Pharmacogenetics of the human drug-transporter gene *MDR1*: impact of polymorphisms on pharmacotherapy

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The blood- and tissue-concentrations, and thus the activity, of many drugs are influenced by factors that are subject to inter-individual variation. Variables that influence blood levels are metabolizing enzymes and transporters. Transporters control drug uptake, distribution and elimination. Transport by efflux pumps such as *MDR1*-encoded P-glycoprotein can influence the bioavailability of drugs. Knowledge of the transporter 'status' might allow for compensation of differences in drug uptake, such as by dose adjustment, which is important for drugs with narrow therapeutic windows. So far, intestinal expression of *MDR1* has been determined by cumbersome methods, such as biopsies, although recently a functional polymorphism has been identified, which discriminates individual high or low-expressor alleles. As a result, clinical trials and therapy can be adapted to the '*MDR1*-status' of individual patients.

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▼ The multidrug resistance gene, *MDRI*, encodes for the protein P-glycoprotein (Pgp), which belongs to the large adenosine triphosphate (ATP)-binding cassette (ABC) protein family, which includes various membrane molecules, all of them possessing ABC domains. Most ABC transporters are composed of two transmembrane domains (TMDs). Each TMD contains six membrane-spanning helices. Cytoplasmic nucleotide-binding (ABC) domains generate energy for the transport process by hydrolysis of ATP. P-glycoprotein is one of the most thoroughly studied proteins among the ABC family, and a significant amount of information has been acquired regarding the structure and function of ABC transporters, based on analyses of Pgp.

MDR1 gene

The *MDR1* gene was initially discovered as the precursor to a protein associated with a major problem of cancer chemotherapy: failure caused by cross-resistance of tumors to many different cytotoxic agents. This phenotype, confirmed by experimental analyses *in vitro* (over-expression of *MDR1* causes resistance in cultured tumor cells), is shared by other members of the transporter family that are closely related to *MDR1* (multidrug resistance associated genes, MRP1-5, Refs 1–11). These transporter molecules protect cells against many drugs that are transport substrates because they act as efflux pumps for xenobiotics, providing a barrier against the entry of various substances. Because of its association with drug resistance and its influence on therapy outcome (leukemia with high PGP levels has poor prognosis[12–14]), great effort has been made to find and develop substances that modulate PGP activity. Chemosensitizers, such as substances that interfere with substrate recognition or ATP hydrolysis, or which act as competitive or non-competitive inhibitors, can restore the sensitivity of tumor cells towards chemotherapy with *MDR1* substrates. Prominent examples of the first generation are verapamil, nifidepine, immunosuppressants (cyclosporin A, FK506) and antiarrhythmic drugs (amiodarone, quinidine[15–19]). Second-generation compounds that are substrates, are PSC833 (Novartis, Basel, Switzerland), GF120918 (GlaxoSmithKline, Stevenage, UK) and XR9576 (Xenova, Slough, UK). Substrates that are recognized and transported by Pgp include a variety of drugs, including chemotherapy drugs, as well as many other medications and metabolites. Box 1 shows a small selection of Pgp substrates from different fields of medicine, including antibiotics, CNS-active drugs, cardiovascular medications and HIV inhibitors.

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Box 1. Selected substrates of P-glycoprotein

Antibiotics	**Immunosuppressants**	**Antiarrhythmics**
Cefotetan	Cyclosporin A	Amiodarone
Cefazolin	Tacrolimus	Quinidine
Ca^{++}Blocker	**Cardiac stimulants**	**CNS treatment**
Diltiazem	Digoxin	Cis-flupenthixol
Verapamil	Nicardipine	Trifluperazine
		Phenytoin
Anti-Neoplastic	**Analgesics**	**Anti-emetics**
Topotecan	Morphine	Ondansetron
Tamoxifen		
Mitoxantrone		

