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A synonymous polymorphism in a common MDR1 (ABCB1) haplotype shapes protein function

King Leung Fung and Michael M. Gottesman*

Laboratory of Cell Biology, Center of Cancer Research, National Cancer Institute, National Institutes of Health, 37 Convent Drive, Room 2108, Bethesda, Maryland 20892-4254 USA

Abstract

The *MDR1* (*ABCB1*) gene encodes a membrane-bound transporter that actively effluxes a wide range of compounds from cells. The overexpression of MDR1 by multidrug-resistant cancer cells is a serious impediment to chemotherapy. MDR1 is expressed in various tissues to protect them from the adverse effect of toxins. The pharmacokinetics of drugs that are also MDR1 substrates also influence disease outcome and treatment efficacy. Although MDR1 is a well conserved gene, there is increasing evidence that its polymorphisms affect substrate specificity. Three single nucleotide polymorphisms (SNPs) occur frequently and have strong linkage, creating a common haplotype at positions 1236C>T (G412G), 2677G>T (A893S) and 3435C>T (I1145I). The frequency of the synonymous 3435C>T polymorphism has been shown to vary significantly according to ethnicity. Existing literature suggests that the haplotype plays a role in response to drugs and disease susceptibility. This review summarizes recent findings on the 3435C>T polymorphism of MDR1 and the haplotype to which it belongs. A possible molecular mechanism of action by ribosome stalling that can change protein structure and function by altering protein folding is discussed.

Keywords

ABCB1; MDR1; polymorphism; haplotype; ribosome stalling

1. Introduction

1.1 Drug transporters as xenobiotic removal pumps

Toxins in the environment are a major threat to many living organisms. Once internalized, toxic compounds must be removed for the organisms to survive. Therefore, humans have developed, inherited and perfected ways to reduce the effect of xenobiotics. At the cellular level, the cell membrane acts as a physical barrier to prevent compounds from entering the cell. However, because some compounds can diffuse through the cell membrane, alternative protective methods have evolved. One of the most important defense mechanisms is to pump xenobiotics out of the cells. Thus, drug transporters are commonly found in the cell membranes of many organisms, from bacteria to mammals, and are responsible for cell protection. However, the existence of these drug transporters often hinders the use of compounds used to treat diseases because they are substrates of these efflux pumps either by affecting the pharmacokinetics of drugs in the body or by limiting accumulation in target cells such as cancer cells.

*Corresponding Author: Michael M. Gottesman, Laboratory of Cell Biology, Center of Cancer Research, National Cancer Institute, National Institutes of Health, 37 Convent Drive, Room 2108, Bethesda, Maryland 20892-4254 USA, mgottesman@nih.gov.

1.2 Localization of MDR1

MDR1 (P-glycoprotein, ABCB1) was the first ABC transporter identified and has become the most studied gene in the field of multidrug resistance [1]. It is a member of the ATP-binding cassette (ABC) transporter superfamily [2]. In addition to MDR1, 47 other ABC transporters have been identified or predicted in the human genome. These transporters are classified into seven groups (A to G) based on sequence similarities [2]. Among these, several (ABCA2, ABCB4, ABCG2, ABCC1-5,6,10) are also able to confer drug resistance (reviewed in [3,4]). Sequence similarities do not predict the role of ABC transporters, suggesting that protein folding and the formation of the substrate binding pocket play important roles in their function. ABC transporters show diverse expression patterns. Human MDR1 is normally found in a tissue-specific manner. It is found in normal cells of the adrenal gland, kidney, liver, colon, jejunum, pancreas, and in capillary endothelial cells in the testis and blood brain barrier [5-7]. MDR1 is also found in the placenta and in the endometrium of pregnant women. It is expressed in a polarized manner in apical cells lining the small intestine and the colon and in the kidney proximal tubules. The expression pattern of MDR1 suggests that its major physiological role of MDR1 is to protect vital organs and the fetus [8] from xenobiotics.

MDR1 is highly expressed in drug-resistant cancer cells, particularly in cancer cells originating from cells that normally express MDR1 [9]. It was first detected on colchicine-resistant Chinese hamster ovary (CHO) cells [10], and has frequently been found in cell culture models of MDR and in clinical samples from tumors that are resistant to chemotherapy. The MDR1 gene is found on chromosome 7, at band p21-21.1 [11], and its cDNA spans about 4.5kb, with 28 exons ranging in size from 49 to 587 bp [12]. The *MDR1* gene encodes a polypeptide with 1280 amino acids which has an apparent molecular weight of 170 kDa. The protein is defined as having two halves, each containing six hydrophobic trans-membrane domains, and an ATP binding domain (Figure 1). The two halves are separated by a flexible linker region, and the two ATP-binding domains are structurally similar. All 12 trans-membrane domains are found in the plasma membrane. Several motifs have been identified in each of the ATP-binding domains, including the Walker-A, Walker-B, A-loop, H-loop, D-loop, Q-loop and the signature motif "LSSGQ" consensus sequences. The ATP-binding domains act as ATPases that hydrolyze ATP to ADP. *In vitro* studies have shown that the ATPase activity can be induced in the presence of MDR1 substrates [13-15]. MDR1 is post-translationally regulated, and contains three N-linked glycosylation sites (N91, N94 and N99) in the first extracellular loop [16]. The post-translationally processed protein is localized in the plasma membrane [17], and a glycosylation-defective mutant does not show altered drug transport [18]. Several phosphorylation sites have also been identified, but studies on mutants have shown that these sites are not responsible for localization or function in cultured cells [16,19].

1.3 Mechanism of MDR1 and its role in drug resistance

Biochemical studies suggested that substrate transport by MDR1 is coupled to ATP hydrolysis [20,21]. There is speculation that MDR1 may work as a flippase so that substrate initially interacts with the inner leaflet of the lipid bilayer and then MDR1 flips the compound to the outer leaflet [22]. Nonetheless, the currently accepted model is the "hydrophobic vacuum pump", in which the substrate directly interacts with the protein's drug binding pocket and is pumped into the extracellular space, assisted by hydrolysis of ATP to ADP (reviewed in [3, 23,24]).

The most significant feature of MDR1 function is its broad substrate specificity. Reviews have categorized MDR1 substrates based on their clinical use [25,26], and by correlating MDR1 expression levels with drug resistance in the NCI-60 cell-line panel [27], it has been predicted that a great number of compounds are candidate MDR1 substrates [28]. These MDR1 substrates have diverse chemical structures and have a wide range of biological functions, e.g., anticancer

drugs, anti-HIV proteases, antihistamines, calcium channel blockers, antibiotics etc. This substrate “promiscuity”, and the absence of a crystal structure for MDR1, makes prediction of MDR1 substrates difficult and therefore attempts to predict MDR1 substrates based on chemical structures have to date been unsuccessful. The substrate binding mechanism and recognition of substrates and inhibitors by MDR1 is complex. It has been found that MDR1 contains a large drug binding pocket that is generally assembled by several trans-membrane domains (TM5, TM6, TM11 and TM12) which determines the substrate specificity of MDR1 [29-31]. The presence of this drug binding pocket is further supported by photoaffinity labeling experiments with MDR1 substrate analogues. However, there are mutations of amino acids in other parts of the transporter that also change substrate specificity, suggesting that drug recognition is a complex process [32,33].

