



Polymorphisms in the ABC drug transporter gene *MDR1*

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ABSTRACT

In addition to genetically variable metabolic enzymes such as Cyp p450 proteins, blood and tissue levels of many drugs are influenced by controlled transport across compartmental boundaries. Major determinants in these transport processes are ATP-dependent efflux pumps such as P-glycoprotein and related proteins (eg MRPs), which can influence the bioavailability and CNS concentrations, as well as disposition of drugs. In addition to its recognized role in the development of multiple chemotherapy resistances, experimental evidence for the relevant influence of the *MDR1* gene encoded P-glycoprotein, on the pharmacology of many other drugs has been gathered by the analyses of knockout mice, as well as in clinical studies. Recently, functional genetic polymorphisms in the *MDR1* gene have been identified which influence the distribution and bioavailability of PGP substrates. *The Pharmacogenomics Journal* (2001) 1, 59–64.

Keywords: drug uptake; drug metabolism; hereditary polymorphisms; multidrug resistance; pharmacogenetics; p-glycoprotein; detoxification

THE FUNCTION OF THE ABC TRANSPORTER *MDR1* IN DRUG RESISTANCE AND PHARMACOLOGY

The multidrug resistance gene (*MDR1*) is the most thoroughly analyzed gene and protein among the ATP-binding cassette (ABC) transporter protein family, which comprises a superfamily of membrane proteins with defined subfamilies (*MDR/TAP*, *ALD*, *MRP/CFTR*, *ABC1*, *White*, *OABP*, *ANSA*, and *GCN20*). Most of these proteins are composed of two transmembranous units (TMDs), composed of six membrane-spanning helices, and cytoplasmatic nucleotide binding domains which bind and hydrolyze ATP to generate energy which in turn drives the transport process. Many structural studies and functional analyses of ABC transporter proteins are based on knowledge obtained on *MDR1*.

Drug Resistance

MDR1 was originally identified as a gene that confers multidrug resistance (MDR) to cancer cells,¹ and later related proteins (MRPs) were discovered as alternative transporter-mediated drug resistance mechanisms.^{2–6} These proteins function as efflux pump for xenobiotics and thus provide a barrier to entry for drugs, and cellular metabolites.^{7–10} This causes one major problem in cancer chemotherapy: development of cross-resistance of tumors to many cytotoxic agents, even to those cells which were never exposed before, which frequently leads to therapeutic failure. Experiments *in vitro*, eg tumor cells grown in tissue culture, show that expression or overexpression of *MDR1* can be causative for this resistance.^{11–14} A selected list of PGP substrates, affected by this mechanism of drug resistance is provided in Table 1. These compounds include not only medicines used for tumor therapy, but also other therapeutics such as HIV-protease inhibitors. A consensus structure for recognition by p-glycoprotein can not be defined, but generally, PGP substrates are hydrophobic and amphipathic.¹⁵ Because of its

Table 1 Selected PGP substrates and inhibitors

| <i>Substrates for MDR1</i> | | |
|----------------------------------|---------------------------|-----------------------------------|
| Anticancer drugs | CA blockers | HIV protease inhibitors |
| Actinomycin D | Diltiazem | Indinavir |
| Daunorubicin | Nicardipine | Ritonavir |
| Doxorubicin | Verapamil | Saquinavir |
| Etoposide | | |
| Mitomycin C | Cardiacs | Morphins |
| Paclitaxel (taxol) | Propafenone | Morphine 6-glucuronide |
| Tamoxifen | Amiodaron | Morphine |
| Topotecan | Quinidine | Loperamide |
| Vinblastine | Digoxin | |
| Vincristine | | |
| Antiallergics | CNS drugs | Peptides |
| Terfenadine | Domperidone | Gramicidin D |
| | Fluphenazine | Valinomycin |
| | Odansetron | N-Acetyl-leucyl-leucyl-norleucine |
| Antibiotics | Perphenazin | |
| Cefazolin | Perphenazine | |
| Cefoperazon | Phenoxazine | Steroids |
| | Phenytoin | Aldosterone |
| Immunosuppressants | | Dexamethason |
| Cyclosporine A | | Hydrocortisone |
| Tacrolimus | | |
| <i>Inhibitors of MDR1</i> | | |
| Tricyclic ring structures | Alkaloids | Neuroleptics |
| Phenoxazine | Colchicine | Phenothiazines |
| Phenothiazine | Reserpine | Thioxanthene |
| Phenoxazone | Staurosporine | Flupentixol |
| Resurfin acetate | | |
| Xanthene | Anti malaria drugs | Peptides |
| Xanthene carboxylic acid | Primaquine | Prenylcysteines |
| Phenanthroline | Chloroquine | |
| Acridine | | |
| Acridine Orange | | |
| Quinacrine | | |

