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Review Article

Functional Implications of Genetic Polymorphisms in the Multidrug Resistance Gene *MDR1 (ABCB1)*

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The multidrug resistance (*MDR1)* gene product P-glycoprotein is a membrane protein that functions as an ATP-dependent efflux pump, transporting exogenous and endogenous substrates from the inside of cells to the outside. Physiological expression of P-glycoprotein in tissues with excretory or protective function is a major determinant of drug disposition and provides a cellular defense mechanism against potentially harmful compounds. Therefore, P-glycoprotein has significant impact on therapeutic efficacy and toxicity as it plays a key role in absorption of oral medications from the intestinal tract, excretion into bile and urine, and distribution into protected tissues such as the brain and testes. There is increasing interest in the possible role of genetic variation in *MDR1* in drug therapy. Numerous genetic polymorphisms in *MDR1* have been described, some of which have been shown to determine P-glycoprotein expression levels and substrate transport. Furthermore, some of these polymorphisms have an impact on pharmacokinetic and pharmacodynamic profiles of drug substrates and directly influence outcome and prognosis of certain diseases. This review will focus on the impact of genetic variation in *MDR1* on expression and function of P-glycoprotein and the implications of this variation for drug therapy and disease risk.

KEY WORDS: polymorphism, drug resistance, MDR1, ABCB1

INTRODUCTION

Many transporters relevant for drug therapy are members of the superfamily of ABC (ATP-binding cassette) transporters, which comprises eight subfamilies that are encoded by separate genes on different chromosomes. The multidrug resistance gene *MDR1* (*ABCB1*) and its gene product P-glycoprotein are the most thoroughly analyzed among ABC transporters. *MDR1* is a large gene, spanning more than 100 kb on chromosome 7, with 28 exons that are spliced into a 4.5-kb mRNA. The encoded P-glycoprotein is a highly conserved member of the ABC transporter family with 12 membrane spanning domains, two nucleotide binding domains, and a molecular weight of approximately 170 kDa (1–4).

It is now evident that P-glycoprotein plays a major role in drug disposition and in protecting the organism against many of the toxic xenobiotics to which it can potentially be exposed in nature. P-glycoprotein confers protection by limiting the uptake of compounds from the gastrointestinal tract and by contributing to their excretion via the liver, kidneys, and intestine. Moreover, P-glycoprotein in the blood-brain barrier and other blood-tissue barriers protects sensitive organs from exposure to toxic compounds that may have entered the bloodstream. (5–8). Substrate specificity of P-glycoprotein is extremely broad, and consequently, P-glycoprotein is a major determinant of drug disposition. Recently, genetic variation in *MDR1* has been identified as a determinant of P-glycoprotein expression and function in normal tissue, thereby contributing to interindividual differences in drug response. This review summarizes currently available data on *MDR1* genetic polymorphisms and their functional consequences and impact on drug treatment and disease course.

TISSUE DISTRIBUTION AND SUBSTRATE SPECIFICITY OF P-GLYCOPROTEIN

The relevance of P-glycoprotein for pharmacological therapy was first recognized in cancer treatment, where it was identified to be one of the main players associated with multidrug resistance. Overexpression of this protein in tumor cells has been shown to decrease intracellular accumulation of chemotherapeutic agents *in vitro*, thereby allowing cancer cells to escape the otherwise cytotoxic effects of these drugs (9). Available evidence suggests that P-glycoprotein also causes drug resistance in clinical tumors, and P-glycoprotein overexpression in cancer has been associated with poor prognosis in affected patients (10–12).

More recently, physiological expression of P-glycoprotein was found in epithelial cells of different tissues with excretory or protective function, including the brush border membrane of enterocytes in the small intestine, the canalicular membrane of hepatocytes, capillary endothelial cells of brain and testis, and the brush border membrane of proximal tubule cells in kidneys (13,14). Furthermore, P-glycoprotein can be detected in hematopoetic cells and in pancreatic, adre-

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Table I. *MDR1* Coding Variants

