Expert Opinion





# Single nucleotide polymorphisms in human P-glycoprotein: its impact on drug delivery and disposition

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Drug efflux pumps belong to a large family of ATP-binding cassette transporter proteins. These pumps bind their substrate and export it through the membrane using energy derived from ATP hydrolysis. P-glycoprotein, the main efflux pump in this family, is expressed not only in tumour cells but also in normal tissues with excretory function (liver, kidney and the intestine). It has a broad specificity of substrates and plays an important role in drug delivery and disposition. Recently, genetic screening of P-glycoprotein has yielded multiple single nucleotide polymorphisms, which seem to alter transporter function and expression. This review discusses the various polymorphisms of this gene and its impact on drug disposition and diseases.

Keywords: drug efflux, drug transport, multi-drug resistance-1, P-glycoprotein, pharmacogenetics, single nucleotide polymorphism

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## 1. Introduction

Nearly 200 proteins are involved in the transport of substrates across biological membranes. Most of these transport proteins belong to the ATP binding cassette (ABC) superfamily, also termed as traffic ATPases. The original concept of multi-drug resistance (MDR) was introduced in 1970s to designate cells resistant to one drug, which developed cross-resistance to unrelated drugs that bear no resemblance in structure or cellular target [1]. MDR was first associated with drug efflux transporters such as P-glycoprotein (P-gp), and multi-drug resistance-associated protein (MRP) in tumour cells. Current evidence suggests that these transporters are present in the normal tissue, and, therefore, may play a role in drug disposition [2]. It is known that ATP hydrolytic energy powers the efflux action of P-gp [3,4]. P-gp is a gene product of the ABCB subfamily (Table 1). Mammalian P-gps display 60 – 65% homology with most P-gps from other species, suggesting that their role in drug trafficking is highly conserved throughout evolution [5-7]. The ABCB subfamily is unique in mammals in that it contains both full and half transporters. Four full transporters and seven half transporters have currently been described as members of this subfamily. ABCB1 (*MDR1*/P-gp) was the first human transporter cloned and characterised through its ability to confer a MDR phenotype to cancer cells. It is an integral membrane protein composed of two homologous halves, each consisting of an N-terminal hydrophobic domain, with six transmembrane segments, which is separated from the hydrophilic domain, containing a nucleoside binding fold, by a flexible polypeptide linker. P-gp encoding genes from hamster, mice and human have also been identified in other species [8,9]. This transporter is encoded by a small multigene family, described as MDR class I, II and III. It is observed that P-gp belonging to all three classes is present in rodents, whereas human cells express P-gp belonging to classes I and III. Transfection studies have shown that the class I and II isoforms confer MDR, whereas the class III isoform represents the phosphatidylcholine translocase

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**Table 1. List of human ABCB genes, their chromosomal location and function.**

| Symbol | Alias | Location | Function |
|---|---|---|---|
| *ABCB1* | P-gp, MDR | 7q21.12 | MDR |
| *ABCB2* | TAP1 | 6p21.3 | Peptide transport |
| *ABCB3* | TAP2 | 6p21.3 | Peptide transport |
| *ABCB4* | P-gp3 | 7q21.12 | PC transport |
| *ABCB5* | | 7p21.1 | |
| *ABCB6* | MTABC3 | 2q35 | Iron transport |
| *ABCB7* | ABC7 | Xq21-22 | Fe/S cluster transport |
| *ABCB8* | MABC1 | 7q36.1 | |
| *ABCB9* | | 12q24.31 | |
| *ABCB10* | MTABC2 | 1q42.13 | |
| *ABCB11* | SPGP | 2q24.3 | Bile salt transport |

ABC: ATP-binding cassette; MDR: Multi-drug resistance; PC: Phosphatidylcholine; P-gp: P-glycoprotein.

responsible for the secretion of phospholipids into bile [10-12]. The subject of this review is to discuss polymorphisms in human *MDR1* (class I) only.