association with drug resistance, determination of PGP over-expression is of prognostic value in certain diseases, eg in leukemia with high *MDR1* levels correlating with poor prognosis,^{16–21} and a considerable amount of effort is being invested in the development of substances that inhibit, or modulate the activity of PGP. Among the various chemosensitizers that have been found to restore the sensitivity of tumor cells towards chemotherapy either by competitive or noncompetitive inhibition, interference with substrate recognition or ATP hydrolysis, are calcium channel blockers (verapamil, nifedipine), immunosuppressants (cyclosporin A, FK506), and antiarrhythmic drugs (amiodarone, quinidine).^{22–26}

Physiological Function

MDR1 is not only expressed in tumor cells, but also in many normal tissues. The function as a cellular efflux pump to control the intracellular concentration of potential harmful

substances is reflected by its polarized cell-specific and organ specific distribution, as well as by its capacity to recognize and transport a broad range of compounds. *MDR1* is mainly expressed on the apical (or luminal) surface of epithelial cells of the lower gastrointestinal tract (jejunum, ileum, and colon). Here, it influences intestinal drug absorption and thus limits oral drug bioavailability.^{27,28} It may also facilitate excretion across the intestinal mucosa.²⁹ PGP is also found in significant amounts in biliary canalicular membrane of hepatocytes,³⁰ and in the brush-border membrane of proximal tubules in the kidney,²⁹ which fits the proposed role of PGP in excretion of xenobiotics and endogenous metabolites into the urine and bile. Other sites where PGP probably has a very important protective function by being expressed at the luminal surface of capillary endothelial cells, in the blood–brain barrier, as well as the placenta.^{31–33} Finally, PGP is expressed in lymphocytes, and expression in the adrenal cortex may suggest an involvement in the secretion and metabolism of steroids.³⁴

MDR1 AND BIOAVAILABILITY: LESSONS FROM KNOCKOUT MICE

The most convincing experimental proof for the important role of PGP in drug absorption, disposition and elimination has been obtained by analyzing knockout mice. Knockouts (disruption) of one or both of the *MDR1* genes (*MDR1a* and *MDR1b*) in mice resulted in viable animals,^{35,36} which however were hypersensitive to xenobiotics. They showed elevated brain uptake and significantly altered pharmacokinetics of many drugs. For example, animals without *MDR1a* had increased bioavailability and, when given intravenously, reduced fecal and urinary clearance of vinblastine, doxorubicin, digoxin, taxol, tri-n-butylmethylammonium, and azidoprocaïnamide methoiodide.^{37–44} Increased drug accumulation was also observed in liver, brain, and gall bladder, tissues which usually express PGP (see above). Thus, PGP contributes significantly to the elimination of drugs through hepatic as well as intestinal secretion. These phenotypes are similar to the effects in humans after application of PGP inhibitors.⁴⁵ Paclitaxel, loperamide, vinblastine, ivermectin, cyclosporin A, and protease inhibitors showed also increased bioavailability in *MDR1a* knockout mice, combined with increased drug accumulation in the brain, liver, intestine.^{38,39,41,43,46–48} Increased bioavailability and reduced barrier function (particularly at the brain) in mice lacking functional *MDR1* genes cause a strongly increased sensitivity towards central nervous toxicity. For example, Ivermectin, which usually barely enters the brain, is present at lethal levels in *MDR1*-deficient mice; similar effects are seen with digoxin and loperamide.^{40,42}