The function of MDR1 as a transporter is similar to other drug transporters. Within the long list of MDR1 substrates, it is evident that some of the substrates (drugs) are also substrates of drug metabolizing enzymes, especially CYP3A4. CYP3A4 is located at 7q22.1, very close to MDR1 on the chromosome, suggesting their inter-dependency during evolution to protect the host organism from toxins by detoxification and extrusion. Some MDR1 substrates, e.g. anti-cancer drugs, can be effluxed by other MDR transporters, suggesting redundancy, or “back up” in transporter function. For example, vincristine is a substrate of MDR1 [34] and MRP1 (ABCC1) [35] while doxorubicin is a substrate of MDR1 [36], MRP1 (ABCC1) [37] and BCRP (ABCG2) [38].

1.4 Knock-out mice show that MDR1 affects pharmacokinetics

MDR1's expression pattern and function suggest that it plays a crucial role in drug absorption, disposition and elimination. Studies using transgenic knockout mice have provided important evidence that loss of *MDR1* affects drug pharmacokinetics [39,40]. Although only one *MDR1* gene in humans confers drug resistance, mice have two (*mdr1a*, *mdr1b*) [41]. Transgenic mice with genetic disruption of *mdr1a*, *mdr1b* or both were generated [39,40]. All of these animals are viable and have no significant defects. However, these mice have higher sensitivity to MDR1 substrates *in vivo*. Sensitivity to ivermectin, a neurotoxin, was increased 100-fold in *mdr1a* knockout mice [39]. The mice also showed increased sensitivity to other drugs such as dexamethasone, digoxin and cyclosporine A. The effect of drug accumulation is greater in organs expressing *mdr1b* in the *mdr1a/1b* double knockout mice [40]. This shows that both genes share common function. Importantly, loss of function of both *mdr1* genes does not increase expression of other drug resistance genes in mice, indicating that change in drug accumulation is due to loss of *mdr1a* and *mdr1b* genes [40]. Clearly, this evidence strongly suggests that pharmacokinetics of many drugs is affected by *mdr1a/mdr1b*.

2. Single nucleotide polymorphisms (SNP) of MDR1

2.1 Characterization of MDR1 polymorphisms

According to the SNP database maintained by the National Center for Biotechnology Information (NCBI), there are more than 50 SNPs in the human *MDR1* coding region. Table 1 summarizes the SNPs in the exons of MDR1 reported in both the NCBI and Ensemble databases. Studying the location of the SNPs has led to several important observations. First, SNPs with more than 1% heterozygosity were found in two-thirds of the twenty-nine total exons. SNPs are found in the *MDR1* transcript from the 5' start site to the 3' untranslated region (UTR). Second, within the coding region, the SNPs result in both synonymous and non-synonymous mutations. However, no nonsense mutations have been found. Third, most of the mutations are translated into amino-acids located in the intracellular region. In the extracellular region, there are 3 SNPs, all located in the first extracellular loop. Only 4 SNPs are found in 2 of the 12 trans-membrane domains (A2505G (TM9), A2506G (TM9), A2587G (TM9) and

A2956G (TM12)). In addition, there are no SNPs that change either glycosylation or phosphorylation sites. No SNPs lead to amino acid changes in the A-loops, D-loops, H-loops, Q-loops, or Walker-A motifs, although two synonymous polymorphisms are found in the first signature motif and one in the first Walker-B motif (Table 1).

The characteristics of the SNPs of MDR1 suggest the preservation of the structural integrity of the gene through evolution. In this protein, 96 amino acids (consisting of 288 nucleotides) are found in the extracellular region. The SNP occurrence rate in this region is 1.04% (3 SNPs among 288 nucleotides). This rate is similar to the SNP occurrence rate (1.3%) in the intracellular region (36 SNPs among 2757 nucleotides). In contrast, the rate of SNP occurrence in the trans-membrane domains is much lower (0.503%, or 4 SNPs among 795 nucleotides). This suggests that the functions of the trans-membrane domains, which anchor the protein in the plasma membrane and form the drug-binding pocket, are well-conserved. In fact, only one SNP has been found with significant heterozygosity in the TM12 domain (Table 1). This SNP affects drug-stimulated ATPase activity but not expression level [42]; the effect of such a mutation is still poorly understood. Taken together, the lower occurrence of SNPs in the trans-membrane domains, the absence of SNPs in the glycosylation and the phosphorylation sites, together with the highly conserved protein sequences of MDR1 in different organisms, support the idea that all of the domains and motifs of MDR1 are critical to its structure and function.

2.2 Intronic polymorphisms

Not much attention has been given to the intronic and non-coding SNPs in MDR1. Current studies focus on the relationship of polymorphisms in the intronic region to diseases. It is suggested that some SNPs (-1517 T>C (promoter), -41 A>G (intron -1), -129 T>C (exon 1b)) may be associated with younger onset age of mood disorder [43]. Recently, a frequently occurring SNP in intron 13 (exon 13+81 C>T) has been found to be associated with sporadic colorectal cancer [44]. Soranzo and colleagues showed that three intronic SNPs (IVS 25+3050 G>T, IVS 25 +5231 T>C, IVS 26 +80 T>C) are associated with drug resistance in epileptic patients [45]. *In vitro* studies on non-coding SNPs are limited. The effect of polymorphisms in the promoter region is not clear *in vitro*. Compared to the 5' UTR, the 3' UTR has more polymorphic sites (Table 1). Some of these SNPs are stably expressed in HEK-293T cells but none of them affect mRNA stability [46]. Given that the 3' UTR sequence could be crucial for miRNA recognition or recognition by binding proteins, the impact of these SNPs on *MDR1* regulation remains to be examined.

