Clin Chem Lab Med 2003; 41(10):1345–1350 © 2003 by Walter de Gruyter · Berlin · New York

CYP3A4 and *MDR1* Alleles in a Portuguese Population[1)]

Isa Cavaco[1,3], J. Pedro Gil[2,3], Eva Gil-Berglund[4] and Vera Ribeiro[1,3]*

[1] Laboratory of Molecular Toxicology, CMQA, Faculty of Sciences and Technology, University of Algarve, Faro, Portugal
[2] Malaria Research Laboratory, Department of Medicine, Karolinska Hospital, Karolinska Institute, Stockholm, Sweden
[3] Center for Molecular and Structural Biomedicine, Faculty of Sciences and Technology, University of Algarve, Faro, Portugal
[4] Medical Products Agency, Uppsala, Sweden

Polymorphisms in cytochrome P450 *CYP3A4* and multidrug resistance (*MDR*)*1* genes coding for the important drug-metabolising CYP3A4 and the ATP-binding cassette (ABC) transporter P-glycoprotein (Pgp) are poorly documented in the Portuguese population. In this study we have determined the frequencies of *CYP3A4* and *MDR1* alleles in Portuguese Caucasians. Both genes were simultaneously analysed as these genes are known to be frequently co-induced and their products to show a pronounced overlap of substrates. *CYP3A4* A-392G (*CYP3A4*1B*), T673C (*CYP3A4*2*) and *MDR1* T-129C, G2677T and C3435T single nucleotide polymorphisms (SNPs) were analysed in 100 individuals from the southern region of the country. We observed a frequency of 4.0% for *CYP3A4*1B*, not significantly different from that reported on other Caucasian European populations. *CYP3A4*2* was found at an allele frequency of 4.5%, constituting the first report of the presence of this allele outside the Finnish population. Significant differences were found concerning the *MDR1* C3435T SNP frequency (64.5%) compared with other European populations, while no differences were found concerning G2677T (47.5%) or T-129C (5%) SNPs. Linkage between the C3435T and G2677T SNPs was observed, although not as evidently as documented in other Caucasian populations. No preferential associations were detected between *CYP3A4* and *MDR1* alleles. Clin Chem Lab Med 2003; 41(10):1345–1350

Key words: Cytochrome P450; *CYP3A4*; *MDR1*; Allele; Polymorphism.

Abbreviations: ABC, ATP-binding cassette; CYP, cytochrome P450; *MDR*, multidrug resistance gene; Pgp, P-glycoprotein; SNP, single nucleotide polymorphism.

Introduction

The cytochrome P450 CYP3A4 and the ATP binding cassette (ABC) transporter P-glycoprotein (Pgp) are two major factors that modulate the exposure of a large range of xenobiotics. CYP3A4 is one of the quantitatively most important drug-metabolising enzymes involved in both hepatic and intestinal metabolism of drugs (1). Pgp acts as a transmembrane transporter in tissues such as the intestine, liver (to the canaliculi), kidney, blood-brain barrier, placenta and testis, limiting the exposure of organs to xenobiotics (2–5). Pgp appears to work in concert with CYP3A4 by improving the presentation of drug to the enzyme in the intestine (6, 7). Both Pgp and CYP3A4 are promiscuous proteins with a large range of substrates and a marked overlap in substrate specificity (8), as well as inducers (9, 10). The transcription of the corresponding genes – *CYP3A4* and multidrug resistance (*MDR*)*1* – is induced by xenobiotics through activation of at least one common nuclear receptor, PXR (pregnane X receptor) (11).

These two proteins have been proposed to work as a "battery" defence against xenobiotics, minimising both their entrance and systemic exposure. Considering only one of these two proteins is increasingly viewed as insufficient for predicting situations of altered drug exposure, since the resulting systemic exposure of CYP3A4/Pgp substrates is usually a consequence of the activities of both proteins.

MDR1 and *CYP3A4* genes are known to harbour a significant number of single nucleotide polymorphisms (SNPs), mainly unveiled in the last 2 years (12, 13). Approximately 30 SNPs have been identified in *CYP3A4* (http://www.imm.ki.se/CYPalleles/), although most of them apparently with no consequences for gene expression or protein activity (13). Two relevant non-intronic variations found in *CYP3A4* among Caucasians are *CYP3A4*1B* (14) and *CYP3A4*2* (15). A 5′-flanking region A-392G transition defines the *CYP3A4*1B* allele (14). Although the functional consequences of this SNP are still under debate, it has been associated with cancer susceptibility (14, 16).

