Polymorphisms of the *MDR1* Gene in the Czech Population

(P-glycoprotein / *MDR1* / polymorphisms / frequencies)

K. PECHANDOVÁ, H. BUZKOVÁ, O. SLANAŘ, F. PERLÍK

Clinical Pharmacology Unit, Department of Pharmacology, 1st Faculty of Medicine, Charles University, Prague, Czech Republic

Abstract. There exists a marked inter-individual variability of P-glycoprotein expression and activity, which can be of clinical importance due to the large number of drugs that are substrates for the transporter. Previously identified polymorphisms in the *MDR1* gene belong to important factors causing this phenomenon. Our aim was to investigate the frequency of major functional SNPs of the *MDR1* gene coding for P-glycoprotein in the Czech population. DNA was isolated from whole blood of 189 healthy, young and unrelated subjects (99 females and 90 males, aged from 23 to 28 years). The genotypes of polymorphic positions C3435T, G2677T/A, C1236T and T-76A were determined by PCR-RFLP. Observed allelic frequencies were 56.5%, 46.0%, 0.53%, 44.5% and 37.6% for the alleles 3435T, 2677T, 2677A, 1236T and -76A, respectively. We have found 64 subjects homozygous for 3435T, 42 for 2677T, 40 for 1236T and 31 for -76A alleles. The allelic distribution complies well with Hardy-Weinberg equilibrium. Allelic frequencies of functionally important *MDR1* variants are in the Czech population similar to that of other Caucasian populations.

Introduction

P-glycoprotein (Pgp) is a large transmembrane protein of 170 kD that functions as an energy-dependent drug-transport pump transporting a variety of compounds extracellularly. It belongs to the large ATP-binding cassette transporter family and is defined as ABCB1. Pgp is composed of 1280 amino acids forming two analogous halves with 43% sequence homology. Two domains interact to form a functional transporter; each part is composed of six transmembrane α-helices and a nucleotide binding domain (NBD) (Fig. 1). NBD is a highly conservative area within the ABC transporter family that is involved in binding of ATP and hydrolysis of ATP to release energy, which is utilized for active uphill transport. The shape of Pgp reminds a cylinder with a maximal height of 8 nm and 10 nm in diameter (Higgins et al., 1997; Rosenberg et al., 1997).

Pgp is a product of the human multidrug resistance 1 (*MDR1*) gene that is localized in chromosome 7 band p21-21. *MDR1* spans 28 exons and cDNA consists of 3843 base pairs (Cascorbi et al., 2001). A number of variations have been found in the sequence of nucleotides. Pgp is widely expressed in tumour cells, but also on the apical surface of epithelial cells of the intestine, biliary canalicular membrane of hepatocytes, on the luminal surface of the capillary cells forming the blood brain barrier, in brush border membranes of proximal tubules in the kidney, in the adrenal cortex and in placenta. This transporter plays a significant role in disposition of drugs, i. e. absorption, distribution, and excretion and might be involved in secretion of steroids (Kerb et al., 2001; Sakaeda et al., 2002; Leonard et al., 2003).

The substrate specificity of the P-glycoprotein is extremely wide. The typical substrates – frequently used drugs are shown in Table 1. It also exports a number of chemically unrelated hydrophobic, amphipathic xenobiotics extracellularly.

Thanks to its localization, function and overlapping substrate specificity with cytochrome P450 Pgp can be considered both as a facilitator of direct elimination of the compounds from the body and a as synergistic part of the enzyme detoxification system. Pgp decreases the load of xenobiotics to intracellularly located metabolic enzymes and thus helps prevent saturation of the enzyme pathways, and it also provides protection from forming possibly toxic metabolites. Therefore, any situation leading to either decreased or increased activity of Pgp including functional genetic polymorphisms may alter either the distribution and elimination of the drugs or their metabolic fate (Kerb et al., 2001).

A clinically relevant example of overexpression and high function of Pgp in cancer cells is one of the mechanisms of multidrug resistance to therapy, which frequently leads to therapeutic failure. Cross-resistance to several agents, even those the patients were never exposed to, is a consequence of the transporter wide substrate specifity (Leonard et al., 2003).

More than fifty single nucleotide polymorphisms (SNPs) have been reported in the *MDR1* gene (Ishikawa et al., 2004). The effect of most of these polymorphisms

Received September 10, 2006. Accepted November 22, 2006.

This study has been supported by grant GA UK19/C/2005.