MDR1-encoded Pgp – a bioavailability gene

Despite the high expression of Pgp in many cancers, where it poses a severe problem because it mediates cells that are resistant towards many chemotherapeutic agents, the physiological function of Pgp is not restricted to tumors. *MDR1* is expressed in many normal tissues. One physiological function of Pgp could be in the adrenal cortex and might involve the metabolism of steroids[20]. In other tissues, Pgp acts as a cellular efflux pump to control the intracellular concentration of substances. Its cell- and organ-specific distribution, and its capacity to transport a broad range of compounds, might render Pgp an effective cellular protector against toxic substances that are Pgp substrates. Box 2 summarizes some key organs where significant levels of Pgp are found. For example, in the lower gastrointestinal tract (jejunum, ileum and colon), Pgp is found on the surface of epithelial cells, influencing intestinal drug absorption and, in some cases, constraining oral drug bioavailability[20–23], as well as possibly facilitating excretion across the intestinal mucosa. It is probable that Pgp has a protective function at the luminal surface of capillary endothelial cells at the blood–brain barrier (BBB) and placenta[24,25], controlling the amount of substances entering the brain or the fetus, respectively. In the liver and the kidney, Pgp is expressed in the biliary canalicular membrane of hepatocytes and in the brush-border membrane of proximal tubules[23], respectively. This distribution supports its role in the biliary and renal excretion of substances.

Box 2. Expression of *MDR1* in human tissues

Low level expression of Pgp:
Many cells/tissues

Marked expression of Pgp:
Lower gastrointestinal tract: epithelial cells of jejunum, ileum and colon
Blood–brain barrier: luminal surface of capillary endothelial cells
Placenta: luminal surface of capillary endothelial cells
Liver: biliary canalicular membrane of hepatocytes
Kidney: brush-border membrane of proximal tubules
Blood: CD56 lymphocytes

High expression of Pgp:
Many drug-resistant tumors

A direct function of Pgp in drug absorption, disposition and elimination has also been demonstrated experimentally in mice. Mice harbour two *MDR1* genes (*Mdr1a* and *Mdr1b*), and knockouts of one or both of these genes were made. Although these mice were viable (i.e. *MDR1* appears not to be an essential gene in mice), the animals were hypersensitive to xenobiotics, with elevated brain-uptake and significantly altered pharmacokinetics of many drugs[26,27]. Increased bioavailability and, after intravenous application, reduced fecal and urinary clearance has been observed in knockout (KO) mice for drugs such as anthracyclins, digoxin, taxol, tri-*n*-butylmethylammonium and azidoprocainamide methoiodide. Accumulation of drugs that are Pgp substrates (see later) was also increased in the liver, the brain and the gall bladder. The increased availability of medications such as paclitaxel, loperamide, vinblastine, ivermectin and cyclosporin, and subsequent drug accumulation in the brain, liver and intestine[28–39] in mice lacking functional *MDR1* genes can result not only in improved therapeutic efficacy, but can also lead to increased sensitivity towards adverse effects.

Variability and polymorphisms in the human *MDR1*-gene

The current model of the SAR of Pgp and its mode of action is that two homologous halves, each with six transmembrane domains and one ABC-domain, recognize substrates, interact with each other and use energy (ATP) for transport. This is the result of the analyses of many variant Pgp molecules that have been recombinantly produced. Using these recombinant technologies, it has become clear that substrate specificity is affected by mutations in TMDs 5, 6, 11 and 12. This indicates the presence of a drug-binding site in this region[40–45]. Also, intact ATP domains and the interaction of these with drug-binding sites are needed for transport function[46–48], (see Ref. 42 for a comprehensive overview).

One important question is whether hereditary variants of *MDR1* account for the inter-individual variability in the pharmacokinetics and pharmacodynamics of drugs. Mickley *et al.*[49] have reported the first evidence of the presence of polymorphisms in the human *MDR1* gene. Single

nucleotide polymorphisms (SNPs) in Exons 21 and 24 (G2677T and G2995A) were observed in a population of tumor patients, in drug-resistant cell lines, in cells from refractory malignant malignomas and in healthy volunteers. A screen of the entire *MDR1* gene for the presence of additional SNPs was undertaken by Hoffmeyer and coworkers[50], and led to the detection of 15 SNPs (Table 1). Whether certain SNPs might be of functional consequence can be predicted, to some degree, from their position within the gene and protein. For example, SNPs that change amino acids, and thus possibly have an effect on protein function, are located at position $A_{61}G$ (replacement of Asn with Asp at position 21 of exon 2 of Pgp), a Phe–Leu change in position 103 next to the second TMD close to a glycosylation site, and a $G_{1199}A$ SNP in exon 11, which causes a Ser–Asn size- and charge-change close to the first ATP-binding domain. Nevertheless, no correlation between these protein-SNPs with altered function or with Pgp activity has been reported, to date. However, another SNP, a $C_{3435}T$ change at a wobble position in exon 26, has been shown to have pharmacological consequences. The *MDR1* genotype at the SNP $C_{3435}T$-position correlates with Pgp expression in the intestine, influencing the uptake of orally administered Pgp substrates. The T-allele, particularly if homozygous, is associated with low intestinal expression of Pgp. Conversely, the corresponding C-allele is associated with increased Pgp levels. Individuals that carry the homozygous low-expressor (T)-allele, approximately 25% of the Caucasian population[51], show increased digoxin plasma-levels because of increased uptake (Fig. 1).