*CYP3A4*2* harbours an exonic SNP (T673C) that has been associated with altered catalytic activity, with a six-fold increase in the K_m for the calcium channel blocker nifedipine (15).

Among the 28 polymorphisms presently known in the *MDR1* gene (12), three positions have been particularly studied and associated with altered Pgp function: C3435T, G2677T and T-129C (17–19). The C3435T SNP at exon 26 represents a synonymous alteration located near the 3′ ATP binding domain-coding sequence of the gene, which has received much attention due to a set of consistent observations of altered protein expression in the duodenal tissue (20). More recently, several stud-

[1)] This article is based on a presentation at the 1st Santorini Biologie Prospective Conference "From Genetic Variations to Risk Prediction and Pharmacogenomics", Santorini, Greece, 25–28 September 2002.

*E-mail of the corresponding author: vmarques@ualg.pt

ies *in vivo* and *in vitro* have shown no differences between the C and T harbouring variants, leading to the hypothesis that this SNP is linked to another alteration in the gene (G2677T), which leads to an Ala893Ser change and would represent the real cause of the observed phenotypes (18, 19). Finally, the T-129C alteration, located in the promoter region, has been shown to significantly alter the expression of *MDR1* in placenta (19).

There is scarce data concerning the frequency of the aforementioned *CYP3A4* and *MDR1* SNPs in southern European populations. Information on the degree of intragenic linkage between the SNPs in positions 3435, 2677 and –129 of *MDR1* is also limited. Finally, due to the overlapping substrate spectra we hypothesise that potentially biased frequencies for *CYP3A4*/*MDR1* variant associations may exist, due to the metabolic relationship of their gene products. This study intended to address these questions in a Portuguese population.

Materials and Methods

Subjects

The group of subjects included in this study consisted of 100 unrelated, healthy Caucasian Portuguese from southern Portugal, recruited from local medical check-ups (45 men and 55 women (age range: 7–89 years, mean = 47 years, SD ± 19).

This study was approved by the Ethical Boards of the involved clinical institutions and followed the recommendations of the Declaration of Helsinki, promulgated in 1964 (http://ohsr.od.nih.gov/helsinki.php3). All peripheral blood samples were obtained upon individual consent.

PCR-RFLP-based genotyping of CYP3A4 and MDR1 SNPs

Genomic DNA was extracted from whole peripheral blood and was amplified by polymerase chain reaction (PCR). DNA (200–700 ng) was used for each amplification. PCR primers used for PCR-restriction fragment length polymorphism (RFLP) were as follows: for *CYP3A4* A-392G (indicative of *CYP3A4*1B*), sense: 5′-(AAT GAG GAC AGC CAT AGA GAC AAG GcC)-3′, antisense: 5′-(CAA TCA ATG TTA CTG GGG AGT CCA AGG G)-3′; for *CYP3A4* T673C (Ser222Pro, indicative of *CYP3A4*2*), sense: 5′-(AGA TTT GAT TTT TTG GAT CCA TTC TTT gTC)-3′, antisense: 5′-(CAA ATC ACT GAA CTG TAT ATT TTA AGT GG)-3′; for *MDR1* C3435T, sense: 5′-(ATG GGC TCC GAG CAC ACC TG)-3′, antisense: 5′-(AGG CAG TGA CTC GAT GAA GGC)-3′; for *MDR1* G2677T, sense: 5′-(GTA CCC ATC ATT GCA ATA GCA)-3′, antisense: 5′-(TTT AGT TTG ACT CAC CTT CCg AG)-3′; for *MDR1* T-129C, sense: 5′-(TCT CGA GGA ATC AGC ATT CAG TCA ATC C)-3′, antisense: 5′-(CTA AAG GAA ACG AAG AGC GGC CTC TG)-3′.

Lower case is indicative of mismatched nucleotides incorporated for the generation of restriction enzyme recognition sequences. PCR was performed in an Eppendorf MasterCycle personal 5332 thermocycler (Eppendorf, Hamburg, Germany). All reagents used for PCR were from Promega (Madison, USA).