## 2. P-glycoprotein

### 2.1 P-glycoprotein: physiological and pharmacological functions

The physiological role of P-gp is to protect cells from harmful toxic substances. The primary function of P-gp in the intestine is to decrease plasma levels of several chemotherapeutic agents by actively secreting them back in the lumen and thus maintaining subtherapeutic intracellular concentrations at target sites. P-gp is also expressed in various normal human tissues, including the lungs, brain endothelial cells, liver, proximal tubule epithelial lining in kidney and intestinal enterocytes [13]. P-gp expressed in the intestine is functionally identical to that present in other epithelial and cancer cells [14,15]; however, the expression levels are much higher in cancer cells than normal cells. P-gp transports many structurally dissimilar drugs that act on different intracellular targets, including anticancer drugs, cardiac drugs, antifungal agents, HIV protease inhibitors, steroids, calcium channel blockers and cytotoxic drugs (Table 2). As many drugs are substrates of P-gp, the degree of expression and functionality of it can directly affect the therapeutic efficacy of such drugs. The substrate specificity of P-gp initially seemed to be similar to that of MRP1, although later studies have revealed that the preferred substrates for MRP1 are organic anions (e.g., drugs conjugated to glutathione, glucuronate, or sulfate) whereas P-gp has a low affinity for such negatively charged compounds [16-18].

Modulation of *MDR1* expression in normal cell types can influence the bioavailability of various drugs [19-22]. In the intestine, the modulation of *MDR1* may control the degree of drug uptake after ingestion [23-25]. High expression levels of P-gp may limit the uptake of drug into the brain [26-29], and lower expression of P-gp activity may lead to abnormal levels in the brain resulting in undesirable toxicity. *MDR1* overexpression may be partially due to gene amplification [30-33] or to other external factors [34-37]. Allelic differences in individual *MDR1* gene sequences may be associated with different expression levels. Expression levels can also be influenced by structural differences in the genome, methylation or acetylation, or other chromatin alterations. *MDR1* activity can be altered when the amino acid sequence of P-gp is changed. Two naturally occurring *MDR1* polymorphisms have been described with significant clinical effects [32,38]. Experiments conducted with an artificially introduced *MDR1* mutation have shown that P-gp reacts sensitively to amino acid alterations. Mutations that change amino acids can alter the substrate specificity of P-gp, the efflux potential, and P-gp inhibition and induction [39-52].

### 2.2 Mechanisms of P-glycoprotein action

The exact mechanism of P-gp action is poorly understood. It is hypothesised that P-gp intercepts compounds as they are passively diffusing through the lipid bilayer membrane. These compounds are then flipped from the inner leaflet of the membrane to the outer leaflet and into the extracellular media ('flip-flop' mechanism) [53]. Transmembrane movement of MDR-type drugs, such as doxorubicin, occurs by the flip-flop mechanism with a lifetime of ~ 1 min rather than by diffusion down a gradient present in the lipid core [54]. Another novel hypothesis suggests that P-gp is an energy-dependent efflux pump only for certain conjugated metabolites (probably sulfates) of the lipophilic anticancer drugs, but not for the parent compounds. According to this hypothesis, P-gp overexpression in most cases is not the 'culprit' but instead an 'accomplice' in P-gp-associated MDR. The culprit is probably the enhanced function of the metabolising enzymes for the lipophilic anticancer drugs [55]. Photoaffinity and mutagenesis studies have revealed that substrates interact with several sites on P-gp that lie within or close to certain transmembrane domains. Distribution of hydrophobic weak bases can be dramatically altered by changing the potential difference and pH gradient across the membrane [56]. Initial work, which suggested that P-gp substrates are predominantly cationic molecules, led to the hypothesis that P-gp does not transport drug molecules but alters drug distribution across membranes indirectly. An alternative explanation, based on the broad substrate specificity of P-gp, suggests that P-gp modifies the pH or membrane potential across the plasma membrane of cells [57]. P-gp functions as both a calcium channel [58] and an ATP channel [59], and it is now accepted that although P-gp regulates the activity of other calcium channels in certain cell types, it does not possess channel activity itself [60]. In addition, the arguments that P-gp acts as an ATP channel have been refuted [4]. Recent observations