Table 1. Single nucleotide polymorphisms (SNPs) in the *MDR1* gene

SNP	Region	Number	Frequency of SNPs[a] [%]			Effect
			Heterozygous	Homozygous Observed	Estimated	
$T-_{12}C$	E1	85	11.8	0	0.4	Non-coding
$G-_{1}A$	E2	188	11.2	0	0.4	Translation initiation
$A_{61}G$	E2	188	17.6	0.5	0.81	$Asn_{21}Asp$
$G-_{25}T$	I4	85	26.0	3.5	2.3	
$G-_{35}C$	I4	85	1.2	0	0.01	
$T_{307}C$	E5	85	1.2	0	0.01	$Phe_{103}Leu$
$C+_{139}T$	I5	85	48.2	16.5	16.8	
$C+_{145}T$	I5	85	2.4	0	0.01	
$G_{1199}A$	E11	85	12.9	0	0.4	$Ser_{400}Asn$
$C_{1236}T$	E12	188	48.9	13.3	14.4	$Gly_{412}Gly$
$C+_{44}T$	I12	188	11.7	0	0.4	
$T-_{76}A$	I16	85	45.9	22.4	20.3	
$A+_{137}G$	I17	85	1.2	0	0.01	
$G_{2677}T$	E21	83[b]	43.4	42.2	38.4	$Ala_{893}Ser$
$G_{2995}A$	E24	36[b]	11.1	0		$Ala_{999}Thr$
$C_{3435}T$	E26	537	47.7	26.4	24.1	$Ile_{1145}Ile$
$C_{3396}T$	E26	188	0.53	0	0.01	Wobble

[a]*MDR1* sequences Genbank (gb) accession numbers AC002457 and AC005068 are defined as wildtype.
[b]Samples are from cell lines and various tissues.
Abbreviations: E, exon; I, intron.
The positions of the identified polymorphisms (column 1) correspond to positions of the *MDR1* cDNA (gb: M14758, codon TTC in exon 10, F335, is missing in that sequence) with the the first base of the ATG start codon set to 1. SNPs that are located in introns are presented as exon+/–n, where n = nucleotides upstream (–) or downstream (+) of the exons according to Chen *et al.*[7]. The predicted ratios of the homozygous genotypes (q^2) were calculated on the basis of the Hardy–Weinberg distribution, using the formulas $p = (2 \times AA + 1 \times Aa)/2N$ and $p + q = 1$, where AA = number of probands homozygous for the wildtype (wt) allele, Aa = number of heterozygotes, N = size of the sample test, p = frequency of the wt allele, q = frequency of the mutated (mut) allele, q^2 = frequency of the genotype homozygous for the mut allele.

Pharmacological implications of transporter pharmacogenetics

Absorption, distribution, metabolism and elimination are major factors that affect the therapeutic efficacy of compounds. Pgp and other ABC transporters are proven to play a role in these processes, by providing a barrier to entry of compounds into the body, as well as controlling their rate of transfer between different tissues and compartments. The discovery of genetic variations that influence the function or expression of Pgp can have a direct impact on the likelihood of intestinal absorption or elimination of compounds, as well as on their ability to penetrate the brain. The functional significance of the recently discovered *MDR1* $C_{3435}T$ polymorphism is just one example: it correlates with reduced Pgp expression[50], which can modulate oral bioavailability of Pgp substrates. Digoxin, a known Pgp substrate[27], is taken up in a *MDR1*-dependent manner[22], and as a result digoxin plasma levels are higher in individuals with a C–T nucleotide exchange in exon 26 ($C_{3435}T$, low-expressor allele). Reduced amounts of Pgp (associated with the homozygous low-expressor genotype) on enterocytes remove less digoxin from the cells, increasing the uptake of digoxin.