cDNA position[a]	NT change	DNA/AA position	AA change	Allele frequency[b]					
				Total (n = 494)	CA (n = 200)	AA (n = 200)	AS (n = 60)	ME (n = 20)	PA (n = 14)
61	A to G	21	Asn to Asp	0.045	0.080	0.025	0.017	0	0
266	T to C	89	Met to Thr	0.002	0.005	0	0	0	0
729	A to G	243	Syn	0.002	0.005	0	0	0	0
781	A to G	261	Ile to Val	0.006	0	0.015	0	0	0
1199	G to A	400	Ser to Asn	0.014	0.025	0.010	0	0	0
1236	C to T	412	Syn	0.385	0.459	0.209	0.685	0.450	0.571
1308	A to G	436	Syn	0.002	0	0.005	0	0	0
1617	C to T	539	Syn	0.002	0.005	0	0	0	0
1985	T to G	662	Leu to Arg	0.002	0.005	0	0	0	0
2005	C to T	669	Arg to Cys	0.004	0	0.010	0	0	0
2547	A to G	849	Ile to Met	0.002	0.005	0	0	0	0
2650	C to T	884	Syn	0.004	0.005	0.005	0	0	0
2677	G to T	893	Ala to Ser	0.308	0.464	0.100	0.450	0.400	0.357
2677	G to A	893	Ala to Thr	0.035	0.036	0.005	0.067	0	0.357
3151	C to G	1051	Pro to Ala	0.002	0	0.005	0	0	0
3322	T to C	1108	Trp to Arg	0.002	0	0.005	0	0	0
3421	T to A	1141	Ser to Thr	0.047	0	0.111	0	0.050	0
3435	C to T	1145	Syn	0.392	0.561	0.202	0.400	0.500	0.500
3751	G to A	1251	Val to Ile	0.002	0	0	0	0.050	0
3767	C to A	1256	Thr to Lys	0.002	0.005	0	0	0	0

[a] cDNA numbers are relative to the ATG site and based on the cDNA sequence from GenBank accession number M14758.

[b] Allele frequencies were calculated for the total population and each individual ethnic group; CA, Caucasian, AA, African American, AS, Asian American, ME, Mexican American, PA, Pacific Islander. n is the number of chromosomes in each ethnic group.

nal, and placental tissue. Its major function is to confer protection by limiting intracellular accumulation of xenobiotics and by controlling tissue exposure to potentially toxic compounds. Substrates of P-glycoprotein show substantial overlap with cytochrome P450 3A4 substrates and include numerous drugs used for treatment of common diseases, such as cardiovascular disease, HIV infection, and malignant tumors (15,16). Consequently, P-glycoprotein is a major determinant of *in vivo* drug disposition and response and is involved in numerous drug interactions. Drug interactions involving P-glycoprotein are especially relevant for drugs with narrow therapeutic indices, where induction or inhibition of transporter function can have a tremendous impact on drug efficacy and safety (5,17,18).

GENETIC VARIATION IN *MDR1*

There is increasing evidence that genetic variation in *MDR1* affects P-glycoprotein function and expression levels. The first genetic polymorphism of *MDR1* to be identified was a G2677T variant isolated from human adrenal, liver, and kidney samples that results in an Ala893Ser change in P-glycoprotein (19,20). More recently, Hoffmeyer *et al.* sequenced *MDR1* exons and flanking intron-exon boundaries in a collection of 188 healthy Caucasian volunteers and identified a total of 15 variant sites. Additional variants have been identified by resequencing *MDR1* in larger sample sets and different ethnic populations (21–28). Table I gives a summary of 19 segregating sites, resulting in 20 coding region variants identified in a population of 247 healthy individuals of different ethnic backgrounds (22). Thirteen of these coding region variants resulted in an amino acid change, and six were silent mutations. With only two exceptions, these coding region variants were located in the intracellular loops of the protein (Fig. 1). It can be expected that additional variants will be detected, although the extensive sampling in the latest study insured identification of all common *MDR1* variants.

As shown in Table I, allele frequencies of *MDR1* variants varied widely between different populations. The power to detect variants was highest in Caucasians and African Americans as sample size was considerably larger in these populations compared to other ethnic groups. There is also increasing data on variant segregation in Asians, whereas data on individuals with other ethnic backgrounds are still limited (22,24,28). Of particular interest is the large discrepancy in allele frequency of the common C1236T, G2677T, and C3435T variants between Caucasians and African Americans (21,22). These three variants are all found at 45–55% fre-

