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GENETIC POLYMORPHISMS OF THE HUMAN *MDR1* DRUG TRANSPORTER*

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■ **Abstract** P-glycoprotein is an ATP-dependent efflux pump that contributes to the protection of the body from environmental toxins. It transports a huge variety of structurally diverse compounds. P-glycoprotein is involved in limiting absorption of xenobiotics from the gut lumen, in protection of sensitive tissues (brain, fetus, testis), and in biliary and urinary excretion of its substrates. P-glycoprotein can be inhibited or induced by xenobiotics, thereby contributing to variable drug disposition and drug interactions. Recently, several SNPs have been identified in the *MDR1* gene, some of which can affect P-glycoprotein expression and function. Potential implications of *MDR1* polymorphisms for drug disposition, drug effects, and disease risk are discussed.

OVERVIEW

ABC Transporters

Translocation of endogenous compounds and xenobiotics across biological membranes not only occurs via passive diffusion, but also by carrier-mediated processes. In recent years, multiple transporter genes and proteins have been identified. Knowledge about cellular and tissue-specific transporter expression, as well as characterization of substrates of individual transporters, leads to better understanding of the role of these proteins for physiological processes and elimination of xenobiotics. The ATP-binding (ABC) superfamily is a large family of

*Abbreviations used in text: ALL, acute lymphoblastic leukemia; CD, Crohn's disease; MDR, multidrug resistance; PBMC, peripheral blood mononuclear cells; SNP, single nucleotide polymorphism; UC, ulcerative colitits.

different transporters expressed in a broad variety of organisms (1). In humans, 48 ABC transporters have been identified (2). ABC transporters are located in the plasma membrane or in intracellular membranes. Conserved ABC motifs (Walker A and Walker B) are involved in ATP binding and hydrolysis (3). In general, ABC superfamily members transport a wide range of substances including ions, sugars, amino acids, glycans, peptides, proteins, phospholipids, toxins, and drugs (1). Human ABC transporters are divided into seven subfamilies, each containing several individual members. These subfamilies are ABC1 (12 members), MDR/TAP (11 members), MRP/CFTR (12 members), ALD (4 members), OABP (1 member), GCN20 (3 members), and White [5 members; (1); http://nutrigene.4t.com/humanabc.htm]. Proteins of the MDR/TAP and MRP/CFTR families appear to be most relevant for disposition of xenobiotics in humans.

THE *MDR1* GENE AND ITS PRODUCT P-GLYCOPROTEIN

The *MDR1* gene (ABCB1) product P-glycoprotein is probably one of the most important ABC transporters for drug disposition in humans. It is assumed to be a protective mechanism against potentially toxic xenobiotics that are ingested with the diet. It was first described in tumor cells where it contributed to the phenomenon of MDR against anticancer agents (4). The *MDR1* gene is located on chromosomal region 7q21 and consists of 28 exons, encoding a 1280–amino acid transporter (~170 kDa). It has two homologous halves, each containing six transmembrane domains and an ATP binding site (5, 6). It is currently assumed that P-glycoprotein acts as a "flippase" or a "hydrophobic vacuum cleaner" that removes its substrates from the membrane lipid bilayer into the extracellular space (6, 7). Mutational analysis was intensively used to investigate P-glycoprotein structure-function relationships. In particular, multiple mutations in *MDR1* regions encoding for transmembrane domains 5, 6, 11, and 12 alter P-glycoprotein substrate specificity (1, 6). However, mutations in *MDR1* regions encoding for other domains such as intra- or extracellular loops or ATP binding sites also alter the transporter's substrate specificity (1, 6).

Substrates of P-glycoprotein

P-glycoprotein transports a wide range of substances with diverse chemical structures, among them anticancer agents, cardiac drugs (e.g., digoxin, quinidine), HIV protease inhibitors, immunosuppressants (e.g., cyclosporine), and ß-adrenoceptor antagonists. A summary of important drugs transported by P-glycoprotein is given in Table 1. Attempts to predict P-glycoprotein-mediated transport of a substance from its chemical structure have not been particularly successful. However, P-glycoprotein substrates in general appear to be lipophilic and amphiphatic (6). Interestingly, most substrates of P-glycoprotein are also metabolized by the major drug metabolizing enzyme CYP3A4 (8). This is of particular importance because

TABLE 1 List of clinically important drugs that are substrates of human P-glycoprotein

Drug	Reference
actinomycin D	(82)
amprenavir	(63)
atorvastatin	(83)
bunitrolol	(84)
celiprolol	(85)
colchicine	(86)
cyclosporine A	(87)
daunorubicin	(88)
dexamethasone	(15)
digitoxin	(89)
digoxin	(15)
diltiazem	(90)
docetaxel	(91)
domperidon	(52)
doxorubicin	(88)
erythromycin	(92)
etoposide	(52)
fexofenadine	(59)
indinavir	(60)
irinotecan	(93)
levofloxacin	(94)
loperamide	(52)
losartan	(95)
lovastatin	(13)
mitomycin C	(96)
mitoxantrone	(96)
morphine	(15)
nelfinavir	(60)
ondansetron	(52)
paclitaxel	(97)
phenytoin	(52)
quinidine	(16)
rifampin	(98)