3. MDR1 polymorphisms and drug pharmacokinetics

3.1 Importance of understanding the role of MDR1 polymorphisms

The presence of MDR1 directly influences drug efficacy and its expression determines the degree of resistance of cancer cells to chemotherapy. Therefore, there is an urgent need to understand the factors that determine the function of MDR1. Since it is a well-conserved gene, research has been focused on the factors that affect its expression (reviewed in [47]). Mutation studies have confirmed that changes in crucial amino acid residues in the trans-membrane domains, ATP-binding domains, Walker-A motifs, Walker-B motifs, or the signature motif affect MDR1 function [48-51]. Mutations may also affect folding pathways or protein conformation [52]. For example, non-synonymous amino-acid substitutions in TM5 or TM6 affect the ability of UIC2, a conformational-sensitive antibody, to bind to MDR1 [52,53]. Therefore, it is important to identify SNPs in MDR1 and examine their effect on protein function.

Polymorphisms of MDR1 in other animal species have been found to result in more severe phenotypes. For example, a 4-bp deletion in the canine MDR1 exon leads to a frameshift

mutation. The collies that carry both mutant alleles become hypersensitive to ivermectin, a deworming agent, because they lack functional MDR1 transporters [54]. Another example is found in a subpopulation of CF-1 mice, comprising 25% of the animals, which display much higher drug sensitivity to ivermectins [55,56]. Genetic analysis revealed that this phenotype is because of an insertion in the *mdr1a* gene that renders it unable to translate Mdr1a protein [57,58]. Although these genetic changes are not found in humans, we cannot rule out the possibility that such mutations exist. Indeed, new SNPs have recently been identified, and the list is expanding. Even though most of the SNPs have low frequencies, they may still affect the function of MDR1, either alone or in conjunction with other SNPs. It is still unknown why the three polymorphisms in the most common human haplotype are in tight linkage and their functional and clinical consequences are not fully understood. Studying MDR1 and its haplotypes could further our understanding of the structure and function of MDR1.

3.2 Effect of SNPs on MDR1 function

The functional consequences of the reported SNPs are not completely clear. Within the list, only a handful of them have been studied *in vitro* [59-64]. A missense mutation found at position 183 is very close to two amino acids before the G185V mutation site. This G185V mutation has been identified in drug-resistant cell lines, but not in humans. This mutation is responsible for increased colchicine resistance [65]. In fact, none of the SNPs tested resulted in a non-functional MDR1. In addition, the consensus of these studies is that single nucleotide mutations of MDR1 *in vitro* do not affect cell-surface expression or level of expression. However, the effect of SNPs on MDR1 function is so far uncertain. A study in our lab showed that common polymorphisms of MDR1 at 61A>G (N21D), 307T>C (F103L), 1199G>A (S400N), 2677G>T (A893S) and 2995G>A (A999T) do not change the transport of four MDR1 substrates when expressed at high levels in human cells [66]. A recent study by Gow and colleagues suggested that all of the SNPs they tested (N21D, S400N, R669C, A893S, A893T, S1141T, V1251I) produced small changes which in most cases are not statistically significant [59]. Another study using a yeast host to express human MDR1 SNPs (M89T, L662R, R669C, A893S, W1108R, S1141T) showed increased resistance to anthracyclines, actinomycin D and valinomycin. However, it is known that yeast cells handle drugs very differently from human cells [61]. Several factors may contribute to the differences in these reports including selection of host and test method. Nonetheless, such discrepancies may reveal small but significant differences in the transport function of MDR1.

4. The synonymous 3435C>T polymorphism and its haplotype

4.1 Elucidating the role of the 3435C>T SNP and its haplotype

Several SNPs in the exonic region of MDR1 occur frequently, indicating their importance during the evolution of MDR1. Much interest has been focused on the polymorphism at 3435, located in the middle of exon 26. This mutation, which changes cytosine to thymine, is found frequently (Supplemental Table 1). It is a wobble mutation that translates to isoleucine, a hydrophobic amino acid residue. This isoleucine is well conserved in different animal species, from humans to primates, mice and pigs. It is found in the second ATP binding domain, which is located between the Q-loop and the second signature motif on the intracellular side of the protein. The association of this SNP with expression of MDR1 was first reported by Hoffmeyer *et al* [67]. In their study, the presence of the homozygous T allele showed reduced MDR1 expression, and the 3435T allele was associated with two-fold reduction of MDR1 expression in the duodenum [67]. Many additional investigations have subsequently been performed on phenotypes associated with the 3435C>T SNP. An important characteristic of this SNP is that its allele frequency varies in different human populations. Supplemental Table 1 summarizes the allele and genotype frequencies of the MDR1 3435C>T SNP in healthy African, Asian, Indian, South American, Jewish, and Caucasian subjects. More studies have been done on

Asians and Caucasians than the other groups. In general, the C alleles are more frequent than T alleles, hence they are considered wild-type. Africans have significantly higher wild-type allele frequency (>74%) and at least 50% carry both C alleles.

The discovery of the synonymous 3435C>T SNP and its association with MDR1 expression led to speculation that other non-synonymous polymorphisms might be linked to the 3435C>T SNP. Linkage analysis, which uses mathematical calculation to estimate the occurrence of multiple SNPs, confirmed that this SNP is associated with several other SNPs in MDR1. These sets of mutations, called haplotypes, have been catalogued in several studies [68-71]. The haplotypes range in number from 3 to 55. The most frequent haplotype consists of the 3435C>T polymorphism combined with 2677G>T/A, and/or 1236C>T. Strong linkage disequilibrium among these SNPs has been commonly found in multiple studies [70,72-76]. In this haplotype, the SNP at position 2677 is a non-synonymous polymorphism. The 2677G>T/A mutation leads to one of two possible amino acid changes but their occurrences are not similar. The occurrence of 2677G>T (A893S) is far more frequent than G2677A (A893T). Another SNP, the 1236C>T polymorphism, is a synonymous polymorphism found in exon 12. The mutation is found in the third position that encodes glycine. The amino acid is found in the intracellular region before the second trans-membrane domain.

Since the frequency of the polymorphism at 3435 varies according to ethnicity, it is no surprise that the haplotype which carries this SNP shows a similar pattern. Table 2 summarizes the haplotype distribution of MDR1 among healthy individuals in different races. It is clear that people of African origin carry predominantly the wild-type (CGC) allele and not the haplotype allele (TTT). In Caucasian people, the frequency of CGC and TTT alleles is approximately the same. It is interesting that the TTT haplotype is the predominant genotype among Asian and Indian populations. Data show that the MDR1 haplotype also varies by ethnic group, resembling observations concerning the 3435C>T SNP. In addition, the frequency distribution forms a pattern, providing strong evidence that the MDR1 genotype gradually changed in the course of human migration, which originated from Africa [77].