PCR products were restricted with the following enzymes: *Bst* NI (Stratagene, La Jolla, USA) (*CYP3A4*, A-392G SNP detection), *Alw*26 I (Promega, Madison, USA) (*CYP3A4*, T673C SNP detection), *Sau*3A I (*MDR1*, C3435T SNP detection), *Alw*21I (*MDR1*, G2677T SNP detection), *Msp*A1 I (*MDR1*, T-129C SNP detection). All digestions were performed in accordance with the recommendations of the commercial suppliers.

Restriction fragments were analysed in 2–2.5% agarose gels (0.1 µg/ml ethidium bromide) and visualised by UV transillumination in a BioRad GelDoc 2000 (Biorad, Hercules, USA).

Although the SNP at position 2677 may consist of a change to a T or an A, our study was limited to the detection of the G2677T change, since the available data shows this alteration as the overwhelmingly most frequent alternative to guanine at this codon among Caucasians (21). Also, functional studies are reported only concerning G2677T (18).

Statistical analysis

Confidence intervals and statistical calculations were performed through the use of the CIA program (22), χ^2 testing was performed with the Microstat® software (Ecosoft Inc., 1984, Indianapolis, IN, USA).

Results

*CYP3A4*1B and CYP3A4*2 SNPs*

The frequencies of *CYP3A4*1B* and *CYP3A4*2* were found to be 4.0% and 4.5%, respectively (Table 1). No individuals were found to be homozygous for these alleles – not a surprising observation as the expected frequency for such an event involving these variants would be below 1%. The detection of the *CYP3A4*2* allele among the Portuguese constitutes the first observation of the presence of this allele outside the Finnish population (15). No individuals were found harbouring simultaneously both SNPs. Together these two alleles account for nearly a tenth of the *CYP3A4* alleles herein analysed among the Portuguese.

MDR1 T-129C, C3435T and G2677T SNPs

MDR1 3435T and 2677T harbouring alleles were found to be present at high frequencies of 64.5% and 47.5% (Table 1), in agreement with previous reports among Caucasians (see Table 2). As for the T-129C SNP, the presence of –129C was observed at a frequency of 5%. One C/C homozygous was identified, an unexpected observation according to the frequency of the C harbouring allele.

The MDR1 intragenic C3435T/G2677T linkage disequilibrium

Linkage between the C3435T/G2677T SNPs was observed, with an association of 3435 C/C with 2677 G/G genotypes more frequent than expected, as 8/31 (25.8%) were found against a theoretically expected 3.7/31 (11.9%) (Table 3). As observed by others (18), we did not find any individual carrying the 3435 C/C associated with 2677 T/T. No other evident trends were observed.

No significant linkage was observed between the T-129C SNP and the other *MDR1* positions analysed here (data not shown).

CYP3A4 and MDR1 allele associations

In the present study the associations between *CYP3A4*1B* or *CYP3A4*2* alleles and the studied *MDR1* alleles were observed at a frequency not significantly

Table 1 *CYP3A4* and *MDR1* SNP frequencies in the studied Portuguese population.

	SNP	Frequency (95% CI)	Genotype	Frequency (95% CI)
CYP3A4	A	0.960 (0.923–0.983)	A/A	0.920 (0.848–0.965)
A-392G (**1B*)	G	0.040 (0.017–0.077)	A/G	0.080 (0.035–0.152)
			G/G	0 (0–0.0362)
CYP3A4	T	0.955 (0.916–0.979)	T/T	0.910 (0.836–0.958)
T673C (**2*)	C	0.045 (0.021–0.084)	T/C	0.090 (0.042–0.164)
			C/C	0 (0–0.0362)
MDR1	T	0.950 (0.910–0.976)	T/T	0.910 (0.836–0.958)
T-129C	C	0.050 (0.024–0.900)	T/C	0.080 (0.035–0.152)
			C/C	0.010 (0.000305–0.055)
MDR1	C	0.355 (0.289–0.421)	C/C	0.120 (0.064–0.200)
C3435T	T	0.645 (0.579–0.711)	C/T	0.470 (0.369–0.572)
			T/T	0.410 (0.313–0.513)
MDR1	G	0.525 (0.456–0.594)	G/G	0.310 (0.221–0.410)
G2677T*	T	0.475 (0.406–0.544)	G/T	0.430 (0.331–0.533)
			T/T	0.260 (0.177–0.357)

n = 100. *The rare G2677A variant was not analysed in this study. Due to its low frequency among Caucasians, we believe that its discrimination would not significantly change the results and conclusions of this work.