(*Continued*)

TABLE 1 (*Continued*)

Drug	Reference
ritonavir	(61)
saquinavir	(60)
sparfloxacin	(99)
tacrolimus	(100)
talinolol	(101)
teniposide	(96)
topotecan	(96)
verapamil	(102)
vinblastine	(103)
vincristine	(104)

P-glycoprotein and CYP3A4 are colocalized in tissues with major function for drug disposition, such as small intestine and liver. CYP3A4 and P-glycoprotein work in these tissues in a coordinate fashion in order to prevent entry of orally ingested xenobiotics into the body (9–12). However, it should be noted that the overlap in substrate specificity between P-glycoprotein and CYP3A4 is not complete. For example, nifedipine and midazolam, well-known substrates of CYP3A4, are not transported by human P-glycoprotein (13).

Drug Interactions Involving P-glycoprotein

Traditionally, most drug-drug interactions have been attributed to inhibition or induction of drug metabolizing enzymes. It is now well established that modification of P-glycoprotein function by concomitantly administered drugs is another important mechanism of drug-drug interactions. For example, in the late 1970s, it was shown that administration of the antiarrhythmic quinidine significantly increases digoxin serum concentrations in humans (14). Because digoxin is not metabolized to a major extent in humans, the mechanism underlying this interaction remained unclear. However, recent studies identified digoxin as a P-glycoprotein substrate and quinidine as a potent inhibitor of P-glycoprotein function (15, 16). On the other hand, induction of P-glycoprotein by rifampin or St. John's wort results in decreased plasma concentrations of its substrates [e.g., digoxin, talinolol; (17–19)]. Because most drugs are substrates of both CYP3A4 and P-glycoprotein and because inhibitors or inducers of P-glycoprotein in most cases also affect CYP3A4 function, the majority of drug-drug interactions are due to alteration of both CYP3A4 and P-glycoprotein function. Whereas factors determining basal promoter activity of *MDR1* are poorly characterized (1, 20), it

is now established that rifampin-mediated induction of P-glycoprotein occurs by a DR4 motif in the upstream enhancer at approximately −8 kb pairs, to which the nuclear receptor PXR binds (21).

GENETIC VARIABILITY

The first evidence on structural variability of the *MDR1* locus was obtained by screening a large number of samples including normal tissues, unselected, and drug-selected cell lines by RNase protection and subsequent sequencing analysis (22). Two SNPs in exon 21 (G2677T) and exon 24 (G2995A) were identified, of which the single base pair mismatch at position 2677 results in an amino acid change from Ala to Ser (23, 24). A first systematic screen of the *MDR1* gene for the presence of additional SNPs was performed by Hoffmeyer et al. (25). All 28 exons, including the core promotor region and exon-intron boundaries, were sequenced using specific oligonucleotide primers for amplification by PCR derived from the original *MDR1* sequence (Gene Bank accession #AC002457 and #AC005068), which was defined as wild-type sequence. Of the 15 identified SNPs, three polymorphisms resulted in protein alterations, one in exon 2 (Asn21Asp), in exon 5 (Phe103Leu), and in exon 11 (Ser400Asn). Seven synonymous mutations are located in introns 4, 6, 12, 16, and 17, close to exon-intron boundaries, and three SNPs at wobble positions with no amino acid changes [one in exon 12 (C1236T) and two in exon 26 (C3435T, C3396T)]. The SNPs at positions A61G (Asn21Asp), C1236T, and C3435T had been reported previously (23, 26). Subsequently, an ethnic screen of 461 German Caucasians for allele and genotype distribution revealed two further rare mutations [2677A (893Thr), 3320C (1107Pro); (27)]. Further SNPs of the *MDR1* gene were identified in Asians, an A to G transversion 41 bases upstream from the initial position of exon 1a (A-41aG) and a C to G transversion at −145 in exon 1a (C-145G) (28), as well as three nonsynonymous mutations A548G (Asn183Ser), C1474T (Arg492Cys), and T3421A (Ser1141Thr) in different ethnic populations (29, 30). Table 2 summarizes all mutations identified until now. Different genotyping methods, conventional PCR-based (RFLP, SSCP) (25, 27–29) and highly automated screening techniques (DHPLC, Light Cycler^{TM}) (31, 32), were established and recently reported.