4.2 Clinical and in vitro studies of MDR1 haplotype

Numerous clinical studies have been performed to examine whether MDR1 polymorphisms, especially the 3435C>T mutation, is a predictive factor in the onset of certain diseases (e.g. cancer, epilepsy, ulcerative colitis) or clinical outcome (immunosuppressant, anti-HIV protease inhibitor, anti-cancer drugs) [78-82]. Although individual studies suggest linkage to 3435C>T, overall analysis of the data suggests that associations may not be statistically significant (reviewed in [83,84]). In addition, the association of the synonymous 3435C>T mutation and protein expression in different organs (e.g., duodenum, intestine, placenta, liver, kidney) has been extensively studied [67,85-90]. Similarly, these studies were not conclusive, with conflicting observations. These observations led to a shift from studying 3435C>T towards the study of the clinical effects of the MDR1 haplotype. Although there have been numerous studies on the 3435C>T mutation, not many have appeared regarding MDR1 haplotypes. In these studies, the sample size of the haplotype is often too low to determine significance. Nonetheless, recent evidence suggests that using the common haplotype (1236C>T, 2677G>T, 3435C>T) may be a better way to detect linkage to a phenotype than just studying only the 3435C>T polymorphism. The effect of the haplotype on disease outcome, including cancer, has been reported. Supplemental Table 2 summarizes recent studies on genotype frequencies and cancer. Similar to the 3435C>T studies, clinical studies showing that the haplotypes influence drug response and disease outcome are yet to be confirmed.

Other than significant ethnic differences, association of the 3435C>T polymorphism with MDR1 expression has not been shown *in vitro*. Cell lines expressing MDR1 with the 3435C>T mutation do not have altered cell surface expression or transport function, compared with cells

with the wild-type gene [64,91] (summarized in Table 3). Clearly the 3435C>T mutation is not the sole reason for lower expression. However, patients carrying the TT genotype at the 3435 SNP site have been reported to have lower MDR1 expression [67]. How does a silent polymorphism at the end of the *MDR1* transcript change its expression? One possibility is that the 3435C>T may be linked to unidentified SNP(s) in the promoter/3' UTR region, which could affect transcription and lower mRNA levels. However, linkage analysis in Japanese patients has not revealed a significant association between the 3435C>T SNP and other polymorphisms in the promoter region [75]. Another explanation is that *MDR1* mRNA stability is changed by the 3435C>T mutation. Wang and colleagues reported that in the liver, the 3435 SNP C allele has higher expression than the T allele [92]. *In vitro* expression in CHO cells in the same study showed that transcripts carrying the T mutation degrade faster than those of the wild-type [92]. However, several lines of evidence suggest that 3435C>T does not alter expression of MDR1. No difference was found in the surface expression of MDR1 wild-type and haplotype in HEK-293 cells using the Flp-In method (a technique which targets the transgene to a specific genomic DNA region) [93]. Expression of the haplotype (which includes the 3435C>T mutation) in several cell lines also yielded comparable expression levels with the wild-type protein [59,64]. Our lab has also constructed stable cell lines which express the MDR1 wild-type and haplotype. The cell lines show no difference in *MDR1* mRNA expression and produce full-length protein. These observations, different from those reported using clinical samples, suggest that mRNA stability does not completely explain the effect of the 3435C>T mutation on protein function.

5. Molecular mechanism of the phenotypic effect of 3435C>T

5.1 3435C>T affects MDR1 function

The frequently occurring 3435C>T mutation has been extensively studied in recent years. Studies using cell lines and patient samples have suggested that this mutation leads to several changes, from mRNA level, protein expression, protein folding to substrate specificity. How does one synonymous mutation change the structure and function of MDR1 in so many ways? Recent reports have focused on the association of this synonymous SNP with changes in MDR1 transport function, and also to changes in drug pharmacokinetics or disease outcome. Our lab has confirmed that, in the MDR1 common haplotype, this mutation is critically responsible for subtle MDR1 function and structural changes [64]. We found that both wild-type and haplotype MDR1 could be expressed in Hela cells by an infection/transfection system. Recombinant wild-type and haplotype cells express comparable amounts of MDR1 protein on the cell surface and exhibit similar transport function. However, inhibition of MDR1 function by cyclosporine A in cells expressing wild-type MDR1 and the haplotype is different. The haplotype including the 3435C>T SNP had altered susceptibility to verapamil but not to rapamycin. We also observed subtle folding differences between wild-type MDR1 and the haplotype. By using the antibody UIC2 and limited tryptic digestion, we confirmed that the conformation of wild-type MDR1 is different from the haplotype. These changes are dependent on the presence of the 3435C>T mutation. Mutation of 3435C>A led to more significant changes in the effect of inhibitors. Our study suggested that synonymous mutations in the MDR1 haplotype produce a subtle but measurable change in substrate binding site conformation. Several researchers have taken another approach, attempting to express wild-type MDR1 and the haplotype in order to mimic the effect found in clinical samples. Human MDR1 can be expressed in various organisms and cell lines. The MDR1 haplotype can also be expressed in cell lines originating from different hosts (LLC-PK1, CHO, Hela, HEK-293, Sf-9) (Table 3). These studies all conclude that the 3435C>T SNP and its haplotype do not affect surface expression of MDR1.

5.2 MDR1 function changed by ribosome stalling

Changes in a protein's function caused by a synonymous polymorphism cannot be explained by the amino acid sequence of the protein. Yet clinical and *in vitro* observations concerning the MDR1 3435C>T SNP strongly suggest that this "silent" polymorphism changes the folding and function of MDR1 [64,67]. Our group suggested that the use of a rarer codon may be responsible for the alternation of kinetics in translation [64]. Therefore the abundance of a tRNA species could influence protein folding dynamics. Recently, Tsai and colleagues have suggested that synonymous SNPs cause ribosome stalling [94]. Proteins are folded through distinct folding pathways. Sufficient translational stalling signals by mutation would lead to distinct minima at the bottom of a folding funnel, and generate a protein with altered function [94].

Current protein folding principles predict that the conformation of wild-type MDR1 and MDR1 with the 3435C>T mutation should be identical since their amino acid sequences are identical. Protein translation is the process by which mRNA is translated into proteins. It is affected by many factors, such as alternate splicing events, mRNA structure, translation kinetics and protein binding. It consists of initiation, elongation and termination. During the initiation step, the 5' region of the mRNA interacts with translation initiation factors that recruit a ribosome complex. This complex runs along the mRNA and begins to synthesize the polypeptide when the first methionine codon is scanned. The next phase, translation elongation, occurs as a continuous series of translocation-pause-translocation events [95]. There are several components that influence the rate of polypeptide synthesis during translation elongation, including codon-anticodon interaction [96], codon context [97], rate of movement of the RNA complex [95], the secondary RNA structure [98] and the abundance of transfer-RNA (tRNA) around the translation machinery. At the end of translation, the ribosome complex ceases protein production when one of the three termination codons (TAA/TAG/TGA) moves into the A site.