**Table 2. List of common substrates, inhibitors and inducers of P-glycoprotein.**

| Drug class | |
|---|---|
| | ***Substrates of P-glycoprotein*** |
| Anticancer | Doxorubicin, daunorubicin, paclitaxel, topotecan, vinblastine, tamoxifen, etoposide |
| Antibiotic | Cefazolin, cefoperazone, erythromycin |
| Calcium channel blockers | Verapamil, diltiazem |
| Central nervous system drugs | Phenytoin, phenoxazine, perphenazine, domperidone |
| Immunosuppressants | Cyclosporin A (low concentration), tacrolimus |
| HIV protease inhibitors | Ritonavir, saquinavir, indinavir |
| Steroids | Dexamethasone, aldosterone, hydrocortisone |
| Other | Morphine, loperamide, quinidine, digoxin, amiodarone |
| | ***Inhibitors of P-glycoprotein*** |
| Alkaloids | Reserpine, colchicine |
| Tricyclics | Xanthene, phenoxazine, acridine |
| Antimalarials | Chloroquin |
| Neuroleptics | Phenothiazine |
| Antifungal | Ketoconazole |
| Immunosuppressant | Cyclosporin A (high concentration) |
| HIV protease inhibitors | Ritonavir (high concentration) |



show that a number of uncharged compounds are good substrates for P-gp, along with many cyclic and linear hydrophobic peptides and ionophores; moreover, the lipophilic cation, tetraphenylphosphonium, can be transported against a steep $H^+$ gradient [61].

### 2.3 How does P-glycoprotein interact with inhibitors?

Many therapeutic agents with varied structural skeletons have been identified as P-gp substrates and inhibitors (Table 2). Inhibitors usually reverse drug resistance when coadministered with the drug of interest, leading to cell toxicity and eventually cell death. Sometimes the ATPase activity is increased to a greater extent by inhibitors than substrates, which either stimulates or inhibits the activity. Previous studies have shown that substrates can equilibrate across lipid bilayers relatively slowly (ranging from minutes to hours), whereas inactivators cross the bilayers too rapidly to be determined experimentally. Thus, for inhibitors, the rate of membrane equilibration is so rapid that flipping of these molecules by P-gp cannot keep pace with this process [62]. The transporter cycle is rendered ineffective despite the turnover rate being high, accompanied by a high rate of ATP hydrolysis.

### 2.4 Tissue distribution of P-glycoprotein

P-gp is expressed in various normal human tissues, including the lungs, brain endothelial cells, liver, proximal tubule epithelial lining in kidney and intestinal enterocytes [13]. In the liver, it is found on the biliary canalicular surface of hepatocytes and the apical surface of small biliary ductules. In the intestine, it is localised on the apical surface of epithelial cells. It is also prevalent in the brush border membranes of the kidney, apical surface of the pancreas, medulla and adrenal glands.

In the eye, P-gp is expressed in retinal capillary endothelial cells [63], retinal pigmented epithelial cells [64], capillary nonpigmented epithelium [65], conjunctival epithelial cells [66], cornea and corneal epithelial cells [67,68], and iris and ciliary muscle cells [63]. Recently, Dey *et al.* has shown that P-gp expressed in corneal epithelium can effectively efflux out drugs from inside the eye resulting in low ocular bioavailability [69]. Poor ocular bioavailability of topically applied drugs has been earlier attributed to precorneal losses. This study clearly suggests a relationship between poor ocular bioavailability and functional expression of P-gp in the corneal epithelium. This observation has high clinical significance in the delivery of topically applied drugs.

Expression of P-gp in the brain is found in the endothelial cells of capillaries of CNS [70] and the choroidal plexus epithelium [27] that forms the blood–brain barrier. A major challenge in drug delivery is to overcome the high efflux potential of P-gp expressed at the blood–brain barrier. P-gp is also expressed in the microvilli border of trophoblasts of human placenta [71,72], which may play a role in chemical transport in the placenta. P-gp is expressed in various amounts in leukocyte lineages with highest expression in $CD56^+$ cells, and moderate to low expression in $CD8^+$, $CD15^+$, $CD4^+$ and $CD14^+$ cells [73]. Finally, P-gp is also expressed in the capillary endothelial cells of the testis.