Detailed linkage disequilibrium analysis of the different SNPs has not yet been described, but haplotype assignment can be performed in part when subjects who are homozygous at a single polymorphic site or multiple sites are identified. Thus, a cosegregation of the silent mutation 3435T in 62% with the T allele of the nonsynonymous exon 21 SNP 2677T and the T allele of the synonymous exon 12 polymorphism T1236C was reported for European Americans (29). Additionally, in a Japanese population ($n = 65$) a strong association, 93.8% ($n = 61$), was reported using placental cDNA between the 3435T allele and the 2677A and T alleles (33). In a Northern Italian population, the extent of linkage disequilibrium

TABLE 2 Summary of *MDR1* genetic variants in different ethnic groups

Location	Position	Allele	Effect	Reference
promotor	5′flanking/−41a	A G		(28)
exon 1a	exon 1a/−145	C G		(28)
exon 1b	exon 1b/−129	T C		(25, 33)
intron 1	exon 2/−4	C T		(29)
intron 1	exon 2/−1	G A	initiation of translation	(25, 27, 29)
exon 2	exon 2/61	A G	Asn21Asp	(25–27, 29)
intron 4	exon 5/−35	G C		(25)
intron 4	exon 5/−25	G T		(25)
exon 5	exon 5/307	T C	Phe103Leu	(25)
intron 6	exon 6/+139	C T		(25, 27)
intron 6	exon 6/+145	C T		(25)
exon 7	exon 7/548	A G	Asn183Ser	(29)
exon 11	exon 11/1199	G A	Ser400Asn	(25, 27, 29)
exon 12	exon 12/1236	C T	wobble (Gly412Gly)	(23, 25, 27, 29)
intron 12	exon 12/+44	C T		(25, 27)
exon 13	exon 13/1474	C T	Arg492Cys	(29)
intron 16	exon 17/−76	T A		(25, 27)
intron 17	exon 17/137	A G		(25)
exon 21	exon 21/2650	C T	wobble (Leu884Leu)	(29)

(*Continued*)

TABLE 2 (*Continued*)

Location	Position	Allele	Effect	Reference
exon 21	exon 21/2677	G		(22, 23, 27, 29)
		T	Ala893Ser	
		A	Ala893Thr	
exon 24	exon 24/2956	*A*	Met986Val	(33)
		G		
exon 24	exon 24/2995	*G*	Ala999Thr	(22)
		A		
exon 26	exon 26/3320	A	Gln1107Pro	(27)
		C		
exon 26	exon 26/3396	C	wobble	(25)
		T		
exon 26	exon 26/3421	T	Ser1141Thr	(29, 30)
		A		
exon 26	exon 26/3435	C	wobble	(23, 25, 29)
		T	(Ile1145Ile)	
exon 28	exon 28/4030	G		(33)
		C		
exon 28	exon 28/4036	A		(23, 33)
		G		

The positions of the polymorphisms correspond to positions of *MDR1* cDNA with the first base of the ATG start codon set to 1 (GenBank accession # M14758). Mutations located in introns are given as position downstream (−) or upstream (+) of the respective exon according to the genomic organization of *MDR1* as described by Chen et al. (105).

between 3435TT subjects and 2677TT carriers appeared to be somewhat less tight (73.3%) (34a). Taken together, the knowledge of the haplotype structure across the entire *MDR1* gene in different populations is of major interest and could shed light on the mechanisms to identify associations between polymorphism represented by each haplotype and expression and function of P-glycoprotein. To take this issue into account, the first *MDR1* nomenclature was proposed by Kim et al. (29). Very recently, a first haplotype profiling using computational algorithms (Arlequin software) has been reported for three ethnic Asian populations (Chinese, Malays, Indians) with regard to four polymorphic sites (34a). These data indicate a strong linkage disequilibrium between the silent 3435 SNP and an unobserved causal SNP, which underlies the observed association between the 3435 polymorphism and functional consequences.

Potential functional consequences of *MDR1* polymorphisms can be deduced from their location within the *MDR1* gene in relation to the domain structure of P-glycoprotein. The A61G mutation (Asn21Asp) results in a net charge change (basic to acidic) close to the N-terminus of P-glycoprotein, which appears to be of minor functional importance if recombinant mutational analyses of P-glycoprotein