Organisms use different codons in order to translate a particular amino acid [99]. Assembly of amino acids occurs within the ribosome complex, which consists of an A-site, P-site and E-site for tRNA docking and amino acid assembly [100]. In the translation initiation, the initiator tRNA (a tRNA bound with methionine) binds to the P-site. Then an aminoacyl-tRNA (a tRNA covalently bound to its amino acid) able to pair with the next codon on the mRNA arrives at the A site. The preceding amino acid covalently links with the incoming amino acid with a peptide bond. The ribosome complex moves downstream by one codon (Figure 2) that pushes the tRNA in the P-site to E-site, and is subsequently released from the large subunit of the ribosome complex. These actions leave a vacant A-site which allows docking of a new aminoacyl-tRNA and formation of a new amino acid in the nascent polypeptide chain. As the ribosome requires tRNA to move along the mRNA, translation elongation could be affected by those two factors. In mRNA, a nucleotide mutation that changes the codon could alter the time required for the aminoacyl-tRNA docking to the A-site. Alternatively, nucleotide mutation could alter the mRNA secondary structures that generate or eliminate new secondary structures such as hairpins or pseudoknots. Any of these changes could stall the ribosome and affect translation.

If the mutation at the MDR1 3435C>T SNP site changes the speed of the ribosome complex during translation, there may be several consequences. First, a ribosome pause causes tRNA dissociation from the ribosome, followed by recruitment of the same aminoacyl-tRNA again from the tRNA pool and continued translation. Second, a ribosome complex may slow down or briefly stop, and then resume. Third, a ribosome sometimes stalls and recognizes a new codon by moving one nucleotide, which is called frameshifting [101]. The last and the worst case is when a ribosome stall site acts as a stop signal. The ribosome complex discontinues running along the transcript and subsequently detaches from the template.

Our current understanding is that 1) the MDR1 haplotype can be fully expressed 2) no truncated *MDR1* mRNA or altered protein sequence has been found, and 3) no putative ribosome frameshift sites have been identified near the 3435C>T SNP site. In fact, some of the above possible consequences would trigger endogenous “surveillance systems”. Generation of prematurely detached mRNA would be degraded rapidly by a mechanism called nonsense-mediated mRNA decay [102]. Degradation of incomplete proteins appears to be an active process requiring specific *trans*-acting factors. Premature termination by ribosome stalling might activate trans-translation, a mechanism found in bacteria. The rare proteins involved in trans-translation interact with stalled translational complexes to release the stalled ribosome and target the nascent polypeptide and mRNA for degradation [103]. In eukaryotes, ribosome pausing can lead to abandonment of translation machinery through a process called mRNA no-go-decay (NGD). NGD could be a result of an mRNA structural change that triggers interaction of the stalled ribosome with the two proteins Dom34p and Hbs1p. The presence of these factors facilitates endonucleolytic cleavage and increases RNA degradation [104].

6. Possible impact of translational pauses in MDR1 haplotype

Synonymous mutation-mediated ribosome stalling may affect domains ahead of the pause sites. The ribosome is a large macromolecular complex consisting of large and small subunits. It runs along the mRNA from 5' to 3' catalyzing polypeptide synthesis from information recorded in mRNA. Formation of the peptide bond is found in the peptidyl transferase center (PTC) in the large subunit [105]. During translation, growing nascent polypeptides must travel through a portion of the ribosome before they are handed over to chaperones or to the protein target machinery. This portion of the structure is called the ribosome tunnel, and the number of amino acids occluded in the tunnel varies from 30 to 72 [106]. The translation rate can be affected by interactions of certain nascent chains with the ribosomal exit tunnel in a regulatory fashion. In bacteria, for example, SecM interacts with SecA at the ribosome exit. Its regulation is achieved by controlled ribosome stalling [107].

Folding of a polypeptide chain into its final conformation starts during translation in the endoplasmic reticulum. Immediately after the amino acid leaves the translation tunnel, the amino acid shapes itself. The folding process is highly organized and affected by many factors including cellular environment and molecular chaperones. Co-translational folding is believed to be an important process during protein formation (reviewed by [108]). MDR1 is known to interact with chaperones such as HSP-90 that are believed to help co-translational folding [109]. Each folding process may involve hundreds of proteins that group as co-chaperones. For example, CFTR (ABCC7) is an important genetic factor that causes cystic fibrosis by deleting a phenylalanine in the coding region. The mutant protein cannot function because it cannot be targeted to the plasma membrane. Modulation of several co-chaperones (e.g., AHA1) could partially rescue folding defects [110]. Like many other proteins, ABC transporters should be folded with the help of chaperones for folding, processing, and targeting.

6.1 Impact of the 3435C>T mutation

If the 3435C>T polymorphism affects co-translational folding in nearby amino acids, the mutation may interfere with translation from 30 to 72 codons before the mutant codon. The 3435C>T SNP site is found in the second ATP binding domain. Analysis of the amino acid sequence indicates that an additional pause signal could interfere with the folding of two motifs. First, it could affect formation of the Q-loop, a key element in the nucleotide binding domain mechanism that shapes the ATP binding domain. In mouse *mdr3*, the Gln471 and Gln1114 (which correspond to Gln475 and Gln1118 in human MDR1 in the first and second Q-loop) are responsible for nucleotide binding domain and substrate binding site communication [111]. In addition, the second Walker-A domain could be affected as well. The Walker-A

domain includes amino acids that bind ATP. Mutation of these amino acid residues (e.g. C1074, K1076) affects MDR1 function by reducing ATP binding affinity, disrupting disulphide bond formation or loss of ATP hydrolysis [112-115].

6.2 Impact of the 1236C>T mutation

The 1236C>T SNP is another synonymous polymorphism in MDR1 that has been studied. It is a synonymous polymorphism which encodes glycine in the first ABC domain, found between the A-loop and Walker-A domain. Several amino acid residues around this SNP (e.g., Y401, S400N) are essential for ATP binding and ATP hydrolysis [42,49]. This mutation in MDR1 appears to change neither protein expression nor protein function. Experiments have also shown that this mutation is not related to changes in mRNA stability [92]. If the 1236C>T SNP generates a ribosome pause site, it might affect co-translational folding of TM6, which is essential for UIC2 antibody recognition and substrate binding [53]. The A-loop (Y401) may not be affected by the stall because it is still occluded within the ribosome tunnel when the machinery interacts with the wobble mutation. The impact of the 1236C>T mutation could result from the use of a rarer codon (from GGC to GGT), similar to the 3435C>T SNP.

