, 5′-AAC GAA TTT GTG CAG GAG A-3′; and reverse, 5′-GTA GTA TTG AGG TTC TTT AAA TCG T-3′. The 115-bp PCR product was digested with *Hin*cII (Fermentas, Vilnius, Lithuania), which recognises the reference sequence (without deletion), resulting in two fragments of 89 and 26 bp.

Allelic frequencies and confidence intervals were assessed using the programme Confidence Interval Analysis (15). χ^2 Testing was performed with GraphPad Software (http://www.graphpad.com/quickcalcs/index.cfm). The GenePop software pack was applied for the evaluation of Hardy-Weinberg equilibrium of the SNPs analysed (http://wbiomed.curtin.edu.au/genepop/).

Results

The most frequent non-wild-type allele observed was *CYP2C8*3*, with a frequency of 19.8%. The *CYP2C8*2*

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Table 1 Frequencies observed for *CYP2C8* genotypes in the Portuguese population (n = 164).

G416A (R139K)	C792G (I264M)	A805T (I269F)	A1196G (K399R)	n	Frequency (95% CI)
–/–	–/–	–/–	–/–	93	0.567 (0.491–0.643)
–/–	–/–	+/–	–/–	3	0.018 (0.004–0.053)
+/–	–/–	–/–	+/–	43	0.262 (0.195–0.330)
+/+	–/–	–/–	+/+	7	0.043 (0.017–0.086)
–/–	+/–	–/–	–/–	7	0.043 (0.017–0.086)
–/–	+/+	–/–	–/–	3	0.018 (0.004–0.053)
+/–	+/–	–/–	+/–	6	0.037 (0.014–0.078)
+/–	–/–	+/–	+/–	1	0.006 (0.0002–0.034)
+/–	+/+	–/–	+/–	1	0.006 (0.0002–0.034)

and *CYP2C8*4* alleles were found in 1.2% and 6.4% of the subjects, respectively (Table 1). Concerning the frequency of individuals homozygous for the mutated alleles, 4.3% were found for *CYP2C8*3* and 2.4% for *CYP2C8*4*, while no **2/*2* subjects were identified.

The 3-bp deletion previously detected in a large SNP survey in Japan was not found among the Portuguese.

The *CYP2C8*2* and *CYP2C8*3* alleles were found to be in Hardy-Weinberg equilibrium ($p = 1$ and $p = 0.8$, respectively). However, *CYP2C8*4* genotypic frequencies are not in agreement with those expected in Hardy-Weinberg equilibrium ($p = 0.0016$), with an excess of homozygous *G* individuals (4 observed vs. 1.378 expected) being present in our population.

Discussion

The frequencies observed for *CYP2C8*2* and *CYP2C8*4* in the Portuguese population are in line with other observations in Europe, although few studies are available (Figure 1).

As for *CYP2C8*3*, the prevalence of nearly 20% found represents the highest value ever documented for this allele, although it is not significantly different from the data available on populations from Western Germany (16), Northeast United Kingdom (6) and Central Spain (17). Complete concordance was observed for the frequencies of Arg139Lys and Lys399Arg substitutions, confirming the structure of the *CYP2C8*3* allele, as described by Dai and coworkers (5).

On the other hand, comparison of our results with data from Scandinavian regions – all with *CYP2C8* frequencies below 10% – shows statistically significant differences. This is true when comparing this study with a report in Sweden ($\chi^2 = 32.206$, $p < 0.01$), a survey in a Finnish population ($\chi^2 = 6.252$, $p < 0.05$), and a recent study performed in the relatively isolated Farøe Islands ($\chi^2 = 34.442$, $p < 0.01$) (18–20). These data suggest the presence of particular prevalences, particularly among certain European populations from peripheral regions.

Although not statistically significant, the differences found between our study and that performed in Central Spain points to a certain singularity of the Portuguese, a trend previously noted when analysing other P450 genes, such as *CYP3A4* (21) and *CYP2D6* (22).

As for the non-detection of the 3-bp deletion previously detected in Japan, our result reinforces the awareness of the particular pharmacogenetic characteristics of the Japanese population, related to its ethnic and geographical characteristics.

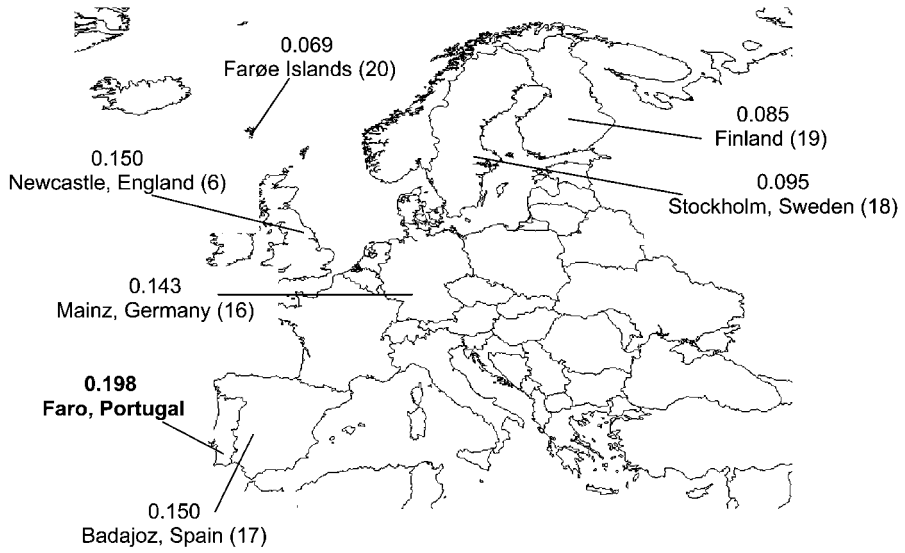

Figure 1 *CYP2C8*3* prevalence in European countries (map modified from http://geography.about.com/library/blank/blxeurope.htm). References are given in parentheses.

Finally, compared with the recent first data in native African populations (14), the present study shows significant differences between these and Caucasian subjects for the three alleles analysed (**2*, **3* and **4*), supporting conclusions from previous comparisons with Afro-American subjects (5).

Possibly related to the endogenous functions of CYP2C8 in the biosynthesis of vasoactive substances (23), the *CYP2C8*3* allele has been associated with a higher risk of acute myocardial infarction (AMI) (24). In this context, the high frequency of this allele among the Portuguese is of some concern, as cardiovascular diseases – including AMI – represent the main cause of death in the country after a gradual increase in incidence in recent decades (Ministry of Health, Portugal, http://www.dgsaude.pt/).

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