Table 2 Comparison of the frequencies of the *MDR1* T-129C, C3435T and G2677T SNPs found in this study with other European populations.

		T-129C		3435 SNP (exon 26)		2677 SNP (exon 21)	
Population	N	T	C	C	T	G	T
Portuguese (this study)	100	0.950	0.050	0.355	0.645	0.525	0.475
Portuguese (23)	100	n.d.	n.d.	0.430	0.570	n.d.	n.d.
Italian (24)	106	0.972	0.028	0.538	0.462	0.561	0.410
German (17)	85	0.941	0.059	n.d.	n.d.	n.d.	n.d.
German (17)	188	n.d.	n.d.	0.519	0.481	n.d.	n.d.
German (21)	461	n.d.	n.d.	0.461	0.539	0.565	0.416
German (25)	537	n.d.	n.d.	0.497	0.503	n.d.	n.d.
Scotland (23)	190	n.d.	n.d.	0.479	0.521	n.d.	n.d.

n = number of individuals, n.d. = no data available.

different from the expected (data not shown). Interestingly, the individual homozygous for the –129C SNP also harboured a *CYP3A4*2* allele, an event only expected to occur at a frequency of 0.17% when taking into account the corresponding allelic frequencies.

Discussion

In this study we have analysed 100 individuals simultaneously for the presence of a subset of functionally documented *CYP3A4* and *MDR1* SNPs. These included two of the most frequent *CYP3A4* alleles found in Caucasians, *CYP3A4*1B* (determined through A-392G detection) and *CYP3A4*2* (determined through T673C detection), as well as three *MDR1* SNPs documented to be associated with altered phenotypes (C3435T, G2677T and T-129C).

Analysis of CYP3A4 allele frequencies

The frequency of *CYP3A4*1B* (4.0%) was shown not to be significantly different from the values found in other European Caucasian populations (15, 26–29). The importance of this variation for *CYP3A4* activity is still controversial. Some *in vitro* studies support a role of this SNP for altered transcription or enzyme expression (30, 31), while others do not (13, 26). This variant has, however, been proposed to be associated with advanced stage prostate cancer risk (14, 16) and to be protective for secondary cancer caused by leukaemia chemotherapy (32). During the completion of this work, some reports were published suggesting these observations are due to a linkage disequilibrium between *CY3A4*1B* and the active wild-type *CYP3A5*1* class of alleles (13, 33). Further studies in the Portuguese population concerning the *CYP3A5* gene are needed for the confirmation of this linkage.

Table 3 Linkage between *MDR1* C3435T and G2677T SNPs in the Portuguese.

		2677 SNP (exon 21) genotype					
		G/G		G/T		T/T	
		O	E	O	E	O	E
3435 SNP	C/C	0.080 (8)	0.037 (3.7)	0.040 (4)	0.052 (5.2)	0.000 (0)	0.031 (3.1)
(Exon 26)	C/T	0.100 (10)	0.146 (14.6)	0.240 (24)	0.202 (20.2)	0.130 (13)	0.122 (12.2)
Genotype	T/T	0.130 (13)	0.127 (12.7)	0.150 (15)	0.176 (17.6)	0.130 (13)	0.107 (10.7)

O: observed and expected, E: frequencies for the combinations of *MDR1* C3435T and G2677T SNP genotypes. The expected numbers of individuals (in parentheses) were calculated on the basis of the observed independent frequencies for each SNP genotype.

Concerning *CYP3A4*2*, this allele is present among the Portuguese in one of the highest frequencies ever assigned to an exonic non-synonymous *CYP3A4* SNP among Caucasians (4.5%). *CYP3A4*2* was previously only found in a Finnish population (2.7%) (15), being reported absent in other studies conducted among Caucasians (29, 34). This observation leads to the suggestion that frequencies above 1% of *CYP3A4*2* may only be found in specific geographic areas and populations.