Enhanced bioavailability leads to increased plasma levels of PGP substrates, and can result in not only greater activity, but also (as seen in the *Mdr1a* and *Mdr1b* KO

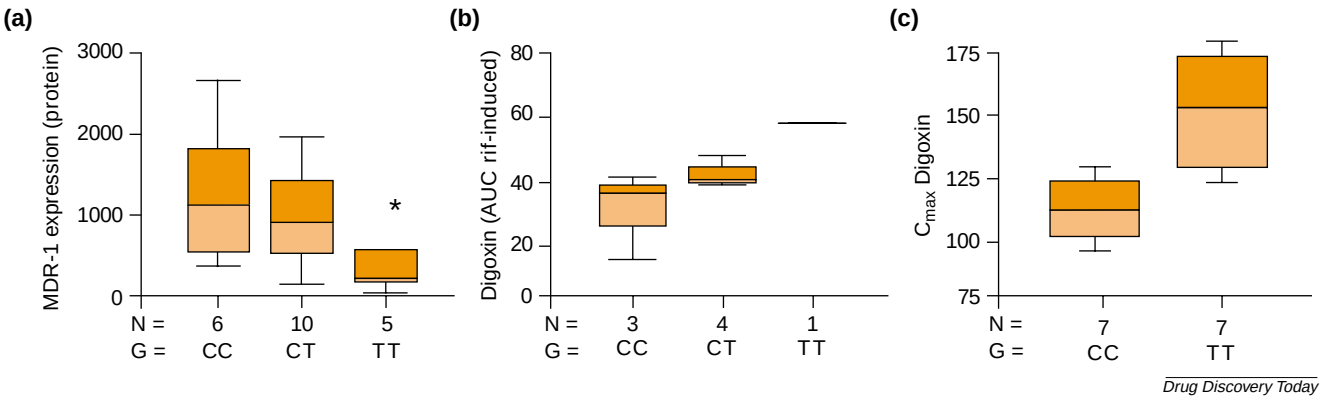

Figure 1. Correlation of the *MDR1* C_{3435}T polymorphism with intestinal P-glycoprotein (Pgp) levels and oral digoxin uptake *in vivo*. The C_{3435}T polymorphism in exon 26 is a wobble, non-promoter single nucleotide polymorphism (SNP) and probably does not directly influence the expression of *MDR1*. This SNP defines an allele; that is, it is linked to one or more other, so far unidentified, changes in regions of the *MDR1* gene that control expression (e.g. in promoter, enhancer or mRNA processing regions). The correlations of genotypes are shown (CC, CT, TT on the X-axis, N = Number of samples analyzed per genotype), with intestinal protein content **(a)** or digoxin uptake, with **(b)** and without **(c)** Rifampicine (Rif) induction. The median value is indicated by the line within the boxplot, extremes are indicated by asterisk. Genetically determined high or low Pgp expression might also affect renal elimination. Pgp-dependent uptake- and elimination-effects might partially antagonize each other. The distribution, metabolism and elimination varies between drugs that are Pgp substrates, emphasizing the importance of analyzing whether transport is a bottleneck for uptake, metabolism or elimination.

mice) greatly increased susceptibility to adverse effects. Consideration of this aspect is particularly valuable for the evaluation of novel compounds in clinical trials. It might be possible to utilize *MDR1* genotyping to reduce the inherent risks of clinical trials and, simultaneously, to explain some adverse effects or abnormal pharmacology observed in some patients. For example, some HIV protease inhibitors were identified as Pgp substrates[52–54], which suggests that different responses between individuals could be caused by both different virus sensitivity and variability in individual Pgp activity. In addition, inter-individual variability of Pgp activity might not only affect blood levels, but also the distribution of drugs to the desired target compartment. In this regard, penetration of the BBB is an important parameter, and insufficient or excessive penetration could account for adverse side effects or inactivity, respectively, of CNS-active medications.

Conclusions

Genetic variability and functional polymorphisms in ABC transporters are relevant pharmacological factors that have to be considered together with drug-metabolizing enzymes, whose activity show a large degree of inter-individual variability[55]. Therefore, combined analyses of the variable activity of metabolizing enzymes and of transporter polymorphisms can be used to understand the individual variability in drug response. This will not only be of advantage for the development of novel drugs (genetically defined volunteer and patient groups reduce the inherent risks and increase the success rates of trials), but might also yield advantages for patients. Furthermore, the diagnosis of such genetic parameters will be the starting point for an individualized drug therapy through the use of genotype-based dose recommendations, to ensure minimal side effects and maximal benefits of therapies.

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