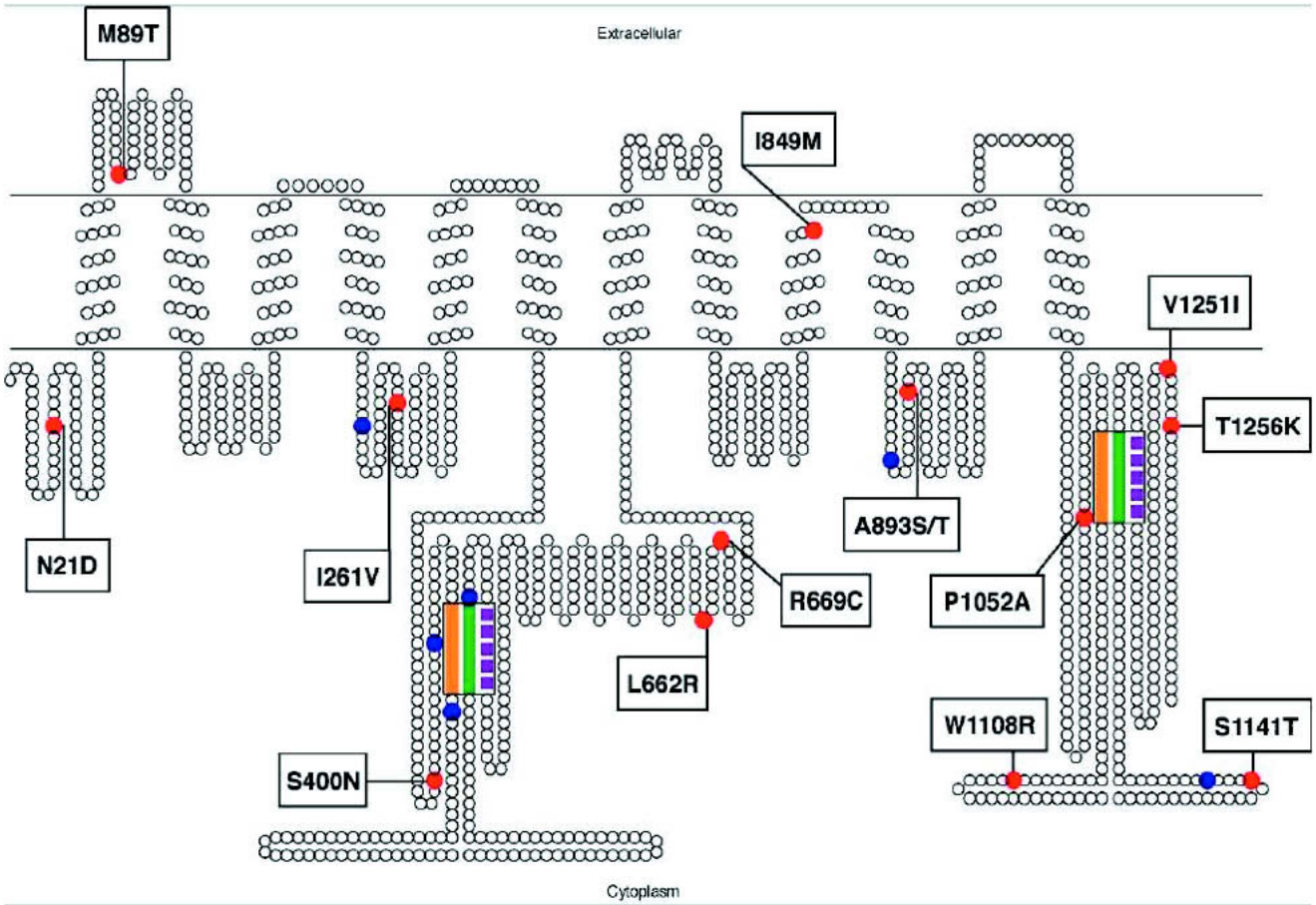

Fig. 1. Secondary structure of P-glycoprotein with coding region SNPs. The transmembrane topology schematic was rendered using TOPO (S. J. Johns and R. C. Speth, transmembrane protein display software, http://www.sacs.ucsf.edu/TOPO/topo.html, unpublished). Non-synonymous amino acid changes are shown in red and synonymous changes are shown in blue. The position of amino acid changes in P-glycoprotein resulting from non-synonymous changes in *ABCB1* are indicated. The Walker A, Walker B, and linker peptide domains comprising the nucleotide binding domains are marked in orange, purple, and green, respectively.

quency in Caucasians but only at 5–10% frequency in the African-American population. In general, the distribution of common *MDR1* variants in Asians, Mexican Americans, and Pacific Islanders shows a similar pattern as in Caucasians (22). Consistent with the age of the African-American population, more distinct and rare variants were detected in this population relative to the other ethnic groups sampled.

HAPLOTYPE STRUCTURE OF *MDR1*

The most complete approach to assigning haplotypes was based on *MDR1* variant identification in 245 DNA samples from individuals of different ethnic origin (22). Bayesian methods were used to statistically infer haplotypes (29,30). Sixty-four haplotypes were inferred, of which 33 were found in 3 or more chromosomes. These 33 common haplotypes represent 20 segregating sites, including 10 intronic, 3 untranslated region, 5 non-synonymous, and 2 synonymous variants. Chromosomes from 98% of Caucasians, 84% of African Americans, 97% of Asian Americans, 100% of Mexican Americans, and 92% of Pacific Islanders were assigned a common haplotype. Haplotype analysis of *MDR1* revealed two major haplotypes, which differed at six segregating sites (22). *MDR1**13 contains three coding variants (C1236T, G2677T, and C3435T) and three intronic variants [intron 13 C(+24)T, intron 9 A(−44)G, and intron 14 A(+38)G] relative to the *MDR1**1 reference haplotype. Figure 2 shows the ethnic distribution of *MDR1**1 and *MDR1**13, which cover 36% of the 490 chromosomes analyzed in this study. The reference haplotype *MDR1**1 is the most prevalent in the African-American population, whereas *MDR1**13 is found at about a 2-fold higher frequency in the Caucasian population than the reference haplotype. There was also great interethnic variability in the total number of haplotypes observed in a population and the ethnic distribution of other haplotypes (22).