Analysis of the frequencies of MDR1 T-129C, C3435T and G2677T SNPs

No significant differences were found between the observed frequencies of the *MDR1* 3435T allele and that reported previously in a study conducted in the central-northern regions of Portugal (Table 2). Also, no significant differences were observed in a population from the Berlin area (21) or from northeast Scotland (23). On the other hand, a comparison with the 3435T allele frequency in other German populations from Stuttgart/Berlin (48.1%) (17) and southwestern Germany (50.3%) (25) showed borderline significant differences ($p = 0.051$ and $p = 0.053$, respectively), while significant differences were found when analysing 3435C (51.9%, $p = 0.025$ and 49.7%, $p = 0.027$, respectively). The same trend was observed (3435T ($p = 0.055$), 3435C ($p = 0.027$)) when comparing our data with a study conducted in the north of Italy (24). These results emphasise the fact that analysis and comparisons of European populations should be done with caution, in particular when conclusions are extrapolated from a regional to a national level, due to the population heterogeneity found in most countries.

The frequency of the G2677T and T-129C SNPs showed no significant inter-population differences when comparing our results with the available data (17, 21, 24). No significant linkage was observed between the T-129C SNP and the other *MDR1* positions analysed here, in agreement with a recent report on a Japanese population (35). Linkage between C3435T and G2677T was observed, mainly concerning an increased association of genotypes 3435 C/C and 2677 G/G. Simultaneously, no association of 3435 C/C with 2677 T/T was found, an event also observed to be uncommon in other studies (24). No other evident trends were observed, including the strong associations between 3435 C/T and 2677 G/T and between 3435 T/T and 2677 T/T, previously reported in other Caucasian populations (18, 24). These results raise the hypothesis that the patterns of linkage between these two *MDR1* SNPs are variable from population to population. If, as hypothesised (18), the main phenotypic effect of the C3435T SNP is actually the result of its frequent linkage disequilibrium with the G2677T SNP – that represents the "active alteration" – the predictive capacity of the C3435T status (17) will be compromised in populations showing different linkage patterns between the two markers. Some recent reports support this view, with studies not finding a correlation between the C3435T genotypes and the bioavailability of documented Pgp substrates (24, 36), or even observing an inverse correlation to the previously reported (17). These results suggest that for molecular epidemiology purposes, at least both SNPs should always be analysed, as has been done recently by Furuno and collaborators (24).

Associations of CYP3A4 and MDR1 alleles

CYP3A4 and Pgp work in concert as a defence battery against systemic exposure of a large number of xenobiotics. The two proteins have overlapping substrate specificity and common regulation systems. Therefore, one may hypothesise that individuals harbouring specific alterations in both genes might be less frequent than expected due to natural selection, possibly caused by dietary exposure to herbal substances such as alkaloids.

Interestingly, in a recent report the genotype of the C3435T SNP on *MDR1* was suggested to correlate to expression of *CYP3A4* in enterocytes, with 3435C/C carriers having significantly higher levels of *CYP3A4* expression than 3435T/T harbouring subjects. This observation was suggested as being the result of a linkage between *MDR1* C3435T and an undetermined genetic variation in *CYP3A4* (37).

The analysis of individual *CYP3A4* genotypes and *MDR1* genotypes revealed no significant associations between specific changes in these two genes.

This result might be due to the particular choice and/or the rarity of the alleles under study. One individual was found simultaneously carrying a *CYP3A4*2*

and an *MDR1* –129C/C association of genotypes. This was surprising as this event was expected to occur at a frequency of approximately 0.17%. Although suggestive of a possible association of genotypes, further studies are needed for the evaluation of the meaning of this observation.

Acknowledgements

Partially supported by Fundação para a Ciência e Tecnologia, Portugal (SFRH/BD/8887/2002 to IC, PRAXIS grant SFRH/BPD/5590/2001 to JPG and Project POCTI/MGI 34699/99). We are indebted to Biocontec, Portugal for their support in this work.

