Review

MDR1 Genotype-related Pharmacokinetics: Fact or Fiction?

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Summary: Multidrug resistant transporter MDR1/P-glycoprotein, the gene product of MDR1, is a glycosylated membrane protein of 170 kDa, belonging to the ATP-binding cassette superfamily of membrane transporters. A number of various types of structurally unrelated drugs are substrates for MDR1, and MDR1 and other transporters are recognized as an important class of proteins for regulating pharmacokinetics. The first investigation of the effects of MDR1 genotypes on pharmacotherapy was reported in 2000; a silent single nucleotide polymorphism (SNP), C3435T in exon 26, was found to be associated with the duodenal expression of MDR1, and thereby the plasma concentration of digoxin after oral administration. In the last 5 years, clinical studies have been conducted around the world on the association of MDR1 genotype with MDR1 expression and function in tissues, and with the pharmacokinetics and pharmacodynamics of drugs; however, there are still discrepancies in the results on C3435T. In 1995, a novel concept to predict in vivo oral pharmacokinetic performance from data on in vivo permeability and in vitro solubility has been proposed, and this Biopharmaceutical Classification System strongly suggested that the effects of intestinal MDR1 on the intestinal absorption of substrates is minimal in the case of commercially available oral drugs, and therefore MDR1 genotypes are little associated with the pharmacokinetics after oral administration. This review summarizes the latest reports for the future individualization of pharmacotherapy based on MDR1 genotyping, and attempts to explain discrepancies.

Key words: MDR1; P-glycoprotein; pharmacogenetics; pharmacogenomics; pharmacokinetics; expression; function; disease susceptibility

Introduction

In 1976, Juliano et al. isolated a 170 kDa glycosylated membrane protein from colchicine-resistant Chinese hamster ovary cells. This glycoprotein appeared unique to sublines displaying altered drug permeability, and was named P-glycoprotein. About 10 years later, a gene was isolated by Chen et al. and Roninson et al. from multidrug-resistant human tumor cells, and it has since been demonstrated to encode human P-glycoprotein and named MDR1. Several isoforms of P-glycoprotein have been identified from humans and rodents as part of the mechanism of multidrug resistance in tumors. Human P-glycoprotein was found to be a phosphorylated and glycosylated protein with 1280 amino acids, consisting of two homologous halves containing six putative hydrophobic transmembrane segments and an intracellular binding site for ATP (Fig. 1). P-glycoprotein is understood to act as a pump which removes its substrate inside the cell to outside, however, another model has been proposed, where P-glycoprotein acts as a flipase carrying its substrate from the inner leaflet of the lipid bilayer to the outer leaflet.

In 1987, Thiebaut et al. found that human P-glycoprotein is expressed also in normal tissues including the liver, kidneys, small and large intestines, brain, testis, muscle tissue, placenta and adrenals, and over the last decade, it has been elucidated that it confers an intrinsic resistance to normal tissues by exporting unnecessary and toxic exogeneous substances or metabolites out of the body. Intestinal...
Fig. 1. Two-dimensional structure of MDR1. Some of the positions corresponding to the polymorphisms in the *MDR1* gene are indicated. MSDs: membrane-spanning domains. NBDs: nucleotide (ATP)-binding domains.

P-glycoprotein located in the brush border membrane of enterocytes limits absorption of the substrates. P-glycoprotein in the capillary endothelial cells of brain and testis also limits the transport of substrates into their extravascular space. P-glycoprotein in the luminal membrane of renal proximal tubules and in the biliary canalicular front of hepatocytes accelerates their secretion into the urine and bile, respectively. In 1992, Tanigawara *et al.*, and subsequently in 1993, Saeki *et al.*, demonstrated that cardiac glycoside digoxin, and the calcineurin inhibitors cyclosporine A (CsA) and tacrolimus (FK506) are the substrates for P-glycoprotein.17,18) These were actually the first reports in which drugs other than anticancer drugs were clarified to be substrates for MDR1, and to date, a number of various types of structurally unrelated drugs have been found to be transported by P-glycoprotein, and P-glycoprotein and other transporters are recognized as an important class of proteins for regulating pharmacokinetics.4–15) Immediately thereafter, P-glycoprotein was found to be a key protein for drug interaction,19) and much experimental evidence has been accumulating over the last decade.20–23) P-glycoprotein belongs to a large group of transport proteins, known as the ATP-binding cassette (ABC) superfamily, that share common structural and functional properties.24–26) More than 40 human ABC transporter genes have been identified, and at present, they are classified into 7 subfamilies, ABCA to ABCG. In this new system of nomenclature, P-glycoprotein is defined as ABCB1. Recently, the term MDR1 has come to be used instead of P-glycoprotein, so MDR1 is used in this review hereafter.