are considered (6). The protein alteration Phe103Leu in exon 5 is located next to the second transmembrane domain on the extracellular side of P-glycoprotein and is in close vinicity to glycosylation sites of P-glycoprotein. The change from a large aromatic to a large lipophilic residue may contribute to a structural alteration of protein by disturbing the side chain packing. The nonsynonymous G1199A SNP in exon 11 (Ser400Asn) results in a significant size change dependent on pH and isoelectric environment of the residue, leading possibly to a charge change in the protein. This SNP is located on the cytoplasmatic side just preceding the first ATP-binding domain. Both polymorphisms, G2677T/A and G2995A, resulting in amino acid exchanges in exon 21 (Ala893Ser/Thr) and exon 24 (Ala999Thr), respectively, are located in the second transmembrane domain, the exon G2995A polymorphism closer to the ABC domain. For Ser893, it can be supposed that certain serine residues in P-glycoprotein are subject to phosphorylation by protein kinase C, resulting in altered protein function (35). In fact, a different multidrug resistance pattern in AdrR MCF-7 cells with Ser893 substitution was described (24). Finally, the C3435T SNP at a wobble position in exon 26 does not alter its encoded Ile amino acid (Ile1145Ile) and is therefore of apparent silent nature. Nevertheless, this polymorphism was associated with altered P-glycoprotein expression and function (see below). The molecular basis of this observation is still poorly understood, but the following mechanisms are conceivable (34a). First, a linkage of the C3435T SNP to other, so far unidentified, mutations elsewhere within the *MDR1* gene, e.g., in the promoter/enhancer or intronic regions, or in another nearby gene can be assumed. Second, silent mutations may yet alter downstream mRNA splicing by allele-specific differences in RNA folding (36) resulting in disrupted exon skipping, a mechanism that has so far probably been vastly underestimated (37). Third, further mechanisms, such as alteration of RNA processing (38) or of translational control, as well as regulatory processes have been described to influence protein expression/function (39, 40a). Finally, a reduced translation efficiency has been discussed to explain how a silent mutation may have functional consequences. In the case of the 3435T allele, an Ile codon is created that is infrequently utilized in the human genome (http://iubio.bio.indiana.edu/soft/molbio/codon/hum.cod or http://www.kazusa.or.jp/codon/).

The only data for retroviral expression of genetic variants of MDR1 are available for the G2677T polymorphism using NIH3T3 GP+E86 cells stably transduced with MDR1-Ser893 or MDR1-Ala893 compared to untransduced cells (29). Whereas P-glycoprotein expression was found to be similar, after incubation with digoxin intracellular concentration was 47% lower for the Ser893 variant than for Ala893, which implies an enhanced efflux transporting ability of the MDR1 Ser893. However, a recent publication characterized the functional consequences of five coding SNPs (Asn21Asp, Phe103Leu, Ser400Asn, Ala893Ser, Ala999Thr) using a vaccinia virus–based transient expression system (40a). Interestingly, cell surface expression and function was not altered even in some common double polymorphisms. The reasons for the contradictory findings between these two publications regarding the G2677T polymorphisms are unclear at the moment (29, 40a).

INTERETHNIC VARIABILITY

Geographic, ethnic, and racial differences in the frequency of variant alleles provide a mechanistic basis for at least some of the observed differences in pharmacokinetics and/or drug effect or toxicity between populations.

Significant ethnic differences exist in the frequency of allele and genotype distribution of the C3435T polymorphism of *MDR1*. Whereas in European and American Caucasians the frequency of individuals homozygous for the C and T allele, respectively, is approximately 25% for each genotype, in Africans the TT genotype has only a frequency of up to 6%. Table 3 summarizes interethnic differences observed for the C3435T SNP in various populations. Data on the allele

TABLE 3 Ethnic distribution of allele and genotype frequencies of the *MDR1* exon 26 SNP C3435T

		Allele frequency		Genotype frequency			
Population	**n**	**C**	**T**	**CC**	**CT**	**TT**	**Reference**
CAUCASIANS							
German Caucasian	188	0.52	0.48	0.28	0.48	0.24	(25)
German Caucasian	461	0.46	0.54	0.21	0.51	0.29	(27)
German Caucasian	537	0.50	0.50	0.26	0.48	0.26	(106)
Caucasian, UK	190	0.48	0.52	0.24	0.48	0.28	(107)
European American	37	0.46	0.54	n.a.	n.a.	n.a.	(29)
Portuguese	100	0.43	0.57	0.22	0.42	0.36	(107)
Northern Italian	106	0.54	0.46	0.26	0.55	0.19	(34)
EAST ASIAN							
Japanese	50	0.57	0.43	0.34	0.46	0.20	(106)
Japanese	100	0.58	0.42	0.35	0.46	0.19	(33)
Japanese	114	0.61	0.39	0.35	0.53	0.12	(51)
Chinese	132	0.53	0.47	0.32	0.42	0.26	(107)
Filipino	60	0.59	0.41	0.38	0.42	0.20	(107)
Saudi	96	0.55	0.45	0.37	0.38	0.26	(107)
SOUTH ASIAN							
Southwest Asians	89	0.34	0.66	0.15	0.38	0.47	(107)
AFRICANS							
Ghanaian	206	0.83	0.17	0.67	0.34	0.00	(107)
Ghanaian	172	0.90	0.10	0.83	0.16	0.02	(106)
Kenyan	80	0.83	0.17	0.70	0.26	0.04	(107)
Sudanese	51	0.73	0.27	0.52	0.43	0.06	(107)
African American	88	0.84	0.16	0.68	0.31	0.01	(107)
African American	41	0.78	0.22	0.61	0.34	0.05	(106)
African American	23	0.74	0.26	n.a.	n.a.	n.a.	(29)

n.a.: data not available.

frequency for other SNPs are limited. In a German population, the 2677T allele was observed in 42% (27) and in Japanese 41.7% (33), whereas in African Americans, the frequency is only 13% (29). Additionally, the frequency of Caucasians homozygous for the wobble mutation at position 1236 (1236TT) is about one-third of the value in Japanese (13.3% versus 37.5%) (25, 28). Moreover, as described above, several mutations are only identified in single ethnic populations. For example, the nonsynonymous SNP at position 3421 was only found in Ghanaians (1.2%) and African Americans (2.4% and 4.3%, respectively), but not in Caucasians (29, 30).