References

1. Thummel KE, Wilkinson GR. In vitro and in vivo drug interactions involving human CYP3A. Annu Rev Pharmacol Toxicol 1998; 38:389–430.
2. Fromm MF. P-glycoprotein: a defense mechanism limiting oral bioavailability and CNS accumulation of drugs. Int J Clin Pharmacol Ther 2000; 38:69–74.
3. Ushigome F, Takanaga H, Matsuo H, Yanai S, Tsukimori K, Nakano H, *et al.* Human placental transport of vinblastine, vincristine, digoxin and progesterone: contribution of P-glycoprotein. Eur J Pharmacol 2000; 408:1–10.
4. Ames BN, Profet M, Gold LS. Nature's chemicals and synthetic chemicals: comparative toxicology. Proc Natl Acad Sci USA 1990; 87:7782–6.
5. Schinkel AH. The physiological function of drug-transporting P-glycoproteins. Semin Cancer Biol 1997; 8:161–70.
6. Benet LZ, Izumi T, Zhang Y, Silverman JA, Wacher VJ. Intestinal MDR transport proteins and P-450 enzymes as barriers to oral drug delivery. J Control Release 1999; 62:25–31.
7. Suzuki H, Sugiyama Y. Role of metabolic enzymes and efflux transporters in the absorption of drugs from the small intestine. Eur J Pharm Sci 2000; 12:3–12.
8. Wacher VJ, Wu CY, Benet LZ. Overlapping substrate specificity and tissue distribution of cytochrome P450 3A and P-glycoprotein: implications for drug delivery and activity in cancer chemotherapy. Mol Carcinog 1995; 13:129–34.
9. Schuetz EG, Beck WT, Schuetz JD. Modulators and substrates of P-glycoprotein and cytochrome P4503A coordinately upregulate these proteins in human colon carcinoma cells. Mol Pharmacol 1996; 49:311–8 .
10. Schuetz E, Strom S. Promiscuous regulator of xenobiotic removal. Nat Med 2001; 7:536–7.
11. Dussault I, Forman BM. The nuclear receptor PXR: a master regulator of "homeland" defense. Crit Rev Eukaryot Gene Expr 2002;12:53–64.
12. Brinkmann U, Eichelbaum M. Polymorphisms in the ABC drug transporter gene MDR1. Pharmacogenomics J 2001; 1:59–64.
13. Lamba JK, Lin YS, Schuetz EG, Thummel KE. Genetic contribution to variable human CYP3A-mediated metabolism. Adv Drug Deliv Rev 2002; 54:1271–94.
14. Rebbeck TR, Jaffe JM, Walker AH, Wein AJ, Malkowicz SB. Modification of clinical presentation of prostate tumors by a novel genetic variant in CYP3A4. J Natl Cancer Inst 1998; 90:1225–9.
15. Sata F, Sapone A, Elizondo G, Stocker P, Miller VP, Zheng W, *et al.* CYP3A4 allelic variants with amino acid substitutions in exons 7 and 12: evidence for an allelic variant with altered catalytic activity. Clin Pharmacol Ther 2000; 67:48–56.
16. Tayeb MT, Clark C, Sharp L, Haites NE, Rooney PH, Murray GI, *et al.* CYP3A4 promoter variant is associated with prostate cancer risk in men with benign prostate hyperplasia. Oncol Rep 2002; 9:653–5.
17. Hoffmeyer S, Burk O, von Richter O, Arnold HP, Brockmoller J, Johne A, *et al.* Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in vivo. Proc Natl Acad Sci USA 2000; 97:3473–8.
18. Kim RB, Leake BF, Choo EF, Dresser GK, Kubba SV, Schwarz UI, *et al.* Identification of functionally variant MDR1 alleles among European Americans and African Americans. Clin Pharmacol Ther 2001; 70:189–99.
19. Tanabe M, Ieiri I, Nagata N, Inoue K, Ito S, Kanamori Y, *et al.* Expression of P-glycoprotein in human placenta: relation to genetic polymorphism of the multidrug resistance (MDR)-1 gene. J Pharmacol Exp Ther 2001; 297:1137–43.
20. Borst P, Elferink RO. Mammalian ABC transporters in health and disease. Ann Rev Biochem 2002; 71:537–92.
21. Cascorbi I, Gerloff T, Johne A, Meisel C, Hoffmeyer S, Schwab M, *et al.* Frequency of single nucleotide polymorphisms in the P-glycoprotein drug transporter MDR1 gene in white subjects. Clin Pharmacol Ther 2001; 69:169–74.
22. Gardner MJ, Altman DG, editors. Statistics with confidence. London: Br Med J, 1989.
23. Ameyaw MM, Regateiro F, Li T, Liu X, Tariq M, Mobarek A, *et al.* MDR1 pharmacogenetics: frequency of the C3435T mutation in exon 26 is significantly influenced by ethnicity. Pharmacogenetics 2001;11:217–21.
24. Furuno T, Landi MT, Ceroni M, Caporaso N, Bernucci I, Nappi G, *et al.* Expression polymorphism of the blood-brain barrier component P-glycoprotein (MDR1) in relation to Parkinson's disease. Pharmacogenetics 2002; 12:529–34.
25. Siegsmund M, Brinkmann U, Schaffeler E, Weirich G, Schwab M, Eichelbaum M, *et al.* Association of the P-glycoprotein transporter MDR1(C3435T) polymorphism with the susceptibility to renal epithelial tumors. J Am Soc Nephrol 2002; 13:1847–54.
26. Westlind A, Lofberg L, Tindberg N, Andersson TB, Ingelman-Sundberg M. Interindividual differences in hepatic expression of CYP3A4: relationship to genetic polymorphism in the 5'-upstream regulatory region. Biochem Biophys Res Commun 1999; 259:201–15.
27. Tayeb MT, Clark C, Ameyaw M, Haites NE, Evans DA, Tariq M, *et al.* CYP3A4 promotor variants in Saudi, Ghanaian and Scottish Caucasian populations. Pharmacogenetics 2000; 10:753–6.
28. van Schaik RH, Wildt SN, van Iperen NM, Uitterlinden AG, van den Anker JN, Lindemans J. CYP3A4-V polymorphism detection by PCR-restriction fragment length polymorphism analysis and its allelic frequency among 199 Dutch Caucasians. Clin Chem 2000; 46:1834–6.
29. Garcia-Martin E, Martinez C, Pizarro RM, Garcia-Gamito FJ, Gullsten H, Raunio H, *et al.* CYP3A4 variant alleles in white individuals with low CYP3A4 enzyme activity. Clin Pharmacol Ther 2002; 71:196–204.
30. Amirimani B, Walker AH, Weber BL, Rebbeck TR. RESPONSE: re: modification of clinical presentation of prostate tumors by a novel genetic variant in CYP3A4. J Natl Cancer Inst 1999; 91:1588–90.
31. Ando Y, Tateishi T, Sekido Y, Yamamoto T, Satoh T, Hasegawa Y, *et al.* Re: Modification of clinical presentation