6.3 Impact of the 2677G>T mutation

Ribosome stalling could be caused by a non-synonymous polymorphism because of a nucleotide change. If a change of the nucleotide sequence at 2677 (A893) results in a ribosome pause, it may affect co-translation of amino acids before TM9 and TM10. There is no apparent functional domain within this segment, which forms an intracellular loop. Nevertheless, mutation studies have identified several amino acids (G830V, I849M) that could change the kinetics of drug-induced ATPase activity [42,116]. Folding of TM10 has an important effect on drug resistance. Adriamycin and actinomycin D but not vinblastine transport is reduced by mutation of the conserved proline residue (P866) within TM10 [50]. Biochemical analysis has confirmed that mutation of A893 from alanine to serine or threonine may alter drug transport [42]. This evidence suggests that the A893 mutation likely affects drug-induced ATPase activity.

This analysis suggests that each of the alleles that make up the complete haplotype may contribute small but significant, additive or synergistic changes that lead to altered protein folding and altered function. Co-translational folding of the two MDR1 ATPase domains is crucial for its function. After successive translation, the two ATP binding domains are folded together to form a large domain that can hydrolyze two ATPs to ADPs in an inter-dependent manner [3,23,117]. While the impact of each of the SNPs in the MDR1 haplotype may be independent, when present together they may produce a much more salient phenotype.

7. Conclusions and perspectives

In MDR1, most non-synonymous SNPs do not change protein function. There is a misconception that all synonymous SNPs are “silent” because they do not change the encoded amino acid. In fact, cumulative evidence suggests that gene functions and onset of human diseases could be affected by synonymous polymorphisms (reviewed in [118,119]). In one MDR1 haplotype, there are two synonymous SNP sites (1236C>T and 3435C>T) that occur frequently and have a distinctive distribution pattern in human populations. The ethnic diversity of MDR1 shows a clear pattern concerning conservation of this haplotype in the course of human migration. Genotyping of the single polymorphism at 3435C>T SNP shows that the C allele is the dominant ancestral allele, carried by most people of African origin. It is unclear what drives the increased frequency of the 3435T genotype and to its associated haplotype in Caucasians and Asians. The existence of synonymous or non-synonymous polymorphisms in human *MDR1* may provide room for modification of MDR1 function in a manner that could

provide selective advantage in some populations exposed to environmental toxins or subject to specific diseases. Evidence shows that the MDR1 haplotype displays folding and substrate specificity differences from the wild-type [64,93]. The haplotype protein structure is changed, and is generally less responsive to several but not all MDR1 modulators [64,93]. These observations lead to an important question: does the 3435C>T mutation, together with the 2677G>T and 1236C>T mutations, somehow improve the function of the protein under a certain set of circumstances? In order to further explore this possibility, the functions of wild-type of MDR1 and its haplotype should be carefully and broadly examined. More biochemical and quantitative analysis should be performed.

We speculate that the altered shape and function of MDR1 comes from changes resulting from a combination of mutations. Most of the known MDR1 SNPs and haplotypes do not affect protein expression *in vitro*, suggesting that MDR1 expression is important for proper function and therefore cell survival. Most of the domains and motifs are important for protein function because the amino acid sequences are conserved in the most common polymorphisms and haplotypes. It seems that MDR1 SNPs do not occur randomly. Polymorphisms included in the haplotype are linked, and have evolved as a group.

Although it has been determined that the 3435C>T mutation plays a key role in modifying the function of MDR1, how this happens on a molecular level is not fully understood. Based on bacterial studies, we can predict that codon usage should have an effect on the rhythm of translation. A single nucleotide change from an abundant codon to a rarer one may change mRNA structure and alter the co-translational folding pathway, or may interrupt ribosome movement because of a low abundance of tRNA. The effect of a synonymous polymorphism may not affect the immediate encoding amino acid itself, but may interfere with amino acids or protein domains nearby. While this hypothesis needs to be further examined, the effect of these mutations is certain, and should no longer be considered “silent”. Anfinsen’s principle [120], which states that the amino acid sequence *per se* governs protein conformation, cannot explain the effect of synonymous polymorphisms. Some may argue that a significant translation pause signal could create a large amount of misfolded protein. However, from the evidence, it seems that the MDR1 haplotype can be properly expressed on the cell surface, but does not activate cellular folding surveillance machinery, as do the N-glycosylation defective or Y490 deletion mutants [18,121]. There is more and more evidence suggesting that folding information is stored at different levels, and mRNA plays an important role in folding dynamics.

The presence of synonymous polymorphisms in drug transporters is not limited to MDR1. In the ABC gene family, several members (*BCRP*, *MRP1*, *MRP2*) are also considered as important multidrug transporters. In *MRP1*, some SNPs (e.g., T1684C and G4002A) also vary according to ethnicity [122], and may be linked to functional changes. A synonymous SNP in *MRP2* (C3972T) has been identified and is found frequently among Japanese [123]. It is a wobble mutation found in the second ATP binding domain where the MDR1 3435C>T SNP is also located. Such a coincidence may suggest the possibility that this *MRP2* SNP may also affect *MRP2* protein function. In addition to drug transporters, drug metabolizing enzymes are important for the detoxification process. In the cytochrome P450 system, which catalyzes oxidative reactions, many new SNPs have been identified (reviewed in [124]). A study with a cohort of 92 docetaxel treated patients found that the CYP3A5 (A22893G) SNP is linked to the MDR1 haplotype [86].

The presence of the 3435C>T polymorphism may also lead to changes in the rhythm of translation. The precise consequence of a translation pause, if it occurs, through this polymorphism is not clear. There are several important questions. How does a translation delay change the global protein structure? What is the minimal requirement to generate a significant

translational pause? Is the pause created by a single polymorphism? What is the relationship of codon usage to a translation pause signal? How does a folding change affect protein function? Study of the important 3435C>T SNP in MDR1 reveals the special nature of human genetic evolution, highlighting the ability of human genes to fine-tune themselves in response to new challenges to human survival.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

ABC	ATP-binding cassette
ATP	adenosine triphosphate
ADP	adenosine diphosphate
CFTR	cystic fibrosis trans-membrane conductance regulator
CYP3A4	cytochrome P450 3A4
CYP3A5	cytochrome P450 3A5
IVS	intervening sequence
MDR1	multidrug resistant 1

SNP	single nucleotide polymorphism
TM	trans-membrane
tRNA	transfer RNA
UTR	untranslated region

Figure 1.

Schematic diagram of MDR1 showing the amino acids affected by SNPs. This is a hypothetical two-dimensional model of human MDR1. Each circle represents one amino acid residue. Red circles represent the amino acids affected by SNPs. Amino acid residues affected by synonymous SNPs are marked with triangles. The SNPs of the MDR1 haplotype (G412, A893 and I1145) are circled. The regions that encode A-loops, D-loops, H-loops, Q-loops, signature motifs, Walker-A and Walker-B motifs are colored in green, and the amino acid positions of these motifs are summarized in the table. Phosphorylation sites and glycosylation are also shown. (Modified from Ambudkar, SV, *et al.*, Biochemical, cellular, and pharmacological aspects of the multidrug transporter, *Annu. Rev. Pharmacol. Toxicol.* (1999):39:361-98)

Figure 2.