Corresponding author: Kristina Pechandová, Department of Pharmacology, 1st Faculty of Medicine, Albertov 4, 128 00 Prague 2, Czech republic. Phone: +(420) 224 968 140; e-mail: pechandova@seznam.cz

Abbreviations: CI – confidence interval, MDR – multidrug resistance, Pgp – P-glycoprotein, SNP – single nucleotide polymorphism.

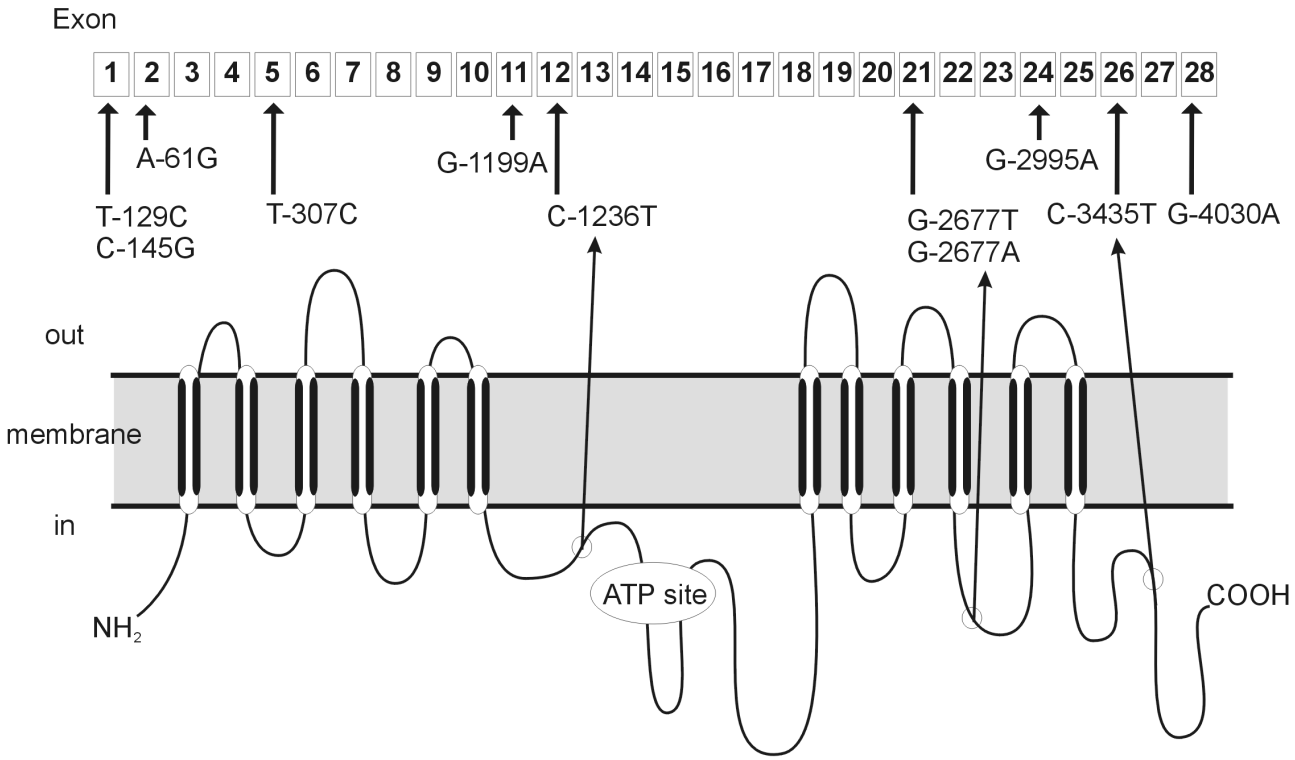

Fig. 1. Schematically depicted primary structure of P-glycoprotein. Arrows represent known SNPs and their relation to the structure of the protein