GENETIC VARIATIONS AND POLYMORPHISMS OF THE HUMAN *MDR1* GENE

Recombinant Variants

With the objective to elucidate the function and molecular mechanism of PGP, many recombinant PGP variants have been generated by site-directed mutagenesis techniques and have subsequently been studied, eg in cell culture as well

as by biochemical assays. The results of this extensive, still ongoing research is a picture of PGP being composed of two homologous halves, each containing six transmembrane domains and one ATP-binding domain, with interaction of both halves being essential to function as a transporter. Functionality of the ATP domains, as well as correct interaction of these with the drug-binding domains are necessary for drug transport.^{49–53} Ambudkar *et al*⁵⁴ have provided a comprehensive overview on these results. Furthermore, most results indicate that substrate specificity can be affected by mutations in transmembrane domains 5, 6, 11 and 12, suggesting the presence of a major drug binding site in this region.^{51,54–58}

MDR1 Polymorphisms

Considering the important role of PGP, as seen experimentally in ko mice, it can be assumed that naturally occurring genetic variants of MDR1 can affect the interindividual variability in pharmacokinetics and the dynamics of many drugs.

Among the first to search for polymorphisms of *MDR1* in humans, applying the search to a population of tumor patients with the objective to identify susceptibility factors predicting individual cancer risk, Mickley *et al*⁵⁹ have reported polymorphisms in exons 21 and 24 (G2677T and G2995A) in drug selected cell lines and in cells from refractory malignant malignomas as well as in healthy volunteers.⁵⁹

A systematic screen of the entire *MDR1* gene for polymorphisms was performed by Hoffmeyer *et al*, in which all 28 exons as well as the core promoter region and exon–intron boundaries were sequenced from genomic DNA of healthy Caucasians.⁶⁰ In that screen, 15 single nucleotide polymorphisms (SNPs) were detected.

All SNPs of the human *MDR1* gene that have been identified so far are listed in Table 2. Some potential functional consequences of polymorphisms may be concluded from their position in the gene and protein (see Figure 1 for the positions of the discovered genetic variations within the *MDR1* gene and how they translate into the domain structure of PGP). For example, the SNP at position A₆₁G replaces Asn with Asp at position 21 of PGP, resulting in a net charge change (basic to acidic) close to the N-terminus of PGP. Recombinant mutational analyses of PGP (see above) did not reveal this region to be of major functional importance, but an impact of this position can certainly not be ruled out.⁵⁴ A phenylalanine to leucine change in position 103 in exon 5 lies next to the second transmembrane domain on the extracellular side. This position is close to a glycosylation site of PGP. The switch from a large aromatic residue to large lipophilic could modify the protein structure by disturbing the side chain packing. The polymorphism G₁₁₉₉A causes an amino acid exchange from serine to asparagine, a significant size change as well as—depending on the pH—possibly a charge change in the protein. This SNP is positioned cytoplasmatic, close to the first ATP-binding domain. The polymorphisms G₂₆₇₇T and G₂₉₉₅A lead in exon 21 and 24, respectively, and change the protein but do not

Table 2 Single nucleotide polymorphisms in the *MDR1* gene

| SNP | Region | n | Frequency of SNPs (% hetero) | Effect |
|---------------------|--------|-----|------------------------------|-------------------------|
| T ₋₁₂ C | E 1 | 85 | 11.8 | noncoding |
| G ₋₁ A | E 2 | 188 | 11.2 | TL initiation |
| A ₆₁ G | E 2 | 188 | 17.6 | Asn ₂₁ Asp |
| G ₋₂₅ T | I 4 | 85 | 26 | |
| G ₋₃₅ C | I 4 | 85 | 1.2 | |
| T ₃₀₇ C | E 5 | 85 | 1.2 | Phe ₁₀₃ Leu |
| C ₊₁₃₉ T | I 5 | 85 | 48.2 | |
| C ₊₁₄₅ T | I 5 | 85 | 2.4 | |
| G ₁₁₉₉ A | E 11 | 85 | 12.9 | Ser ₄₀₀ Asn |
| C ₁₂₃₆ T | E 12 | 188 | 48.9 | Gly ₄₁₂ Gly |
| C ₊₄₄ T | I 12 | 188 | 11.7 | |
| T ₋₇₆ A | I 16 | 85 | 45.9 | |
| A ₊₁₃₇ G | I 17 | 85 | 1.2 | |
| G ₂₆₇₇ T | E 21 | 83 | 43.4 | Ala ₈₉₃ Ser |
| G ₂₉₉₅ A | E 24 | 36 | 11.1 | Ala ₉₉₉ Thr |
| C ₃₄₃₅ T | E 26 | 537 | 47.7 | Ile ₁₁₄₅ Ile |
| C ₃₃₉₆ T | E 26 | 188 | 0.53 | wobble |