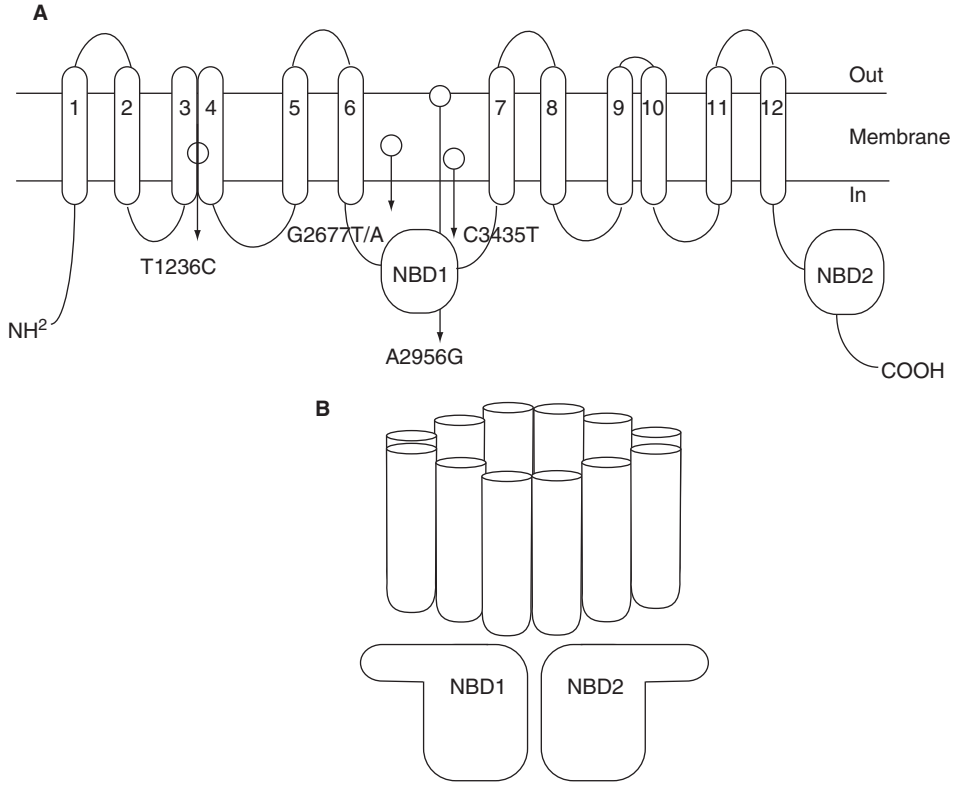


**Figure 1. Polymorphisms in the human *MDR1* gene.** A few representative SNPs are circled with their corresponding polymorphisms.
NBD: Nucleotide binding domain; SNP: Single nucleotide polymorphism.



## 3. *MDR1* polymorphism

Variable P-gp expression or function alters the extent of absorption, distribution, elimination and excretion. It is also fairly reasonable to hypothesise that genetic variability or mutation in this gene will alter drug disposition and function; thus, there is clinical relevance to finding *MDR1* polymorphisms and studying its impact on drug delivery and disposition. Single nucleotide polymorphisms (SNPs) are isolated nucleotide differences between individuals and are the most common class of variations in humans. SNPs are single base changes and occur at a frequency of > 1% in a population. A single base change that occurs in < 1% of the population is termed as mutation. SNPs are normally classified as coding SNPs (in the translated region of the gene), noncoding SNPs (outside the translated region such as promoter region) and random SNPs. Coding SNPs can be further classified as synonymous (when single base substitutions do not cause a change in the resultant amino acid) and nonsynonymous (when single base substitutions cause a change in the resultant amino acid). SNPs may occur at any position in the gene structure, and based on location can be classified as intronic, exonic or promoter region. A complete list of all SNPs can be found at the National Center for Biotechnology Information website [201].

The human *MDR1* gene is composed of 28 exons. In recent years 29 SNPs have been identified in the *MDR1* gene (Figure 1). Genetic polymorphisms were first identified by Kioka *et al.* [74] from cancer cells studied *in vitro*. Mickley *et al.* [38] reported mutations in normal cells involving G2677T/A and G2995A. Hoffmeyer *et al.* [75] analysed all the 28 exons of the *MDR1* gene including the promoter region and intron–exon boundaries. He reported 15 SNPs, with 6 SNPs in the coding region of the gene. Several other groups have also screened the entire *MDR1* coding region to look for SNPs [38,76-84]. Kim *et al.* [79] has identified *MDR1* variants in Caucasian and African-American populations. Nine of those mutations alter the amino acid sequence of P-gp. The A61G SNP, which is located near the N-terminus of P-gp, leads to an amino acid exchange from Asn to Asp. The G1199A SNP is located in the cytoplasmic loop close to the first ATP-binding domain and changes the amino acid from Ser to Asn (Ser400Asn). As seen in Table 3, 19 SNPs are located in exonic regions and 11 SNPs are nonsynonymous [85]. SNPs at exon 21 in position 2677, located in the second transmembrane domain, can result in two distinct amino acid changes: Ala893Ser (G2677T) and Ala893Thr (G2677A). The G2995A mutation is also located in the second transmembrane domain but closer to