MDR1 haplotype structure has also been analyzed in three different Asian populations (28). Haplotypes considered only the three high frequency coding variants, C1236T, G2677T/A, and C3435T. A total of 10 haplotypes were found in this analysis, 3 of which were not identified in other studies (21,22). In agreement with the analysis by Kroetz and coworkers, the 1236T/2677T/3435T haplotype was the most common haplotype in Chinese, Indian, and Malay populations (31–49% frequency). The corresponding haplotype with reference nucleotides at these three positions (1236C/2677G/3435T) was also detected at similar frequencies in these three Asian populations (18–28%). Interestingly, a 1236T/2677G/3435C haplotype was found at 23–35% frequency in Chinese and Malay populations but only at 1.7% frequency in an Indian population.

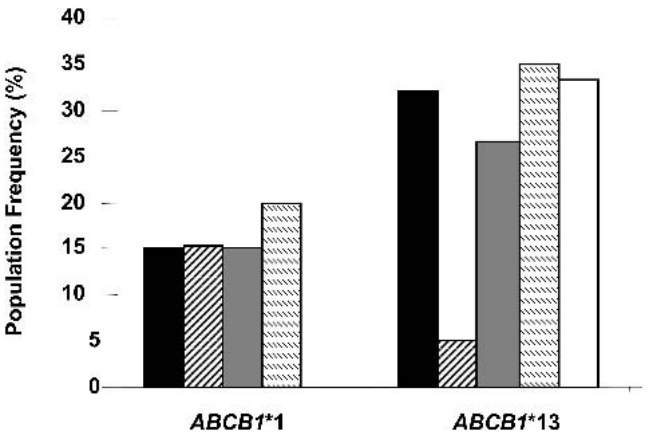

Fig. 2. Ethnic distribution of *MDR1**1 and *MDR1**13. The ethnic distribution of the two major *MDR1* haplotypes are shown. Haplotypes were called for 100 Caucasians (black bars), 99 African Americans (diagonal lines), 60 Asian Americans (gray), 10 Mexican Americans (hatch marks), and 6 Pacific Islanders (white bars). Data from Kroetz *et al.* (27).

IMPACT OF *MDR1* GENETIC VARIATION ON P-GLYCOPROTEIN EXPRESSION AND FUNCTION *IN VITRO*

The earliest data on the functional impact of genetic variation in the *MDR1* gene were derived from *in vitro* experiments in cancer cells grown under selective pressure (Table II). Kioka *et al.* compared the sequence of full-length MDR1 cDNA isolated from human adrenal gland with MDR1 cDNA obtained from colchicine-selected multidrug-resistant cultured cells (19). The colchicine-selected cells exhibited a Gly185Val substitution in P-glycoprotein, resulting in increased resistance to colchicine but no apparent effect on sensitivity to adriamycin and vinblastine. In this case, a single nucleotide polymorphism in *MDR1* resulted in a change in the pattern of P-glycoprotein substrate specificity. A P-glycoprotein variant with a deletion of a phenylalanine at amino acid residue 335 has also been identified in a multidrug-resistant human sarcoma cell line isolated by coselection with doxorubicin and the cyclosporine analog PSC-833 (valspodar) (31). Cells expressing the ΔPhe335 variant exhibited an altered phenotype compared to the reference protein, with decreased resistance to vinca alkaloids, loss of resistance to dactinomycin, and decreased transport of rhodamine 123 and cyclosporin A. However, these functional effects were substrate-dependent, as resistance to doxorubicine and paclitaxel was retained. These results indicate that Phe335 is an important binding site for P-glycoprotein substrates and inhibitors.

Numerous site-directed mutagenesis studies have shown that the introduction of nucleotide changes in highly conserved regions of *MDR1* has a major impact on P-glycoprotein function and expression (32). The cystic fibrosis transmembrane conductance regulator gene (CFTR), another member of the ABC family of transporters, shares conserved sequence motifs with *MDR1* and other ABC genes in the regions coding for nucleotide binding sites. Naturally occurring CFTR mutations introduced at analogous positions in the human MDR1 cDNA resulted in defective processing of mRNA and a nonfunctional P-glycoprotein, whereas a functional multidrug transporter was obtained when the amino acid substitution was introduced in less conserved regions of the gene (33).