Whether these differences are of any functional relevance remains to be determined. In the case of the C3435T polymorphism in which the T allele is associated with reduced P-glycoprotein expression [see below; (25)], a selective advantage of the CC genotype can be supposed because P-glycoprotein plays an important role in defense against several toxins including bacterial and viral particles. Overdominance of a genotype as a consequence of natural selection by infectious diseases has been demonstrated for other polymorphisms, such as the glucose-6-phosphate dehydrogenase gene (41). In the case of P-glycoprotein, it can be assumed that the much higher frequency of the P-glycoprotein high expression 3435CC genotype in Africans compared to Caucasians or Japanese may be protective for developing gastrointestinal-tract infections, which are endemic in tropical countries.

These interethnic variabilities could have an impact on drug disposition. For example, drug disposition of cyclosporine and tacrolimus is mainly affected by CYP3A4 and P-glycoprotein. Moreover, intestinal P-glycoprotein has been shown to determine oral clearance of cyclosporine (42). Comparison of pharmacokinetics of oral cyclosporine in Black and White renal transplant recipients showed a significantly lower bioavailability of cyclosporine in Blacks (mean 30.9%) than in Whites or Hispanics [mean 39.6% ($p = 0.0009$) and 42.1% ($p = 0.0003$), respectively] (43). Furthermore, the mean dosage requirement of the immunosuppressant tacrolimus was 96% higher in Black recipients compared to Whites or Asians ($p < 0.001$) (44). Racial differences in the clearance of cyclosporine as well as tacrolimus were also found among healthy African Americans and White volunteers (45, 46). In this context, it is interesting to note that several studies indicate a poorer outcome for Africans after renal transplantation than for White patients, although the immunosuppressive therapy including cyclosporine or tacrolimus is similar. Hence, the high frequency of the high expression *MDR1* genotype CC in Africans/African Americans versus Caucasians may be of clinical relevance.

MDR1 POLYMORPHISMS AND P-GLYCOPROTEIN EXPRESSION IN HUMANS

P-glycoprotein Expression

The only polymorphism identified so far that affects P-glycoprotein expression in different human tissues is the silent mutation at position 3435 in exon 26 (C3435).

P-glycoprotein expression levels in upper small intestine of healthy volunteers and patients were determined by quantitative immunohistochemistry and Western blot analysis (17, 25). Carriers homozygous for the T-allele had on average more than twofold lower intestinal MDR1 expression levels compared to the CC genotype. Additionally, the subject with the lowest and highest intestinal P-glycoprotein level had the TT and CC genotype, respectively. A similar relationship between P-glycoprotein expression in human kidney and the C3435T polymorphism was found using quantitative immunohistochemistry. Subjects with the TT genotype had on average a significantly (1.5-fold) lower P-glycoprotein expression compared to the CC genotype group ($p = 0.0065$) (47). The specific expression of P-glycoprotein in placenta of women from Japan was significantly correlated to the T-129C mutation (allele frequency: 8.3%) in exon 1b with twofold lower expression levels in heterozygous samples than in homozygous TT subjects ($p = 0.002$) (33). A trend was observed for the polymorphism at position 2677, with lowest P-glycoprotein expression levels in homozygous mutant individuals, intermediate expression in heterozygous subjects, and highest in the wild-type group. With respect to the C3435T SNP, a similar (nonsignificant) trend was reported (CC > CT > TT), however, with a large standard deviation for each genotype group.

MDR1 mRNA Expression

On the level of total cellular MDR1 mRNA expression obtained from PBMC, two independent studies confirmed an association toward lower values in 3435TT subjects as compared to higher levels in individuals with the CT and CC genotype. Whereas these results were not statistically significant in healthy volunteers [$p = 0.33$; (31)], patients with HIV-1 infection before commencing antiretroviral therapy, who were carriers of the 3435TT genotype, showed significantly lower mRNA levels compared to heterozygotes and subjects homozygous for CC ($p = 0.02$) (48). These findings were confirmed by fluorescence-activated cell-sorter analysis of P-glycoprotein expression in PBMCs. In contrast to these studies, mRNA quantification in duodenal enterocytes from 13 healthy Japanese individuals showed higher mean expression levels of MDR1 mRNA in homozygous carriers for 3435T as compared to subjects with a CT or CC genotype (49). Assuming a linkage disequilibrium between the C3435T polymorphism and the genetic variant G2677T, these findings corroborate the in vitro results of retroviral expression of the Ser893 variant (2677T) by Kim et al. (29) leading to an enhanced activity of P-glycoprotein. Consequently, racial differences in the relation between C3435T and C2677T should be considered as a possible explanation for seemingly contradictory findings. Furthermore, tissue specific expression of MDR1 mRNA cannot be completely ruled out as an additional mechanism for different results in PBMCs and intestinal mucosa.