**Table 3. Genetic polymorphisms in human *MDR1*.**

| Location | Position | Mutation | Effect |
|---|---|---|---|
| Promoter | 5'/-41 | A→G | Noncoding |
| Exon 1a | Exon 1a/-145 | C→G | Noncoding |
| Exon 1b | Exon 1b/-129 | T→C | Noncoding |
| Intron 1 | Exon 2/-4 | C→T | Noncoding |
| Intron 1 | Exon 2/-1 | G→A | Initiation of translation |
| Exon 2 | Exon 2/61 | A→G | Asn21Asp |
| Intron 4 | Exon 5/-35 | G→C | |
| Intron 4 | Exon 5/-25 | G→T | |
| Exon 5 | Exon 5/307 | T→C | Phe103Leu |
| Intron 6 | Exon 6/+139 | C→T | |
| Intron 6 | Exon 6/+145 | C→T | |
| Exon 7 | Exon 7/548 | A→G | Asn183Ser |
| Exon 11 | Exon 11/1119 | G→A | Ser400Asn |
| Exon 12 | Exon 12/1236 | C→T | Silent base change |
| Intron 12 | Exon 12/+44 | C→T | |
| Exon 13 | Exon 13/1474 | C→T | Arg492Cys |
| Intron 16 | Exon 17/-76 | T→A | |
| Intron 17 | Exon 17/+137 | A→G | |
| Exon 21 | Exon 21/2650 | C→T | Silent base change |
| Exon 21 | Exon 21/2677 | G→T<br>G→A | Ala893Ser<br>Ala893Thr |
| Exon 24 | Exon 24/2956 | A→G | Met986Val |
| Exon 24 | Exon 24/2995 | G→A | Ala999Thr |
| Exon 26 | Exon 26/3320 | A→C | Gln1107Pro |
| Exon 26 | Exon 26/3396 | C→T | Silent base change |
| Exon 26 | Exon 26/3421 | T→A | Ser1141Thr |
| Exon 26 | Exon 26/3435 | C→T | Silent base change |
| Exon 28 | Exon 28/4030 | G→C | |
| Exon 28 | Exon 28/4036 | A→G | |

The positions of the polymorphism are from the first base of the ATG start codon set to 1. Mutations located in introns are given as positive downstream (-) or upstream (+) of the respective exon.
MDR: Multi-drug resistance.



the second ATP-binding domain. A SNP in exon 26 (C3435T) is associated with altered protein expression, although the encoded amino acid (Ile) does not change. The silent C3435T was also found to be linked to the G2677 polymorphism and to the synonymous polymorphism C1236T [79]. Caucasian subjects with the CC genotype had twofold higher P-gp expression in the small intestine than subjects with the TT genotype.

Studies have shown that SNPs at exon 21 (G2677T/A) and exon 1b (T129C) may be associated with altered function and expression of P-gp [79,81]. In these studies, P-gp levels in human placentae from patients with TT genotype in exon 1b (T129C) were found to be significantly higher in comparison with the group with CT genotype. Studies by Tanabe *et al.* [81] have demonstrated a clear relationship between SNPs in exon 26 (C3435T) and exon 21 (G2677T/A), suggesting that functional differences in P-gp attributed to synonymous SNPs (exon 26) may be due to nonsynonymous SNPs (exon 21). Recently, the existence of such distinct haplotypes has been confirmed by other groups [77,83,84,86-93]. Increased MDR1 mRNA levels in a Japanese population with 3435TT polymorphism as compared with CC and CT groups have recently been reported. MDR1 mRNA levels in mononuclear cells from healthy volunteers showed a genotype-dependent (CC > CT > TT) trend similar to the expression of P-gp in small intestine and placenta [94]. In addition, the same

genotype-dependent population showed a similar trend in P-gp function (CC > CT > TT) in $CD56^+$ NK cells. The functionality of P-gp efflux was determined by rhodamine 123 efflux in the presence or absence of a known P-gp inhibitor. These results have also been confirmed in HIV-positive patients who showed the same trend in mRNA and protein expression levels [95].