The first functional data on naturally occurring genetic variation in *MDR1* considered the effect of these polymorphisms on allelic expression. Two single nucleotide polymorphisms, G2677T and G2995A, were identified that resulted in Ala893Ser and Met999Val changes in P-glycoprotein, respectively. In normal cells and unselected cell lines, the frequency of expression of both alleles was similar, whereas in drug-selected cell lines and in samples of relapsed malignant lymphoma, expression was shifted toward overrepresentation of

Table II. Functional Impact *in vitro* of *MDR1* Variants

Amino acid change	Functional effect of the variant allele	Reference
Val185Ser	Increased colchicine resistance	[30]
ΔPhe335	Decreased resistance to vinca alkaloids; no resistance to dactinomycin	[31]
Lys536Gln, Gly534Asp, Lys536Arg, Ser532Arg, ΔTyr490	Defective RNA processing	[33]
Ala893Ser	Acquired overexpression of one allele in drug-resistant cells	[20]
Ala893Ser	Decreased digoxin efflux	[19]
Asn21Asp, Phe103Leu, Ser400Ala, Ala893Ser, Ala893Thr	No effect on P-glycoprotein cell surface expression and substrate specificity	[69]
Ala893Ser	No difference in calcein-AM transport	[27]
Ala893Ser/Thr	No difference in transport of verapamil, digoxin, viblastine and cyclosporine A	[35]

one allele. Although in this study the functional impact of the amino acid changes was not directly investigated, the deviation in the drug-exposed cells and relapsed tumor cells from the segregation pattern observed in normal or drug-naive tumor cells was interpreted as acquired change, which might offer a selection advantage to the tumor cells (20).

At least five P-glycoprotein variants have been functionally characterized in heterologous expression systems. A vaccinia virus expression system was used to examine the Asn21Asp, Phe103Leu, Ser400Ala, Ala893Ser, and Ala893Thr P-glycoprotein variants. In all cases, the cell surface distribution and substrate specificity of these variant transporters were similar to reference P-glycoprotein, suggesting no functional impact of these variations (34). However, most substrates used in this study were labeled with bulky fluorescent bodipy groups, which might affect the substrate specificity for P-glycoprotein. Both transient and stable expression of the common Ala893Ser variant has failed to identify significant differences in the transport of calcein-AM, verapamil, digoxin, vinblastine, or cyclosporin A relative to the reference protein (22,35). In both of these cases, the nonsynonymous G2677T variant was studied in the context of the C1236T and C3435T variants found in the common *MDR1* haplotypes. In contrast, digoxin efflux in mammalian cells retrovirally transduced with MDR1 cDNAs encoding either the Ser893 or the reference Ala893 P-glycoprotein showed significantly decreased intracellular digoxin concentration for the Ser893 variant, suggesting increased P-glycoprotein function (21). The lack of concordance among these functional studies might reflect differences in the heterologous expression systems, P-glycoprotein substrates, and functional assays that were used in the various studies. Clearly, additional studies examining the kinetics of transport by these P-glycoprotein variants are necessary before a consensus can be reached about the functional effects of these *MDR1* variants. The establishment and validation of a standard experimental system will be essential for meaningful interstudy comparisons of the consequences of different *MDR*1 variants on P-glycoprotein function and expression.

IMPACT OF *MDR1* GENETIC POLYMORPHISM ON TISSUE EXPRESSION AND FUNCTION OF P-GLYCOPROTEIN

Only a limited number of studies have investigated the association between *MDR1* genetic variation and tissue levels of P-glycoprotein (Table III). The levels of intestinal P-glycoprotein were reported to be lower in healthy Caucasian volunteers homozygous for the synonymous C3435T variant relative to those with the reference genotype (25). It must be noted, however, that the molecular mechanism by which this synonymous (C3435T) variant influences P-glycoprotein expression is unclear. Analysis of placental P-glycoprotein expression in Japanese women indicated that individuals with the –129TC genotype had lower levels relative to those with the –129TT genotype; however, there was no significant association between 2677 and 3435 genotype and P-glycoprotein levels (24). The effect of this 5′-untranslated region variant (T-129C) on expression is consistent with the known effects of untranslated regions on protein expression.

In CD56-positive natural killer cells, individuals with the 3435TT genotype had lower levels of MDR1 mRNA and decreased rhodamine 123 efflux compared to those with the reference genotype (36). However, such an association could not be confirmed in a subsequent study, which failed to show an association between the G2677T and C3435T polymorphisms with rhodamine efflux in peripheral blood lymphocytes (37).

IMPACT OF *MDR1* GENETIC POLYMORPHISM ON DRUG DISPOSITION

Digoxin is the most extensively studied P-glycoprotein substrate with respect to the effect of *MDR1* genetic variation on intestinal bioavailability (Table III). Consistent with the decreased levels of intestinal P-glycoprotein, plasma levels of digoxin were significantly higher in individuals with the 3435TT genotype relative to the 3435CC individuals (25). Steady-state digoxin AUC values were also reported to be higher (38) and digoxin renal clearance was lower (39) in volunteers with the 3435T allele. However, decreased intestinal absorption of digoxin (40,41) and lack of effect (17,42) have also been reported for the 3435TT genotype. Possible explanations for these discordant results include heterogeneity in the *MDR1* haplotype structure within the study populations and the relatively small sample sizes used for these studies.