Table 1. Substrates of P-glycoprotein

Anticancer drugs	**Imunosupressants**	**Calcium channel blockers and metabolites**
Actinomycin	Cyclosporin A	Diltiazem
Docetaxel	Tacrolimus	Mibefradil
Doxorubicin	Rapamycin	Verapamil
Daunorubicin		N-dealkylverapamil
Epirubicin	**Steroids**	N-dealkynorverapamil
Etoposide	Aldosterone	
Irinotecan	Dexamethasone	**H1-antihistamines**
Mitomycin C	Estradiol	Fexofenadine
Mitoxantron	Hydrocortisone	Terfenadine
Paclitaxel (Taxol)		
Tamoxifen	**Antiemetics**	**H2-antihistamines**
Teniposide	Domperidone	Cimetidine
Topotecan	Ondansetron	Ranitidine
Vinblastine		
Vincristine	**Hypolipidemics**	**Opiates**
Vindesine	Atorvastatin	Morphine
	Lovastatin	Loperamide
Cardiotonics antiarrhythmics		
Digoxin	**Antibiotics**	**Others**
Digitoxin	Erythromycin	Amitriptyline
Chinidin	Levofloxacin	Colchicine
Amiodarone	Sparfloxacin	Debrisoquine
		Emetine
HIV protease inhibitors	**β blockers**	Fenytoin
Amprenavir	Celioprolol	Losartan
Indinavir	Talinolol	Rifampin
Nelfinavir		
Saquinavir		
Ritonavir		

on the Pgp function or their clinical impact is in most cases unknown, but some of the SNPs are known to be of functional relevance and can also alter the pharmacokinetics of substrate drugs. Notably, the C3435T polymorphism in exon 26 and polymorphism G2677T/A in exon 21 have been studied repeatedly. Digoxin, the most extensively studied substrate drug of Pgp, displays genotype-dependent pharmacokinetics, both at the steady state and after single administration. The plasma levels of this compound are significantly higher in homozygous 3435TT carriers in comparison with 3435CC subjects (Hoffmeyer et al., 2000). Similarly, fexofenadine exposure has been shown to be higher in individuals carrying variant alleles in positions 3435 and 2677. However, some other studies gave contradictory results indicating no effect of these polymorphisms in the positions 3435 and 2677 on fexofenadine pharmacokinetics (Kim et al., 2001; Cavaco et al., 2003). Due to this controversy about the clinical consequences, detection of these SNPs has not yet been introduced into routine practice but already represents an inevitable procedure during early phases of new drug development.

The aim of our work was to assess the frequency of the major known variant alleles and genotype distribution of C3435T, G2677T/A, C1236T and T-76A polymorphisms in the Czech population.

Material and Methods

Subjects

A randomly selected sample of 189 unrelated healthy volunteers from the Czech population were enrolled in the study (90 males and 99 females, aged from 23 to 28 years). All subjects gave their written informed consent. The investigation was performed in accordance with the principles of the Declaration of Helsinki and with the approval of the local ethics committee.

Venous blood containing EDTA (7 ml) was collected and DNA was isolated by a standard phenol-chloroform method (Cascorbi et al. 2001).

The genotypes were determined using a modified analysis by Cascorbi et al. (2001). DNA amplification was done in a MyCycle thermocycler (BioRad Laboratories, Hercules, CA). The reaction mixture consisted of 60 ng of DNA template, 8 nM primers (the sequence was identical to the primers used by Cascorbi et al., 2001), PCR buffer, 2.5 mM $MgCl_2$, 0.2 mM dNTP and 0.5 U of Taq DNA polymerase. Water was added to a final volume of 25 µl. PCR amplification consisted of initial denaturation for 2 min at 94°C followed by 35 cycles, denaturation at 94°C for 30 s, annealing at 60°C for 45 s and extension at 72°C for 1 min. Terminal elongation ran at 72°C for 7 min. Restriction by enzymes *Xap*I**,** *Bse*NI, *Bsh*NI, and *Bsp*1431 produced DNA fragments that were separated on 3.5% agarose gel and visualized after ethidium bromide staining on an ultraviolet transilluminator.

Statistical analysis

Statistical significance ($P < 0.05$) was assessed by χ^2 test for two degrees of freedom. Expected distribution of genotypes was calculated by Hardy-Weinberg equilibrium.

Results

We analysed samples of 189 subjects to detect the polymorphism G2677T/A in exon 21 leading to amino acid exchange, and four SNPs without influence on the amino acid exchange were detected. The allelic and genotype frequencies of *MDR1* variants are given in Table 2.