Lastly, rare SNPs located in exon 7 (A548G, Asn183Ser), exon 13 (C1474T, Arg492Cys) and exon 26 (A3320C, Gln1107Pro) leading to amino acid changes have been reported [76,79,96,97].

### 3.1 Ethnic differences in *MDR1* polymorphisms

Three common haplotype combinations seem to be observed across all ethnic groups. SNPs in exon 12 (C1236T), exon 21 (G2677T/A) and exon 26 (C3435T) have been studied in detail. Of these three SNPs, C3435T is considered to be the most widely studied. Interethnic differences of the C3435T polymorphism are given in Table 4, which shows that the 3435C allele was found to be more frequent in African populations in comparison with Caucasian and Asian populations [79,98,99]. Because the C allele is always associated with higher P-gp expression, it can be said that the observed higher frequency of the CC phenotype may be due to a selective advantage of this genotype against gastrointestinal tract infections that are common in tropical countries [98].

The 2677A genotype (exon 21) is significantly more common in Japanese populations as compared with other populations; however, it is uncertain whether the functional expression of the 2677A allele is linked to drug disposition. A new mutation in exon 26 (T3421A) leading to an amino acid exchange has been reported in African Americans [97]. Another report indicates that the variant 1236T allele of exon 12 is more frequently observed in Asian subjects as compared with Caucasians [81,84,100]. Some rare mutations such as the C-4T polymorphism in African Americans (allele frequency 4%) and the exon 1a-145C→G polymorphism in Japanese population have been detected [80,81]. None of these polymorphisms occur in the Caucasian population. Clearly, additional studies are required to determine the functional consequence of the 2677A allele to drug disposition in different ethnic subjects.

### 3.2 *MDR1* polymorphism and drug disposition

Hoffmeyer *et al.* reported a higher digoxin plasma concentration after oral administration in Caucasian subjects having the 3435TT allele [75]. They reported a twofold reduction in P-gp expression in the TT group in comparison with subjects with the CC genotype. However, another group reported higher areas under the curve (AUC) values after a single oral dose of digoxin in Japanese subjects with the 3435CC genotype compared with the 3435CT and the 3435TT groups [87,101]. The reasons for such discrepancies are not clear but may be due to interethnic differences in SNPs. Recently, further studies have confirmed higher digoxin levels with the T allele rather than the C allele [91,102,103], although these studies did not measure the expression of P-gp. Another study reported no differences in digoxin levels in healthy Caucasian subjects between the 3435T allele and the 3435C allele [78]. Such conflicting and controversial clinical data has also been reported for other P-gp substrates such as cyclosporin A [100,104-107], tacrolimus [82,83,92,108-113] and fexofenadine [79,114].

P-gp functionality and cytochrome P450 (CYP) isozyme expression are often studied together because most drug effluxed by P-gp are also substrates for CYP systems. A study involving phenytoin (P-gp substrate) was conducted to determine the effect of C3435T polymorphism on phenytoin disposition [115,116]. After a single dose of phenytoin there was no significant difference in phenytoin plasma concentration in the TT genotype in comparison with healthy individuals with either the CT or CC genotypes. The study also found that the CC genotype is significantly more common in volunteers with low phenytoin plasma concentrations. It seems that C3435T polymorphism may have some effect on phenytoin plasma concentration. However, the most important determinant in phenytoin plasma concentration is the CYP2C9 genotype.

The immunosuppressant, cyclosporin A is also a substrate of P-gp and CYP3A4. The effects of the C3435T polymorphism on cyclosporin trough concentrations were studied in 124 kidney transplant patients [105]. No differences in cyclosporin trough concentrations were found between the CC, CT and TT genotypes. This group also reported that there was no association of these genotypes with renal function or the incidence of acute rejection.