The impact of translational delay on co-translational folding. Left: Amino acid sequence (from 958 to stop codon) of *MDR1*. Domain sequences are in bold. Putative amino acids (ranging from 30 to 72) occluded by the ribosome complex when the A-site interacts with the isoleucine codon (Ile1145) are underlined. Underlined domains include the Q-loop and Walker-A domains. Right: Translation pause caused by synonymous mutation leads to a change in co-translation folding. When the ribosome complex runs along the mRNA, it decodes the information on the transcript by recruiting aminoacyl tRNA to the A-site, where the tRNA anticodon base pairs with its complementary sequence on the mRNA. The amino acid that the aminoacyl tRNA carries is covalently bound to the growing polypeptide. The nascent chain passes through a tunnel in the ribosome complex. Interaction with chaperone proteins that aid the folding process starts from the tunnel exit. This process is believed to be precise and highly organized, determining the fate of the protein. Properly folded protein will be transferred to its correct destination. Unfolded proteins detected by “surveillance” proteins are targeted for degradation. A synonymous mutation may create a translation pause, which slows down the ribosome. A significant delay could interrupt the timing of chaperone-protein interaction and produce a protein with a slight conformation change. The change could be subtle, and the resulting molecule would not be considered as an unfolded or improperly folded protein. The translation pause may also affect the folding pathway of adjacent motifs.

Table 1

Single nucleotide polymorphisms in the *MDR1* (ABCB1) gene

Exon no.	mRNA position	Wild-type allele	Variant allele	Amino acid position	Amino Acid residue	Amino acid change	Function	Ref.	Neighbor domains
1	-129	T	C	UT	UT	UT	Non-coding		5' UTR
2	43	A	G	15	Asn	Asp	Missense		Intracellular, before TM1
2	49	T	C	17	Phe	Leu	Missense		Intracellular, before TM1
2	61	A	G	21	Asn	Asp	Missense	[59,60]	Intracellular, before TM1
3	131	A	G	44	Asn	Ser	Missense		Intracellular, before TM1
4	240	C	A	80	Ala	Gly	Missense		Extracellular between TM1 and TM2
4	267	T	C	89	Met	Thr	Missense		Extracellular, close to N-glycosylation sites
5	307	T	C	103	Phe	Leu	Missense	[60]	Extracellular, close to N-glycosylation sites
7	548	A	G	183	Asn	Ser	Missense		Intracellular, close to G185 residue, near TM3
8	729	A	G	243	Glu	Glu	Synonymous		Intracellular between TM4 and TM5
8	738	G	A	246	Ala	Ala	Synonymous		Intracellular between TM4 and TM5
8	782	A	G	261	Ile	Val	Missense		Intracellular between TM4 and TM5
11	1199	G	A	400	Ser	Asn	Missense	[42,59,60]	Intracellular, before first A-loop
12	1236	C	T	412	Gly	Gly	Synonymous	[64]	Intracellular between first A-loop and Walker A motif
12	1308	A	G	436	Thr	Thr	Synonymous		Intracellular, after first Walker A motif
13	1474	C	T	492	Arg	Cys	Missense	[42]	Intracellular between first Walker-A motif and signature motif
14	1617	C	T	539	Ile	Ile	Synonymous		Intracellular, within signature motif
14	1662	G	C	554	Leu	Leu	Synonymous		Intracellular, within Walker-B motif
14	1696	G	A	566	Gly	Lys	Missense		Intracellular between first D-loop and H-loop

Exon no.	mRNA position	Wild-type allele	Variant allele	Amino acid position	Amino Acid residue	Amino acid change	Function	Ref.	Neighbor domains
15	1777	C	T	593	Arg	Cys	Missense		Intracellular, between first H-loop and linker region
15	1794	C	T	598	Ile	Ile	Synonymous		Intracellular, between first H-loop and linker region
15	1795	G	A	599	Aa	Thr	Missense		Intracellular, between first H-loop and linker region
16	1985	T	G	662	Leu	Arg	Missense	[61]	Intracellular, within linker region, next to phosphorylation site S661
16	2005	C	T	669	Arg	Cys	Missense	[42,59,61]	Intracellular, within linker region, between two phosphorylation sites (S667, S671)
20	2401	G	A	801	Val	Met	Missense		Intracellular, between TM8 and TM9
21	2485	A	G	829	Ile	Val	Missense		Intracellular, between TM8 and TM9
21	2505	A	G	835	Val	Val	Synonymous		Within TM9
21	2506	A	G	836	Ile	Val	Missense		Within TM9
21	2587	A	G	849	Ile	Met	Missense		Within TM9
21	2650	C	T	884	Leu	Leu	Synonymous		Intracellular, between TM10 and TM11
21	2677	G	T	893	Ala	Ser	Missense	[42,59,61,64]	Intracellular, between TM10 and TM11
21	2677	G	A	893	Ala	Thr	Missense	[42,59,64]	Intracellular, between TM10 and TM11
24	2956	A	G	986	Met	Val	Missense	[42]	Within TM12
24	2995	G	A	999	Ala	Thr	Missense	[42,60]	Intracellular, between TM12 and second Walker-A motif
24	3084	G	A	1028	Pro	Pro	Synonymous		Intracellular, between TM12 and second Walker-A motif
25	3151	C	G	1051	Pro	Ala	Missense	[42]	Intracellular, between TM12 and second Walker-A motif

Exon no.	mRNA position	Wild-type allele	Variant allele	Amino acid position	Amino Acid residue	Amino acid change	Function	Ref.	Neighbor domains
26	3320	A	C	1107	Gln	Pro	Missense		Intracellular, between second Walker-A motif and Q-loop
26	3322	T	C	1108	Trp	Arg	Missense	[61]	Intracellular, between second Walker-A motif and Q-loop
26	3396	C	T	1132	Ala	Ala	Synonymous		Intracellular, between second Q-loop and signature motif
26	3421	T	A	1141	Ser	Thr	Missense	[59,61]	Intracellular, between second Q-loop and signature motif, close to C3435
26	3435	C	T	1145	Ile	Ile	Synonymous	[64]	Intracellular, between second Q-loop and signature motif
28	3747	C	G	1249	Gly	Gly	Synonymous		Intracellular, after second H-loop
28	3751	G	A	1251	Val	Ile	Missense	[59]	Intracellular, after second H-loop
28	3767	C	A	1256	Thr	Lys	Missense		Intracellular, after second H-loop
28	4282	T	C	UT	UT	UT	Non-coding		3' UTR
28	4350	A	T	UT	UT	UT	Non-coding		3' UTR
28	4407	G	A	UT	UT	UT	Non-coding		3' UTR
28	4454	A	G	UT	UT	UT	Non-coding		3' UTR
28	4513	A	C	UT	UT	UT	Non-coding		3' UTR
28	4577	G	A	UT	UT	UT	Non-coding		3' UTR
28	4823	T	C	UT	UT	UT	Non-coding		3' UTR