^a*MDR1* sequences gb:AC002457 and AC005068 are defined as ‘wildtype’ E: exon, I intron.

The positions of the identified polymorphisms correspond to positions of the *MDR1* cDNA (gb:M14758, codon TTC exon 10, F335, is missing), the first base of the ATG start codon is 1. Intron SNPs are labeled as *n* nucleotides upstream (–) or downstream (+) of the exons according to Chen *et al*.⁷

affect expression levels of MDR1.⁵⁹ Both genetic polymorphisms are in the 2nd transmembrane domain, the exon 24 SNP close to the ATP-binding domain.

Pharmacological consequences have been shown for one SNP: C₃₄₃₅T, at a wobble position in exon 26, correlates with intestinal PGP levels and influences the uptake of orally administered PGP substrates. Individuals homozygous for this polymorphism (TT) showed significantly lower duodenal PGP expression, higher *in vivo* activity of PGP and increased digoxin plasma levels.⁶⁰ The C₃₄₃₅T polymorphism is a noncoding, non-promoter SNP and thus it is unlikely that it directly influences the expression of the MDR1 gene. It can be assumed that this SNP defines an allele, ie it is linked to one or more other so far unidentified changes in regions of the *MDR1* gene that control expression, eg in the promoter or enhancer region, or in sequences that are important for mRNA processing.

PHARMACOLOGICAL IMPLICATIONS OF MDR1 POLYMORPHISMS

MDR1 and other ABC transporters play an important role in absorption, distribution, and elimination of many drugs and xenobiotics: PGP serves as barrier against entry of compounds into the body, as well as from entering tissues. Furthermore, PGP participates in removal of drugs from the organism. Because of that, genetic variations that alter protein function or expression of PGP can substantially affect intestinal absorption, the elimination, and the penetration of drugs into brain, germ cells, and the fetus. Already ident-