The C3435T polymorphism has also been studied with respect to anti-HIV drugs. It is known that all HIV protease inhibitors are excellent P-gp substrates [117-121]. These drugs are transported by P-gp, and their intestinal absorption and CNS penetration are dependent on P-gp. Fellay *et al.* [95] reported reduced levels of MDR1 mRNA and P-gp protein expression in peripheral blood mononuclear cells of HIV patients with the TT genotype in comparison with the CT and CC genotype. In addition, patients with the TT genotype have higher CD4 cell counts after starting antiretroviral treatment in comparison with the CT and CC genotype groups [95]. As P-gp protein levels reduced in the TT genotype, HIV protease inhibitors could penetrate more into the infected cells (lymphocytes) that increased CD4 cell count. This study also reported a C3435T-dependent plasma concentration for efavirenz (a non-nucleoside reverse transcriptase inhibitor), which is not a P-gp substrate. It had the same trend as the HIV protease inhibitor with the highest P-gp expression, with the CC genotype followed by CT and TT genotype.

### 3.3 *MDR1* polymorphism in the placenta

P-gp plays an important role in various biological barriers. It is believed that the placenta has the ability to block the transfer of xenobiotics across the human placenta, and that the P-gp in trophoblasts contributes to the function of this barrier. Tanabe *et al.*

**Table 4. Interethnic differences in allele frequencies of the C3435T polymorphism.**

| Population, subtype | N | C | T | Ref. |
|---|---|---|---|---|
| Caucasian, US | 37 | 0.46 | 0.54 | [79] |
| Caucasian, UK | 200 | 0.47 | 0.53 | [142] |
| Caucasian, UK | 190 | 0.48 | 0.52 | [99] |
| Caucasian, Germany | 537 | 0.50 | 0.50 | [98] |
| Caucasian, Germany | 461 | 0.46 | 0.54 | [76] |
| Caucasian, Germany | 188 | 0.52 | 0.48 | [75] |
| Caucasian, Italy | 106 | 0.54 | 0.46 | [89] |
| Caucasian, New Zealand | 160 | 0.47 | 0.53 | [143] |
| Caucasian, Russia | 290 | 0.46 | 0.54 | [93] |
| Caucasian Spain | 408 | 0.52 | 0.48 | [144] |
| Asian, Japanese | 114 | 0.61 | 0.39 | [101] |
| Asian, Japanese | 117 | 0.62 | 0.38 | [87] |
| Asian, Japanese | 50 | 0.57 | 0.43 | [98] |
| Asian, Chinese | 132 | 0.53 | 0.47 | [99] |
| Asian, Chinese | 104 | 0.60 | 0.40 | [84] |
| Asian, Malay | 92 | 0.49 | 0.51 | [104] |
| Asian, Indian | 87 | 0.37 | 0.63 | [100] |
| Asian, Indian | 93 | 0.38 | 0.62 | [104] |
| Asian, Filipino | 60 | 0.59 | 0.41 | [99] |
| African, Ghanaian | 172 | 0.90 | 0.10 | [98] |
| African, Kenyan | 80 | 0.83 | 0.17 | [99] |
| African, Sudanese | 51 | 0.73 | 0.27 | [99] |
| African American | 88 | 0.84 | 0.16 | [99] |
| African American | 41 | 0.78 | 0.22 | [98] |
| African American | 23 | 0.74 | 0.26 | [79] |

N denotes the size of the population.

[81] observed a linkage (association of genes that lie near each other) between SNPs in the 5′-flanking region and exon 1b, and a strong association between C3435T and G2677T/A in 100 human placental samples. In addition, all placental cDNA and genomic DNAs had at least one mutation in the *MDR1* gene. This result was consistent with an earlier report in which 21 healthy Caucasian volunteers had at least one mutation in the *MDR1* gene [75]. The frequency of homozygosity for the mutant alleles was calculated on the basis of Hardy–Weinberg distribution and compared with Caucasian subjects. It was found that the frequency of TT genotype did not differ for exon 26, but the CC genotype for exon 1b and exon 12 did. Nine SNPs were found in the placental study. SNPs T129C (in the promoter region) and G2677T/A correlated with placental P-gp expression.