Table 2
Allele and genotype frequencies of the MDR1 haplotype (1236C>T, 2677G/T, 3435C>T) according to ethnicity

Author	Year	Ethnicity	Ref	Sample	Haplotypes catalogued	CGC	TTT	others
African								
Kim RB	2001	African American	[63]	23	15	69.50%	6.50%	24.00%
Kroiz DL	2003	African American	[70]	99	55	43.60%	8.70%	47.70%
Tang K	2004	African American	[69]	39	13	71.96%	6.49%	21.55%
Allabi AC	2005	Beninese	[125]	109	3	79.30%	4.50%	16.20%
South American								
Estrela RC	2008	Brazilian (Black)	[126]	100	8	58.90%	15.80%	25.30%
Estrela RC	2008	Brazilian(Intermediate)	[126]	114	8	55.40%	21.70%	22.90%
Estrela RC	2008	Brazilian (White)	[126]	106	8	48.40%	35.90%	15.70%
Wielandt AM	2004	Chilean (<i>Pascuense</i>)	[127]	35	8	65.00%	14.00%	21.00%
Wielandt AM	2004	Chilean (Mapuche)	[127]	51	8	25.00%	6.00%	69.00%
Wielandt AM	2004	Chilean (Mestizo)	[127]	59	8	53.00%	7.00%	40.00%
Asian								
Kroiz DL	2003	Asian American	[128]	60	13	21.60%	28.20%	50.20%
Tang K	2002	Chinese	[73]	104	10	23.16%	38.92%	37.91%
Tang K	2004	Chinese	[69]	48	13	25.93%	35.05%	39.02%
Xu P	2008	Chinese	[129]	207	12	16.80%	35.70%	47.50%
Li D	2007	Chinese (Han)	[130]	165	12	20.10%	33.30%	46.60%
Li D	2007	Chinese (Kazakh)	[130]	108	12	21.90%	34.50%	43.60%
Li D	2007	Chinese (Uygur)	[130]	161	12	30.10%	45.30%	24.60%
Yi SY	2004	Korean	[131]	232	10	18.60%	32.20%	49.20%
Choi JH	2007	Korean	[132]	29	7	20.70%	41.40%	37.90%
Indian								
Tang K	2002	Indian	[73]	68	9	17.56%	31.38%	51.06%
Tang K	2004	Indian	[69]	33	13	23.45%	56.09%	20.46%
South Asian								

Author	Year	Ethnicity	Ref	Sample	Haplotypes catalogued	Genotype		
						CGC	TTT	others
Tang K	2002	Malay	[73]	93	6	28.54%	49.47%	22.00%
Tang K	2004	Malay	[69]	43	13	22.70%	32.92%	44.38%
Jewish								
Kimchi-Sarfaty C	2008	Ashkenazi (Israel)	[133]	101	8	31.70%	23.60%	44.70%
Caucasian								
Jeannesson E	2007	French	[76]	222	12	44.00%	35.30%	20.70%
Kim RB	2001	Caucasian	[63]	37	15	43.30%	40.60%	16.10%
Krotz DL	2003	Caucasian	[70]	100	25	32.50%	42.00%	25.50%
Potonik U	2008	Caucasian	[44]	355		45.00%	37.00%	18.00%
Tang K	2004	Caucasian	[69]	38	13	37.69%	42.19%	20.12%
Wasilewska A	2007	Poland (Children)	[134]	135	12	42.80%	41.70%	15.50%
Potonik U	2004	Slovenian	[135]	355	12	40.10%	23.10%	36.80%

Table 3

Cell lines expressing MDR1 and its common haplotype

Author	Year	Ref.	Host cell line	Method	Expression level	Haplotypes tested	Compounds used for assay	Remarks
Kroetz D	2003	[70]	HEK-293	Transient	NA	1236T-267T-3435T	Calcein-AM	No difference between CCC haplotype and TTT haplotype
Morita N	2003	[136]	LLC-PK1	Stable	No difference	2677A-3435C	Verapamil Vinblastine, Cyclosporin A Digoxin	2677G>T/A or 3435C>T and haplotype had no effect on MDR1 function
						2677A-3435T		
						2677G-3435C		
						2677G-3435T		
						2677T-3435C		
Wang D	2005	[92]	CHO	Transient	No significant difference in protein synthesis	2677T-3435T	Actinomycin D	TTT haplotype showed lower mRNA stability, and is dependent on 3435C>T mutation
						1236C-2677G-3435C		
						1236T-2677T-3435T		
						1236T-2677T		
						1236T-3435T		
Kimchy-sarfaty C	2007	[64]	Hela	Transient	No difference	2677T-3435T	Rhodamine 123, Bodipy-FL-verapamil, digoxin rapamycin Bodipy-FL-Paclitaxel	TTT haplotype alter protein conformation. Protein function is different depending on substrate use
						1236T-2677T		
						1236T-3435T		
						1236T-2677T-3435T		
						1236T-2677T-3435A		
Hung CC	2008	[93]	Flp-In HEK-293	Stable	No difference	1236T-2677A	Rhodamine 123 Calcein-AM Phenylethanol, Cyclosporin A Phenobarbital Valproic acid Lamotrigine Carbamazepine Gabapentin Levetiracetam	Certain inhibitors against Rhodamine 123 transport are less effective in double haplotype and TTT haplotype
						1236T-2677T		
						1236T-3435T		
						2677A-3435T		
						2677T-3435T		
Salama NN	2006	[91]	LLC-PK1	Stable	No apparent difference	1236T-2677A-3435T	Rhodamine 123, Vincristine Vinbalsatine	Single or haplotype mutations had
						1236T-2677T-3435T		
						1236T-2677T		

Author	Year	Ref.	Host cell line	Method	Expression level	Haplotypes tested			Compounds used for assay	Remarks
						1236T-3435T	2677T-3435T	1236T-2677T-3435T		
GOW JM	2008	[59]	HEK-293T	Transient	No difference	1236T-2677T-3435T			Calcein-AM, Cyclosporin A, Bodipy-FL-paclitaxel	TTT haplotype with N21D mutation reduce MDR1 function with BODIPY-FL-paclitaxel but not Calcein-AM. TTT haplotype showed no difference compared to control