of prostate tumors by a novel genetic variant in CYP3A4. J Natl Cancer Inst 1999; 91:1587–90.
32. Felix CA, Walker AH, Lange BJ, Williams TM, Winick NJ, Cheung NV, *et al.* Association of CYP3A4 genotype with treatment-related leukemia. Proc Natl Acad Sci USA 1998; 95:13176–81.
33. Kuehl P, Zhang J, Lin Y, Lamba J, Assem M, Schuetz J, *et al.* Sequence diversity in CYP3A promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. Nat Genet 2001; 27:383–91.
34. Eiselt R, Domanski T, Zibat A, Mueller R, Presecan-Siedel E, Huestert E, *et al.* Identification and functional characterization of eight CYP3A4 protein variants. Pharmacogenetics 2001; 11:447–58.
35. Horinouchi M, Sakaeda T, Nakamura T, Morita Y, Tamura T, Aoyama N, *et al.* Significant genetic linkage of MDR1 polymorphisms at positions 3435 and 2677: functional relevance to pharmacokinetics of digoxin. Pharm Res 2002; 19:1581–5.
36. von Ahsen N, Richter M, Grupp C, Ringe B, Oellerich M, Armstrong VW. No influence of the MDR-1 C3435T polymorphism or a CYP3A4 promoter polymorphism (CYP3A4-V allele) on dose-adjusted cyclosporin A through concentrations or rejection incidence in stable renal transplant recipients. Clin Chem 2001; 47:1048–52.
37. Goto M, Masuda S, Saito H, Uemoto S, Kiuchi T, Tanaka K, *et al.* C3435T polymorphism in the MDR1 gene affects the enterocyte expression level of CYP3A4 rather than Pgp in recipients of living-donor liver transplantation. Pharmacogenetics 2002; 12:451–7.

Received 3 December 2002, revised 11 June 2003, accepted 20 June 2003

Corresponding author: Vera Ribeiro, Laboratory of Molecular Toxicology, Faculty of Sciences and Technology, University of Algarve, Campus de Gambelas, 8000-117 Faro, Portugal
Phone: +351 289 800900, Fax: +351 289 819403,
E-mail: vmarques@ualg.pt