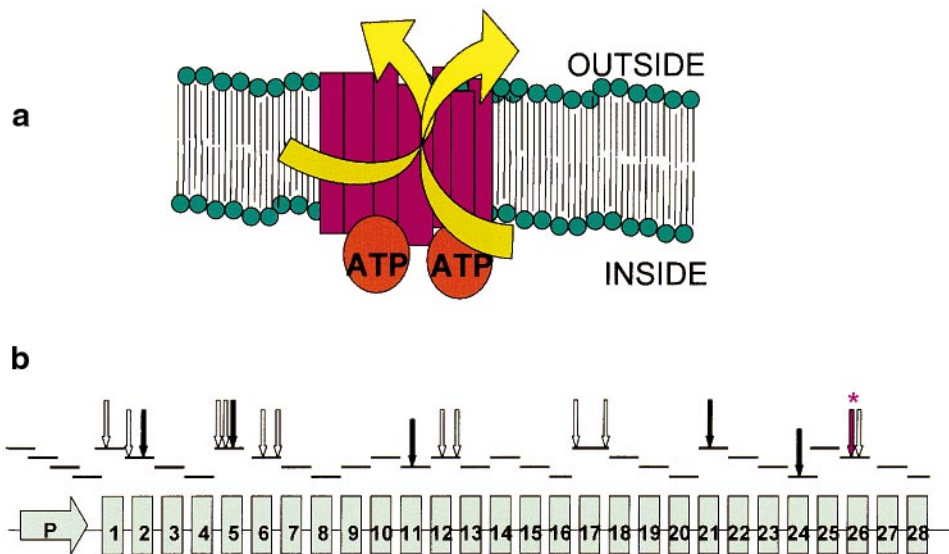


Figure 1 Schematic structure of PGP and polymorphisms in the human *MDR1* gene. (a) PGP is an integral membrane protein which transports compounds from membranes and (after membrane transfer) from the cytoplasm of cells to the outside. The transport process is energized by ATP hydrolysis. (b) Polymorphisms are indicated by arrows in relation to the exon-intron-composition of the human *MDR1* gene and the corresponding structure of the PGP protein. (*) depicts the C₃₄₃₅T, the SNP with phenotypical consequences on uptake of digoxin. Noncoding polymorphisms are represented by white arrows and amino acid changes by black arrows.

ified polymorphisms, such as the C₃₄₃₅T SNP, correlate with lower intestinal PGP expression,⁶⁰ and this in turn does directly affect the oral bioavailability of PGP substrates. For example, the uptake of digoxin, a known PGP substrate,³⁶ is influenced by intestinal PGP expression.²⁸ Consequently, digoxin plasma levels have been demonstrated to be significantly elevated in volunteers with a C > T nucleotide exchange in exon 26 (C₃₄₃₅T) of the *MDR1* gene, which is shown in Figure 2. The explanation for this observation is

that less PGP (associated with the 3435T/T-genotype) on the apical surface of the intestinal villus enterocytes remove less digoxin from the cells, resulting in increased bioavailability. Increased digoxin plasma levels may put individuals to increased risk to encounter adverse effects. However, since digoxin is actively secreted by the renal proximal tubule in a PGP-dependent manner,⁶¹ genetically determined high or low PGP expression may also increase or reduce the renal elimination at the same time. Because the specific distribution, metabolism and elimination (liver, kidney) may be different for various drugs that are PGP substrates, it is important to consider whether or not drug transport is a bottleneck for uptake, metabolism or elimination of the compound in question. Moreover, genetical variable PGP activity can influence the penetration of drugs not only into the blood, but also into compartments where the drug should have its mode of action. Examples that penetration of the blood–brain barrier may affect therapeutic outcome or neurotoxic adverse side effects are found not only in cancer therapy, but also for neuroleptic medications. Also, new HIV protease inhibitors are PGP substrates.^{62–64} Thus, variable responsiveness despite adequate blood levels and sensitive viruses, may be caused by individual differences in PGP activity.

PGP and other ABC drug transporters influence not only the distribution and elimination of various compounds, but they also serve as a cellular defence system against the harm of exo- and endogenous compounds. Because ABC transporters do act in concert with compound-metabolizing enzymes,^{65–67} the analysis of polymorphisms of drug transporters may be applied to further improve risk assessment and the prevention, early diagnosis, and intervention of

| | | |
|----------------------------------|-------------|-------------|
| MDR1 C3435T genotype | <p>C-C</p> | <p>T-T</p> |
| intestinal PGP levels | <p>high</p> | <p>low</p> |
| intestinal digoxin uptake | <p>low</p> | <p>high</p> |

Figure 2 Correlation of the *MDR1* C3435T polymorphism with intestinal PGP levels and oral digoxin uptake *in vivo*.



clinical disease. Thus, in addition to drug metabolizing enzymes as already well recognized components of inter-individual variability of the pharmacology of medications,^{68,69} polymorphisms that affect the expression and/or activity of MDR1, and—if identified—also of other ABC drug transporter genes such as MRP1 and MRP2, will enable us to understand the extensive variability between different individuals in drug response. Genetic testing of such parameters will, in turn, allow a more individualized drug therapy, with the goal to minimize or eliminate adverse effects and thus maximize therapeutic benefits.

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DUALITY OF INTEREST

Dr Ulrich Brinkmann is employed as vice president at EPIDAUROS, a pharmacogenetics company which has applied for protection of intellectual property regarding pharmacogenetic applications of selected MDR1 polymorphisms.

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