Similar controversy also exists regarding the influence of the *MDR1* genotype on the disposition of other P-glycoprotein substrates. In healthy Caucasian and African-American volunteers, individuals with the 2677GG/3435CC genotypes had higher fexofenadine AUC values than individuals homozygous for the variant alleles in these two positions (2677TT and 3435TT), consistent with increased P-glycoprotein function in individuals with the variant genotypes (21). A similar finding was made when the 2677 and

Table III. Impact of *MDR1* Genetic Variation on P-Glycoprotein Expression and Drug Pharmacokinetics

Population	Nucleotide/amino acid change	Substrate	Functional effect of the variant allele	Reference
Caucasian volunteers	C3435T	Digoxin	Decreased intestinal P-glycoprotein expression; increased digoxin AUC after single dose	[23]
Japanese volunteers	C3435T	Digoxin	Decreased digoxin AUC after single dose	[40]
Caucasian volunteers	C3435T	Digoxin	Higher digoxin AUC under steady state conditions	[38]
Japanese volunteers	G2677T (Ala 893Ser) + C3435T	Digoxin	Higher digoxin AUC and digoxin renal clearance after single dose	[39]
Caucasian volunteers	C3435T	Digoxin	No change in single dose digoxin pharmacokinetics	[42]
Caucasian volunteers	C3435T	Rhodamine 123	Decreased rhodamine 123 efflux from CD56 positive cells	[36]
Healthy bone marrow donors	C3435T, G2677T (Ala893Ser), T-129C	Rhodamine 123	No difference in rhodamine 123 efflux from hematopoietic stem cells	[70]
Caucasian volunteers	G2677T (Ala893Ser), C3435T	Rhodamine 123	No difference in rhodamine 123 efflux from CD 56 positive cells	[37]
Caucasian and African American volunteers	G2677T (Ala893Ser) + C3435T	Fexofenadine	Decreased fexofenadine AUC after single dose	[19]
Caucasian volunteers	C3435T	Fexofenadine	No change in fexofenadine pharmacokinetics	[43]
Caucasian volunteers	G2677T (Ala893Ser), C3435T	Talinolol	No change in single dose talinolol pharmacokinetics	[51]
Caucasian volunteers	C3435T	Nelfinavir	Decreased nelfinavir serum levels	[50]
Renal transplant patients	C3435T	Tacrolimus	Higher tacrolimus blood levels	[46]
Renal transplant patients	C3435T	Cyclosporine A	Higher cyclosporine A clearance	[45]
Renal transplant patients	C3435T	Cyclosporine A	No difference in transplant survival or cyclosporine through levels	[48]
Renal transplant patients	C3435T	Cyclosporine A, tacrolimus	No difference in cyclosporine and tacrolimus dose requirement	[49]
Renal transplant patients	T-129C, C1236T, G2677T/A (Ala893Ser/Thr), C3435T	Tacrolimus	Higher tacrolimus dose requirement in carriers of the 2677T/A alleles	[44]
Pediatric heart transplant patients	G2677T/A (Ala893Ser/Thr), C3435T	Tacrolimus	Higher tacrolimus blood levels at 6 and 12 months	[47]
Japanese women	T-129C	—	Increased placental P-glycoprotein expression levels	[22]
Caucasian volunteers	C1236T + G2677T/A (Ala893Ser/Thr) + C3435T	Loperamide	No difference in single dose pharmacokinetics	[57]

3435 polymorphisms were analyzed separately, with AUC values being highest for individuals carrying the reference alleles. However, in a separate study, the *MDR1* C3435T variant had no effect on fexofenadine disposition (43).

The contribution of *MDR1* genetic polymorphisms has also been extensively studied for the calcineurin inhibitors cyclosporine and tacrolimus, which show large interindividual differences in oral bioavailability. Though two studies in renal transplant patients found cyclosporine and tacrolimus dose requirement to be higher in individuals homozygous for the 3435T allele (44,45), two other studies investigating tacrolimus steady-state dose requirement found an opposite effect, with plasma levels being lower in the 3435CC group after 3, 6, and 12 months (46,47). A recent study investigating the effect of genetic polymorphisms in *CYP3A4, CYP3A5,* and *MDR1* on the pharmacokinetics of cyclosporine and tacrolimus also found no evidence supporting a role for the *MDR1* C3435T polymorphism in dose requirement of the two drugs, consistent with previous reports regarding cyclosporin A trough levels and MDR1 genotype (48,49).