MDR1 POLYMORPHISMS AND P-GLYCOPROTEIN FUNCTION IN HUMANS

Several studies addressed the association of *MDR1* genotypes with disposition of P-glycoprotein substrates in humans. These investigations were based on the initial observation that *MDR1* genotype 3435TT is associated with a lower intestinal P-glycoprotein expression in humans in comparison to individuals with the 3435CT or CC genotypes (25). Thus, in individuals with lower intestinal P-glycoprotein concentration the extent of drug absorption from the gastrointestinal (GI) tract should be higher and result in increased plasma levels in comparison to the remainder of the population. Accordingly, Hoffmeyer et al. (25) detected significantly higher maximum digoxin plasma concentrations in seven healthy volunteers with the 3435TT genotype (+38%), compared to seven individuals with the 3435CC genotype during steady-state conditions (0.25 mg/day). Similarly, a reduced digoxin oral clearance (−26.6%) was reported for Korean patients with the one or two T alleles at position 3435 using a population pharmacokinetic approach (50). However, administration of a single oral dose of digoxin to healthy Japanese subjects resulted in a lower AUC_{0-4h} (−20.4%) in individuals with the 3435TT genotype compared to the CT and CC groups (51). The antiepileptic drug phenytoin is primarily metabolized by polymorphic CYP2C9. Moreover, it is transported to some extent by P-glycoprotein (52). Accordingly, Kerb et al. (53) identified the *CYP2C9* genotype as a major determinant of phenytoin disposition in humans. In addition, the *MDR1* 3435 CC genotype was more common in volunteers with low phenytoin plasma concentrations ($p \leq 0.01$).

So far, no clear relationship was observed between the *MDR1* genotype and the disposition of other P-glycoprotein substrates [e.g., the antihistaminic drug fexofenadine; (29, 54, 55)]. Moreover, *MDR1* 3435 polymorphism was not associated with altered disposition of cyclosporine, talinolol, and loperamide (56–58). An interesting finding was also reported for nelfinavir plasma concentrations in relation to the *MDR1* genotype (48). In patients on antiretroviral therapy, nelfinavir plasma concentrations (but also plasma concentrations of the non-P-glycoprotein substrate efavirenz) were highest with the CC genotype compared to the CT and TT groups. In the latter case, it was speculated that an indirect effect of the *MDR1* genotype on nelfinavir disposition could explain these findings (48). For example, low intestinal P-glycoprotein expression increases concentrations of nelfinavir in enterocytes, subsequently leading to induction of other intestinal transporters and/or drug metabolizing enzymes (CYP3A4).

Taken together, in spite of several studies relating the 3435T polymorphism with low tissue expression of P-glycoprotein, there is not a clear trend toward higher plasma concentrations of all P-glycoprotein substrates investigated so far. This could be due to the following confounding factors. (*a*) The effect of the *MDR1* 3435 polymorphism on P-glycoprotein tissue levels is rather modest (approximately twofold). (*b*) Disposition of most P-glycoprotein substrates is also determined by other factors, such as metabolism (e.g., nelfinavir or cyclosporine

metabolism via CYP3A4) or active transport [e.g., fexofenadine uptake via OATP-A; (59)]. (*c*) In addition, modification of drug disposition may occur by exogenous factors (e.g., diet, drugs), which could explain different results, even if the same drug is investigated. (*d*) Due to the presence of multiple SNPs in the *MDR1* gene and pronounced interethnic differences in frequencies of some of these polymorphisms (see above), a detailed haplotype analysis should be performed, rather than determination of one SNP. (*e*) Not all pharmacokinetic parameters are likely to be determined to a major extent by modulation of intestinal P-glycoprotein expression [AUC for the first few hours after oral drug administration or C_{max} are preferable over trough concentrations; (42, 56)].

In accordance with the above-mentioned lower MDR1 mRNA and P-glycoprotein expression in lymphocytes (31, 48), ex vivo investigations using $CD56^+$natural killer cells revealed a significantly lower P-glycoprotein function in cells obtained from healthy volunteers with the 3435TT genotype in comparison to the CT and CC groups ($p = 0.0015$) (31). The potential relevance of these findings is discussed in the following paragraph.