Table 2. Positions, observed and expected frequencies of MDR-1 variants in the Czech population

Location	Position	Allele	Effect	Frequency (%)	Genotype	Frequency		
						Observed (%)	95% CI	Expected (%)
Exon12	cDNA1236	C	Wobble	55.5	C/C	31.7	25.1-38.4	30.6
		T		44.5	C/T	47.1	39.9-54.2	49.4
					T/T	21.2	15.3-26.9	19.9
Intron16	Exon17–76	T	?	62.4	T/T	41.3	34.3-48.3	38.9
		A		37.6	T/A	42.3	35.3-49.4	45.8
					A/A	16.4	11.1-21.7	13.7
Exon21	cDNA2677	G	893Ala	53.4	G/G	29.6	23.1-36.1	28.6
		T	893Ser	46.0	G/T	47.1	40.0-54.2	49.2
		A	893Thr	0.53	T/T	22.2	16.3-28.1	21.2
					G/A	1.1	0.5-1.56	0.57
					A/A	0.0	0.0-0.9	0.0
					T/A	0,0	0.0-0.9	0,0
Exon26	cDNA3435	C	Wobble	43.5	C/C	21.2	15.3-26.9	18.9
		T		56.5	C/T	44.9	37.9-52.1	49.1
					T/T	33.9	27.1-40.6	31.9

The variant allele of C3435T polymorphism in exon 26, which affects the expression level and function of MDR-1, was detected with allelic frequency of 56.50%. We found 64 subjects homozygous for the variant allele. In exon 21, namely at the cDNA position 2677, two variant alleles (substitutions G>T; G>A) are known to lead to amino acid sequence change (Ala839Ser; and Ala839Thr, respectively). The wild-type allele 2677G appeared with frequency of 53.44% and variant allele 2677T of 46.03%. Fifty-six and 42 subjects were homozygous for 2677G and 2677T alleles, respectively. No carrier of homozygous 2677A allele was found, although heterozygous genotypes containing the 2677A allele were found in two subjects.

Forty subjects were homozygous carriers of the abundant exonic silent mutation in exon 12 with allelic frequency 44.5% and 31 subjects were homozygous for variant -76A allele in intron 16 (allelic frequency 37.6%).

The observed genotype frequency distribution did not show a significant deviation from the expected ones calculated by using Hardy-Weinberg equilibrium. The expected allelic frequencies fit into the 95% confidence interval (CI) in all variants.

Relative frequencies of all possible combined C3435T and G2677T/A genotypes are described in Table 3. The wild-type sequence in both these SNP sites was found in 36 subjects, while homozygous presence of a variant sequence in both positions was carried by 40 subjects.

Table 3. Relative frequencies of possible combined C3435T and G2677T/A genotypes

C3435T exon 26 genotype	G2677T exon 21 genotype		
	G/G	G/T	T/T
C/C	**0.19**	0.02	0
C/T	0.1	**0.34**	0.01
T/T	0.01	0.12	**0.21**

Discussion

The *MDR1* gene belongs to highly polymorphic regions. In this study we assessed frequencies of the most common polymorphisms with possible functional significance in the Czech population.

The first genetic polymorphism identified in the *MDR1* gene was G2677T (Mickley et al., 1998). This polymorphism is unique for the three possible variants at the same gene locus resulting in amino acid changes (Ala893Ser/Thr).

Later in 2000, the first systematic screening of *MDR1* polymorphisms was performed by Hoffmeyer et al. The authors discovered that a silent polymorphism in exon 26 at the position 3435 of cDNA was associated with a decreased protein expression in the duodenum as well as with decreased ability to transport digoxin (Hoffmeyer et al., 2000).

Significant linkage between the wild-type–wild-type sequence and variant-variant sequence in positions C3435T and G2677T has been observed (Kim et al., 2001). Our results are in agreement with this observation. Individuals being either wild-type or variant homozygotes or heterozygotes simultaneously in both SNPs represent approximately 75% of the population. This linkage disequilibrium can be the cause of the discrepancies in previously published pharmacokinetic studies, if being based on single SNP analysis only.

We further investigated the distribution of two common polymorphisms, C1236T and T-76A, in exon 12 and 17, respectively. Both SNPs represent synonymous

Table 4. Genotype frequencies of MDR-1 variants in the Caucasian and Asian populations