### 3.4 Other multi-drug resistance gene polymorphisms

Besides *MDR1*, mutations have also been studied in various other transporter and receptor genes. SNP analysis of organic anion transporter polypeptide-2 (OATP-2) has been reported [122]. *In vitro* experiments with cultured cells expressing the wild-type and mutated OATP-2s showed that some of the SNPs are linked with decreased cell activity. SNP analysis of MRP2 in healthy Japanese subjects has revealed at least six kinds of SNPs. Among them, C24T (promoter region), G1249A (exon 10) and C3972T (exon 28) are most frequently observed [80]. The SNP associated with G1249A also changes the amino acid residue from Val to Ile (Val417Ile); however, C3972T is a 'silent mutation' with no change in amino acid sequence. Absence of MRP2 is believed to be responsible for a disease condition called Dubin-Johnson Syndrome in humans, which is characterised by conjugated hyperbilirubinaemia and the formation of dark pigments in the liver [123-127]. MRP2 is important in determining disposition of anionic drugs.

Breast cancer resistance protein (BCRP/ABCG2), found in many normal human tissues, contributes to the disposition of many drug substrates [128,129]. It was recently shown that BCRP transports some sulfate conjugates [130]. At least seven SNPs in BCRP have been identified in healthy individuals and cell lines [131]. These are G34A, C376T, C421A, G1098A, G1322A, T1465C and C1515. Although it has been reported that the amino acid replacement at 482 alters the substrate specificity of BCRP [132-134]; alterations in the substrate specificity due to SNP variations has not yet been reported. Marked ethnic differences in the frequency of C421A SNP of BCRP have also been reported [135,136]. Regarding the disposition of antitumour drugs, it has also been reported that BCRP1($^{-/-}$) haematopoietic stem cells are sensitive to mitoxantrone-induced toxicity [137,138]. These data suggests that the ability to protect stem cells from some toxic xenobiotics may be lower in subjects who have C421A and G1322A SNPs in the *BCRP* gene. Studies have also suggested that oral absorption of topotecan (antitumour drug) is restricted by the intestinal expression of BCRP [128]. After oral administration of topotecan and GF-120918 (inhibitor of *MDR1* and BCRP), the bioavailability of topotecan in plasma was significantly increased in both normal and *MDR1* ($^{-/-}$) mice. Kondo *et al.* [139] have reported that SNPs C421A and G1322A are associated with reduced expression of BCRP; in particular, G1322A SNP is also associated with altered cellular localisation.

## 4. Conclusion

MDR in epithelial cells is associated with the over-expression of certain MDR proteins. Drug efflux pumps (P-gp) are known to limit the absorption of therapeutic agents. The role of P-gp for drug disposition and effects has been clearly demonstrated. The recent identification of multiple SNPs in the *MDR1* gene provides an important basis to study these mutations on P-gp expression and function. There is evidence that some of these mutations may alter P-gp expression in humans, which may change drug disposition for certain diseases. In the future, more knowledge of these mutations will

provide for a better understanding of transporter-mediated drug delivery.

## 5. Expert opinion

The discovery and characterisation of variations in the *MDR1* gene and diagnostic tests of different *MDR1* alleles in human individuals may provide a potent tool for drug therapy. Some mutations (e.g., C3435T) are considered important polymorphism from drug therapy perspectives. Further identification of *MDR1* variations may be useful for the development of new and novel inhibitors that specifically modulate the activity of the individual types of *MDR1*. The feasibility of using such *MDR1* variants with their potential therapeutic application has recently been demonstrated [48,140].

It is clear that P-gp plays an important role in drug disposition by providing a barrier for the entry of xenobiotics as well as controlling their rate of transfer into different tissues. In contrast to the vast knowledge of drug metabolising enzymes, only limited information is available to explain the contradictory findings of the observed functional differences between *MDR1* polymorphisms and P-gp expression or function. Interindividual and interethnic difference in P-gp expression exist, which may lead to confusing results and unexplained correlations. Although the amount of available data are limited, some of these polymorphisms affect drug disposition in general by altering P-gp expression; however, P-gp expression and function is not only determined by polymorphisms in the *MDR1* gene but also by other medications (e.g., rifampin, St. John's Wort [25,141]).

Hence, further studies are required to correlate the polymorphism of P-gp and altered expression and function. Studies with various *MDR1* haplotypes will give us a better understanding of P-gp function. Finally, a thorough knowledge of exogenous factors, interethnic differences in P-gp expression and drug disposition will lead to better drug delivery strategies.



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