The effects of the *MDR1* C3435T variant on plasma drug levels has also been studied for nelfinavir and talinolol (Table III). Nelfinavir trough levels (expressed in percentiles) is lowest in individuals with the 3435TT genotype (50), whereas talinolol pharmacokinetics were unaffected by *MDR1* 3435 genotype (51). The discordant results with various substrates supports additional studies to understand the role of *MDR1* variation in the disposition of P-glycoprotein substrates.

IMPACT OF *MDR1* GENETIC VARIATION ON EXPRESSION AND FUNCTION OF P-GLYCOPROTEIN IN THE BLOOD-BRAIN BARRIER

Most of the aforementioned studies characterized the impact of *MDR1* genetic polymorphism on intestinal P-glycoprotein expression, which is one of the determinants of drug absorption and has a major impact on pharmacokinetic profiles. A second major site of P-glycoprotein expression and function are the capillary endothelial cells of the blood-brain barrier. Impairment of P-glycoprotein function in the blood-brain barrier was associated with severe neurotoxic side ef-

fects of drugs that can otherwise not cross this border (8,52,53). The 3435T allele was recently shown to be a risk factor for occurrence of nortriptyline-induced postural hypotension, which might be due to increased cerebral concentrations of nortriptyline in these patients (54) (Table IV). Furthermore, increased tacrolimus neurotoxicity in liver transplant patients was associated with the G2677T variant (55), which is in tight linkage disequilibrium with the C3435T variant. Surprisingly, despite this significant linkage disequilibrium, *MDR1* G2677T is a positive predictor whereas C3435T is a negative predictor for the development of tacrolimus neurotoxicity.

A recent study investigated the impact of the C3435T polymorphisms on disposition and brain entry of the P-glycoprotein substrate loperamide, as an indirect measure of P-glycoprotein function in the blood-brain barrier (Table IV). Brain entry was studied by measuring respiratory depression in response to an increased level of CO_2, as previously established in P-glycoprotein chemical inhibition studies (56). No significant differences in loperamide plasma levels or the extent of respiratory depression could be found between the 3435CC and 3435TT genotypes (57). These data indicate that the *MDR1* C3435T polymorphism is not a determinant of disposition and brain exposure of loperamide. In the same study, a post hoc analysis considering the two major *MDR1* haplotypes, *MDR1**1 and *MDR1**13, could not detect haplotype-related differences in loperamide pharmacokinetics or respiratory response (57). This is not surprising, as it was the primary goal of this study to investigate the impact of the C3435T polymorphism on loperamide respiratory response, and therefore the statistical power was not calculated to detect haplotype-related effects. However, the study nicely illustrates the diversity of *MDR*1 haplotypes found in a sample selected for a common *MDR1* polymorphism. Haplotypes were assignable for 13 out of 16 individuals participating in the study, and these 13 individuals carried 9 different haplotypes. These observations further underscore the notion that populations selected based on a particular *MDR*1 polymorphism might still be very heterogeneous, which is a likely explanation for the discrepant finding in different *in vivo* studies.

IMPACT OF *MDR1* GENETIC VARIATION ON DISEASE COURSE

A recent focus has been on the association of *MDR1* genetic variation with disease (Table V). Several conditions have been investigated where an impaired cellular barrier function at the level of the small intestine or the blood-brain barrier is likely to contribute to disease pathogenesis. Although there were no statistically significant associations between the *MDR1* T-129C, G2677T, and C3435T variants and Parkinson's disease, there was a trend toward higher frequency of the 3435TT genotype in early onset Parkinson's disease patients (36.0%) compared to late onset patients (22.9%) and controls (18.9%) (58). It has been hypothesized that the 3435TT patients have lower P-glycoprotein expression and/or an impaired blood-brain barrier function and therefore are more susceptible to neurotoxic xenobiotics. This hypothesis is supported by the finding of a 5-fold increased risk to develop Parkinson's syndrome after exposure to pesticides in patients harboring the 3435T allele (59). Furthermore, patients with drug-resistant epilepsy were more likely to have the 3435CC genotype than the 3435TT genotype, pointing toward a more efficient barrier function in the capillary endothelial cells and around the epileptogenic focus in drug-resistant patients (60). At the intestinal and renal level, patients with ulcerative colitis and non–clear cell renal carcinoma had significantly increased frequencies of the 3435T allele, which again supports a role for P-glycoprotein in maintaining effective tissue barriers and protecting the body from potential environmental and metabolic toxins (61,62).