Location/ Position	Exon 26 C3435T			Exon 21 G2677T/A						Exon 12 T1236C			Intron 16 ex17/T-76A		
Ethnic	CC	CT	TT	GG	GT	GA	TT	AA	TA	CC	CT	TT	TT	TA	AA
Caucasian															
Czech C. (N = 189)	21.2	44.9	33.9	29.6	47.1	0.5	22.2	0	0	31.7	47.1	21.2	41.3	42.3	16.4
German C. (N = 461)	20.8	50.5	28.6	30.9	49.2	2.0	16.1	0	1.8	34.4	49.2	16.4	28.4	50.8	20.8
Polish C. (N = 122, 139)	42.0	41.0	0.17*	33.8	46.8	0.7	17.3	0	1.4	35.0	46.8	18.0		N.a.	
Portuguese C. (N = 100)	12.0	47.0	41.0	31.0	43.0	N.a.	26.0	N.a.	N.a		N.a.			N.a.	
Russian C. (N = 290; 59)	21.4	48.6	30.0	30.3	44.9	4.1	18.3	0	2.4	24.0	56.0	20.0		N.a.	
UK C. (N = 190)	24.0	48.0	28.0			N.a.					N.a.			N.a.	
Asian															
Chinese (N = 96)	25.0	43.8	31.3	16.7	33.3	8.3	26.0	1.0	14.6	8.3	39.6	52.1*		N.a.	
Indian (N = 87)	18.4	36.8	44.8	13.8	31.0	8.1	41.4	0	5.8*	13.8	37.9	48.3*		N.a.	
Japanese (N = 114, 48)	35.0	53.0	12.0 *			N.a.				14.6	47.9	37.5*		N.a.	

N.a. – Not assessed

* $P < 0.05$ compared with the Czech population

alterations. Another linkage disequlibrium was noted between SNP C1236T in exon 12 and G2677T, as well as C3435T polymorphisms, in exon 21 and 26, respectively.

The frequencies of major *MDR1* polymorphisms vary among different populations (Table 4). The homozygous 3435C/C genotype ranged from 12.0% in Portuguese to 42.0% in Polish populations (Jamroziak et al., 2002, Bernal et al., 2003; Cavaco et al., 2003). The difference between our and Polish population was statistically significant. The frequency in most other Caucasians was similar (20.8 – 24.0%) to our findings (Cascorbi et al., 2001; Gaikovitch et al., 2003). Statistically significantly lower frequency of wild-type 3435C and variant 1236T alleles was further observed in Japanese compared to Caucasians.

Studies in the African populations reported far more frequent distribution of the 3435C allele (Ghanaian 84.0%, Kenyan 83.0%). It is assumed that overdominance of this homozygous C/C genotype may be a consequence of natural selection representing an advantage against gastrointestinal tract infections (Mickley et al., 1998; Schaeffeler et al., 2001).

The A allele in exon 21 and the T allele in exon 12 are significantly more common in the Asian populations (Table 5) than in Czechs and other Caucasians (Chowbay et al., 2003)

The knowledge about frequency of functionally important SNPs in the *MDR1* gene in the Czech population is substantial information for designing future pharmacokinetic and pharmacodynamic studies conducted with Pgp substrates. It will allow increasing validity of the studies together with decreased economic costs and ethical risks for the participating individuals. The practising clinicians should be further aware of the high frequency of functional polymorphisms in our population, especially when considering treatment with drugs – substrates possessing a narrow therapeutic window. Digoxin is one of the example drugs, where exceptional cautiousness is essential in individuals with decreased activity of Pgp in order to prevent development of serious adverse drug reactions. In the future perspectives, routine testing for the SNPs can become a useful tool for optimizing the dosing and therapy of the drugs that are substrates for Pgp.

References

Bernal, M. L., Sinues, B., Fanlo, A., Mayayo, E. (2003) Frequency distribution of C3435T mutation in exon 26 of the MDR1 gene in a Spanish population. *Ther. Drug Monit*. **25**, 107-111.

Cascorbi, I., Gerloff, T., Johne, A., Meisel, C., Hoffmeyer, S., Schwab, M., Schaeffeler, E., Eichelbaum, M., Brinkmann, U., Roots, I. (2001) Frequency of single nucleotide polymorphisms in the P-glycoprotein drug transporter MDR1 gene in white subjects. *Clin. Pharmacol. Ther.* **69**, 169-174.

Cavaco, I., Gil, J. P., Gil-Berglund, E., Ribeiro, V. (2003) CYP3A4 and MDR1 alleles in a Portuguese population. *Clin. Chem. Lab. Med.* **41**, 1345-1350.

Gaikovitch, E. A., Cascorbi, I., Mrozikiewicz, P. M., Brockmoller, J., Frotschl, R., Kopke, K., Gerloff, T., Chernov, J. N., Roots, I. (2003) Polymorphisms of drug-metabolizing enzymes CYP2C9, CYP2C19, CYP2D6, CYP1A1, NAT2 and of P-glycoprotein in a Russian population. *Eur. J. Clin. Pharmacol.* **59**, 303-312.