MDR1 POLYMORPHISMS AND CLINICAL OUTCOME

HIV

All currently marketed HIV protease inhibitors are P-glycoprotein substrates (60–63). Studies with P-glycoprotein knockout mice revealed that intestinal P-glycoprotein has a major impact on bioavailability of HIV protease inhibitors (60). P-glycoprotein expressed in the blood-brain or blood-testis barriers limited accumulation of these drugs in the CNS and testis, respectively (60, 64). The presence of a substantial barrier to the drugs' distribution into these tissues suggests that the ability to achieve therapeutic brain or testis concentrations is limited, creating a potential sanctuary for virus replication. A similar scenario is possible for $CD4^+$ cells, which are a major target of the HIV virus. In lymphocytes, P-glycoprotein expression is highly variable, with approximately 25% of $CD4^+$ cells displaying considerable activity (65). Interestingly, penetration and antiviral activity of indinavir, saquinavir, and ritonavir are diminished in HIV-1 infected cells with high P-glycoprotein expression (62). Moreover, *MDR1* genotype–related differences in P-glycoprotein function have been described in $CD56^+$ natural killer cells using rhodamine efflux. The 3435 TT genotype was associated with a significantly reduced P-glycoprotein function in $CD56^+$ natural killer cells as well as reduced P-glycoprotein expression in PBMCs (31, 48). Because P-glycoprotein is expressed in $CD4^+$ subpopulations, intracellular concentrations of HIV protease inhibitors and antiretroviral efficacy are affected by variable P-glycoprotein expression. Indeed, in a recent study it was shown for the first time that *MDR1* genotype was significantly related to response to antiretroviral treatment. The 3435 TT genotype was associated with a significantly greater increase in CD4-cell count and a trend toward a more pronounced decrease in viral load compared to patients

with the 3435 CT or CC genotypes six months after antiretroviral therapy was started (48). It is important to determine whether this effect persists for a longer period than six months in order to investigate particular antiretroviral regimens containing different HIV protease inhibitors, which are affected by *MDR1* genotype, and to prove that genotyping of the patients before initiation of treatment will help to further improve the outcome in HIV infected patients (e.g., by additional treatment with selective P-glycoprotein inhibitors).

Childhood ALL

P-glycoprotein is an essential part of the blood-brain barrier. Several P-glycoprotein substrates (doxorubicin, vincristin, etoposide) are used in treatment protocols for childhood ALL. In spite of considerable progress in treatment of childhood ALL, central nervous system relapses still occur in approximately 3%–5% of the patients. The *MDR1* genotype could contribute to a patient's risk for the development of CNS relapse due to a potentially lower P-glycoprotein expression in the blood-brain barrier of individuals with the 3435 TT genotype. Therefore, a better CNS penetration of some anticancer agents may occur in spite of similar plasma concentrations (66). In accordance with this hypothesis, patients (at intermediate or high risk for treatment failure) with the 3435 CT or TT genotype had a significantly lower rate of CNS relapse compared to the CC group (66).

Nortriptyline-Induced Postural Hypotension

Data obtained with P-glycoprotein knockout mice indicate that nortriptyline may be a P-glycoprotein substrate (67). Accordingly, Roberts et al. (68) tested the hypothesis whether side effects of the antidepressant nortriptyline might be associated with the *MDR1* 3435 polymorphism. Indeed, the presence of two T alleles at position 3435 of the *MDR1* gene was associated with a higher risk for the occurrence of postural hypotension in nortriptyline-treated patients in comparison to patients with the 3435 CC or CT genotype (68).

MDR1 POLYMORPHISMS AND DISEASE SUSCEPTIBILITY

Although the physiological role of P-glycoprotein is not fully understood, it is conceivable that transporter proteins, such as P-glycoprotein, prevent intracellular accumulation of potentially toxic substances and metabolites (69). Because the exposure of epithelial cells depends on the entry and efflux of xenobiotics via transporter proteins, it is plausible to hypothesize that genotype-dependent P-glycoprotein expression may contribute to a certain disease susceptibility. This may either indicate that *MDR1* as marker polymorphism causes disease or that this marker is closely linked to a disease locus.

Renal Epithelial Tumor

Except for hereditary diseases (e.g., von Hippel-Lindau syndrome), genetic factors contributing to the development of renal epithelial tumors remain elusive. Because P-glycoprotein mediates active secretion of its substrates into urine and may be involved in the clearance of carcinogens or water-soluble metabolites via the brush border of the proximal tubular lumen, P-glycoprotein may have a protective role as a renal biological barrier protein. In a first case-control study including patients with clear cell renal cell carcinoma (CCRCC; $n = 179$) and non-CCRCC ($n = 33$), a significant association between the 3435T allele frequency and the occurrence of tumors was found [$p = 0.007$; (47)]. Additionally, a second case-control study established the T-allele as a risk factor, especially for non-CCRCC (OR 2.3, $p = 0.0005$), with the highest risk for homozygote TT carriers (OR 21.7, $p < 0.001$). These data indicate that different expression levels of P-glycoprotein may be associated with the susceptibility to develop rare renal epithelial tumors by virtue of the C3435T polymorphism.

Inflammatory Bowel Disease

To date, the exact etiology of IBD, CD, and UC is still unknown. There is evidence that microbial and genetic factors as well as the immune system play a major role in pathogenesis of IBD (70, 71). Genome-wide screening and candidate-gene analysis have been used to search for IBD-susceptibility genes. Although UC and CD share some of these genes, separate genes appear to be associated with disease severity, extent steroid response, and steroid requirements in patients with UC (70).