A possible role of *MDR1* genetic polymorphisms in response to anticancer treatment has also been investigated. In patients with acute myeloid leukemia (AML), homozygosity for the T allele in position 2677 was associated with significantly shorter relapse times and worse survival rates compared to heterozygosity in this position (63). In contrast, an independent study reported lower MDR1 expression, significantly decreased overall AML-survival, and a high probability of relapse in patients with the *MDR1* 3435CC genotype compared to those with the 3435TT genotype (64). Consistent with this study, the 3435C genotype was also associated with resistance to preoperative chemotherapy in locally advanced breast cancer (65)

The importance of P-glycoprotein in the disposition of drugs used in antiretroviral therapy has led to investigation of *MDR1* genetic variation in HIV populations. Though the T-129C, G2677T/A, and C3453T polymorphisms do not influence the risk of HIV infection *per se* (66), the C3435T polymorphism was found to predict immune recovery after initiation of antiretroviral treatment. Maximal immune recovery was observed in patients with the *MDR1* 3435TT genotype, which might reflect enhanced penetration of antiretroviral drugs in these cell populations due to lower cell surface P-glycoprotein expression (50). This is in line with a recent publication describing a trend to earlier virological failure in the *MDR1* 3435CC genotype (67). However, *MDR1* genotype–related differences were not observed in response to antiretroviral therapy in drug-naive HIV-positive patients (68). Though the results of many of these studies in specific disease populations are intriguing, additional studies in large populations with consideration of *MDR1* haplotypes will be necessary before conclusions can be made about the signifi-

Table IV. Impact of *MDR1* Genetic Variation on Function of P-Glycoprotein in the Blood Brain Barrier

Population	Nucleotide/amino acid change	Functional effect of the variant allele	Reference
Patients with major depresssion	C3435T	Increased risk for the development of postural hypotension	[54]
Liver transplant patients	G2677T (Ala893Ser), C3435T	Increased tacrolimus neurotoxicity in 2677T carriers; decreased tacrolimus neurotoxicity in 3435T carriers	[55]
Caucasian volunteers	C1236T + G2677T/A (Ala893Ser/Thr) + C3435T	No difference in CNS effects of loperamide	[57]

Table V. Impact of *MDR1* Genetic Variation on Disease Development and Drug Response

Population	Nucleotide/amino acid change	Functional effect of the variant allele	Reference
Ulcerative colitis	C3435T	Increased susceptibility for ulcerative colitis	[61]
Renal epithelial cell cancer and healthy controls	C3435T	Increased susceptibility for renal epithelial tumor	[62]
Parkinson's disease	C3435T	Trend towards higher frequency of 3435T genotype in early onset Parkinson's disease	[58]
Parkinson's disease	C3435T	Increased susceptibility for pesticide induced Parkinson's syndrome	[59]
Drug resistant epilepsy	C3435T	Lower incidence of drug resistant epilepsy	[60]
AML	G2677T	Shorter relapse time, lower survival rates after chemotherapy for homozygous carriers of the G or the T in this position	[63]
AML	C1236T + G2677T (Ala893Ser) + C3435T	Higher overall survival and longer relapse time	[64]
Breast cancer	C3435T	Decreased resistance to preoperative chemotherapy	[65]
HIV	C3435T	Decreased nelfinavir plasma levels, increased CD4 recovery	[50]
HIV	C3435T	Trend to later virological failure	[67]
HIV	C3435T	No difference in antiretroviral treatment response	[68]

cance of *MDR1* genetic variation on disease development, progression, and response to drug therapy.

SUMMARY

Given its impact on pharmacokinetic and pharmacodynamic effects of drugs, great effort has been applied to identify genetic variation in the *MDR1* gene that might explain interindividual differences in P-glycoprotein expression and function. *MDR1* genetic polymorphisms have been identified in large populations of individuals with different ethnic backgrounds, with sufficient power to detect even relatively uncommon variants. Some of these variants were shown to have an impact on P-glycoprotein expression and function in cancer cells and normal tissue, but for most of these variants, the *in vivo* functional impact remains to be established. To date, the G2677T non-synonymous variant and the synonymous C3435T variant have been associated with a clinical phenotype, but the results of these studies remain controversial. Linkage disequilibrium of the C3435T polymorphism with other coding region variants has underscored the importance of understanding haplotypes to describe function. Recently, extensive haplotype analysis allowed the identification of common haplotypes, which makes it possible to use a haplotype approach for future functional studies. The functional characterization of genetic variation in the *MDR1* gene will provide a very powerful tool to optimize drug therapy for substrates with a narrow therapeutic range, such as cardiovascular or anticancer drugs. It might also be useful to predict therapeutic outcome in certain types of cancer or HIV infection. It is, however, clear that we are in an early stage of defining the pharmacological impact of pharmacogenomic research and that a broader genomic approach will be required to elucidate the impact of genetic variation for most medications. From this perspective, characterization of the functional impact of *MDR1* genetic polymorphisms will add to a network of genes that are involved in drug metabolism, transport, and response, which will make it ultimately possible to more accurately optimize drug response and safety in individual patients.

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