Goreva, O. B., Grishanova, A. Y., Domnikova, N. P., Mukhin, O. V., Lyakhovich, V. V. (2004) *MDR1* gene C1236T and C6+139T polymorphisms in the Russian population: associations with predisposition to lymphoproliferative diseases and drug resistance. *Bull. Exp. Biol. Med.* **138**, 404-406.

Higgins, C. F., Callaghan, R., Linton, K. J., Rosenberg, M. F., Ford, R. C. (1997) Structure of the multidrug resistance P-glycoprotein. *Semin. Cancer Biol.* **8**, 135-142.

Hoffmeyer, S., Burk, O., von Richter, O., Arnold, H. P., Brockmoller, J., Johne, A., Cascorbi, I., Gerloff, T., Roots, I., Eichelbaum, M., Brinkmann, U. (2000) 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* **97**, 3473-3478.

Chowbay, B., Cumaraswamy, S., Cheung, Y. B., Zhou, Q., Lee, E. J. (2003) Genetic polymorphisms in MDR1 and CYP3A4 genes in Asians and the influence of MDR1 haplotypes on cyclosporin disposition in heart transplant recipients. *Pharmacogenetics* **13**, 89-95.

Ishikawa, T., Hirano, H., Onishi, Y., Sakurai, A., Tarui, S. (2004) Functional evaluation of ABCB1 (P-glycoprotein) polymorphisms: high-speed screening and structure-activity relationship analyses. *Drug Metab. Pharmacokinet.* **19**, 1-14.

Jamroziak, K., Balcerczak, E., Mlynarski, W., Mirowski, M., Robak, T. (2002) Distribution of allelic variants of functional C3435T polymorphism of drug transporter MDR1

Table 5. Allelic frequencies of MDR-1 variants in the Asian populations

Population	C3435T		G2677T/A			T1236C	
	wt	var	wt	var T	var A	wt	var
Chinese	46.9	53.1	37.5 *	49.9 *	12.5 *	28.1 *	71.9 *
Indian	36.8	63.2	33.4 *	59.8 *	6.9 *	32.8 *	67.3 *
Japanese	61.5 *	38.5 *	N.a.	N.a.	N.a.	38.5 *	61.5 *

N.a. – Not assessed, wt-wild-type

* $P < 0.05$ compared with the Czech population

gene in a sample of Polish population. *Pol. J. Pharmacol.* **54**, 495-500.

Kerb, R., Hoffmeyer, S., Brinkmann, U. (2001) ABC drug transporters: hereditary polymorphisms and pharmacological impact in MDR1, MRP1 and MRP2. *Pharmacogenomics* **2**, 51-64.

Kim, R. B., Leake, B. F., Choo, E. F., Dresser, G. K., Kubba, S. V., Schwarz, U. I., Taylor, A., Xie, H. G., McKinsey, J., Zhou, S., Lan, L. B., Schuetz, J. D., Schuetz, E. G., Wilkinson, G. R. (2001) Identification of functionally variant MDR1 alleles among European Americans and African Americans. *Clin. Pharmacol. Ther.* **70**, 189-199.

Leonard, G. D., Fojo, T., Bates, S. E. (2003) The role of ABC transporters in clinical practice. *Oncologist* **8**, 411-424.

Mickley, L. A., Lee, J. S., Weng, Z., Zhan, Z., Alvarez, M., Wilson, W., Bates, S. E., Fojo, T. (1998) Genetic polymorphism in MDR-1: a tool for examining allelic expression in normal cells, unselected and drug-selected cell lines, and human tumors. *Blood* **91**, 1749-1756.

Rosenberg, M. F., Callaghan, R., Ford, R. C., Higgins, C. F. (1997) Structure of the multidrug resistance P-glycoprotein to 2.5 nm resolution determined by electron microscopy and image analysis. *J. Biol. Chem.* **272**, 10685-10694.

Sakaeda, T., Nakamura, T., Okumura, K. (2002) MDR1 genotype-related pharmacokinetics and pharmacodynamics. *Biol. Pharm. Bull.* **25**, 1391-1400.

Schaeffeler, E., Eichelbaum, M., Brinkmann, U., Penger, A., Asante-Poku, S., Zanger, U. M., Schwab, M. (2001) Frequency of C3435T polymorphism of MDR1 gene in African people. *Lancet* **358**, 383-384.