Evidence for the role of microbial factors in the pathogenesis of UC comes from experimental animal models of intestinal inflammation (72), but the majority of IBD mouse models are very susceptible to immunological dysregulation due to cytokine imbalance or T cell defect (72). Recent findings with *mdr1a*-P-glycoprotein deficient mice (mdr1a−/−), which are immunologically normal, indicate that an intestinal epithelial barrier defect may contribute to spontaneously severe colitis, which resembles human UC (73, 74). The development of spontaneous colitis in *mdr1a*−/− mice could be prevented when treated with oral antibiotics. Based on the assumption that interindividual variability of intestinal P-glycoprotein expression is linked to the *MDR1* C3435T SNP in exon 26 and the TT-genotype is associated with significantly lower intestinal P-glycoprotein expression, the hypothesis of whether this genetic variant may predispose to UC was tested (75). Compared to sex-matched controls, the 3435T allele frequency was significantly higher in UC patients ($n = 149$; 56.7% versus 48.3%; $p = 0.049$; OR 1.4; 95% CI, 1.02–1.94). Interestingly, no differences were found between CD patients and controls ($n = 126$; $p = 0.66$; OR, 0.9; 95% CI, 0.6–1.3). An overrepresentation of patients homozygous for TT (30.9% versus 22.8%, $p = 0.045$) was likewise found only for UC patients, with a twofold increased overall risk (OR 2.03; 95% CI, 1.04–3.95). The higher frequency of both the 3435T allele and TT genotype in patients with UC corroborates the experimental findings from the *mdr1a*-knockout

mouse model. The results support the notion that P-glycoprotein plays an important role in the defense against intestinal bacteria and suggest that the *MDR1* gene is associated with susceptibility to develop UC.

Parkinson's Disease

Epidemiological studies suggest that both genetic and environmental factors (e.g., neurotoxic xenobiotics) play a role in the development of Parkinson's disease (PD) (76). Therefore, host factors that contribute to variability in uptake and distribution of xenobiotics in the brain can be expected to modulate individual risk, especially because P-glycoprotein has been characterized as a neuroprotective barrier protein in endothelial cells of brain capillaries (77). In a pilot case-control study investigating *MDR1* polymorphisms in relation to the risk for development of PD, the frequency of the 3435 TT genotype was highest in the early-onset PD group (36.0%), second highest in the late-onset PD group (22.9%), and lowest in controls [18.9%, $p = 0.08$; (34a)]. These data provide some evidence that the *MDR1* polymorphism, via altered P-glycoprotein expression in the blood-brain barrier would affect the intracellular concentrations of potentially neurotoxic substances leading to an increased susceptibility factor for PD and/or lead to earlier onset of disease symptoms.

SUMMARY AND FUTURE PERSPECTIVES

P-glycoprotein plays a major role in drug disposition and drug efficacy by providing a barrier for the entry of orally ingested compounds into the body as well as controlling their rate of transfer into different tissues. So far, several SNPs have been identified in the *MDR1* gene that might alter P-glycoprotein expression and function in humans. In contrast to the extensive knowledge of molecular mechanisms of polymorphically expressed drug metabolizing enzymes (e.g., cytochrome P450 2D6), only limited data are available to explain contradictory findings of the observed functional differences when *MDR1* mutations were correlated with P-glycoprotein expression and/or function. Although the data indicate that intestinal P-glycoprotein expression is affected on average twofold by a certain *MDR1* genotype [C3435T; (25)], pronounced intersubject variability exists (up to 10-fold). Moreover, there is an apparent discrepancy in approximately 5%–10% of individuals who are homozygous for the putative P-glycoprotein high (or low) expression genotype 3435CC (or TT) but show a decreased (or increased) P-glycoprotein expression/function (25, 31). The reasons for these observations are unknown; however, it can be speculated that a strong linkage disequilibrium of the wobble mutation C3435T exists with a so far unidentified SNP. Additionally, confounding factors, such as environmental alterations (e.g., dietary salt exposure) (78, 79) as well as comedications [e.g., St John's wort, rifampin; (17, 19)], can modify P-glycoprotein expression, especially if surgical specimens obtained from patients were used for phenotype-genotype correlation studies. Interindividual differences

in transcriptional control of human *MDR1* (20, 21), including genetic variants of transcription factors (e.g., PXR) (80, 81), may also contribute to variability of P-glycoprotein expression.

Future studies need to elucidate the molecular mechanisms of the association of certain *MDR1* genotypes with altered P-glycoprotein expression and function. Moreover, more detailed studies relating *MDR1* haplotypes with P-glycoprotein expression and function will help to improve our understanding of variable P-glycoprotein function. Finally, a better knowledge of the major impact of environmental factors as well as interethnic differences in P-glycoprotein function is necessary for investigations on an association of *MDR1* genotype with P-glycoprotein expression, drug disposition, and risk for certain diseases.

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