The first investigation of the effects of MDR1 genotypes on pharmacotherapy was reported by Hoffmeyer *et al.* in 2000.27) A silent single nucleotide polymorphism (SNP), C3435T in exon 26, was found to be associated with the duodenal expression of MDR1, and thereby the plasma concentration of digoxin after oral administration.27) In the past 5 years, clinical studies have been conducted around the world on the association of MDR1 genotype with MDR1 expression and function in tissues, and with the pharmacokinetics and pharmacodynamics of drugs; however, as summarized in recently published reviews,4–15) there are still discrepancies in the results on C3435T. This review summarizes the latest reports for the future individualization of pharmacotherapy based on MDR1 genotyping, and attempts to explain discrepancies.

**MDR1 Polymorphisms**

*MDR1* is located on human chromosome 7 at q21.1 in a 600 kb NruI fragment,28) and the *MDR1* coding region is contained in a 120 kb Xhol fragment.29) This gene extends over more than 100 kb containing 28 introns, 26 of which interrupt the protein-coding sequence. MDR1 mRNA has a size of 4.7 kb, thus its coding region accounts for less than 5% of the total. In 1989, the first report on the polymorphisms of the *MDR1* gene was presented by Kioka *et al.*30) Two amino acid substitutions, Gly185Val and Ala893Ser, were detected in MDR1 isolated from normal human adrenal glands, and the latter was suggested to reflect a genetic polymorphism. Polymorphism in the promoter region was reported in 1994, in a study in which osteosarcomas were used and the correlations with the responsiveness to chemotherapeutic agents were discussed.31) In 1998, Mickley *et al.* detected two polymorphisms, G2677T in exon 21 and G2995A in exon 24, based on an RNase protection analysis.32) The first systemic screening for MDR1 polymorphisms was performed by Hoffmeyer *et al.* in 2000.27) All 28 exons and the core promoter region
Table 1. Representative genetic polymorphisms in MDR1

<table>
<thead>
<tr>
<th>Position</th>
<th>Location</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1a/-41G</td>
<td>intron</td>
<td>noncoding</td>
</tr>
<tr>
<td>C-145G</td>
<td>exon 1a</td>
<td>noncoding</td>
</tr>
<tr>
<td>T-129C</td>
<td>exon 1b</td>
<td>noncoding</td>
</tr>
<tr>
<td>C-4T</td>
<td>exon 2</td>
<td>noncoding</td>
</tr>
<tr>
<td>G-1A</td>
<td>exon 2</td>
<td>noncoding</td>
</tr>
<tr>
<td>A61G</td>
<td>exon 2</td>
<td>Asn21Asp</td>
</tr>
<tr>
<td>G5/-25T</td>
<td>intron</td>
<td>Phe103Leu</td>
</tr>
<tr>
<td>G5/-35C</td>
<td>intron</td>
<td>Arg492Cys</td>
</tr>
<tr>
<td>T307C</td>
<td>exon 5</td>
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</tr>
<tr>
<td>C6/+139T</td>
<td>intron</td>
<td>silent</td>
</tr>
<tr>
<td>C6/+145T</td>
<td>intron</td>
<td>silent</td>
</tr>
<tr>
<td>A548G</td>
<td>exon 7</td>
<td>Asn183Ser</td>
</tr>
<tr>
<td>G1199A</td>
<td>exon 11</td>
<td>Ser400Asn</td>
</tr>
<tr>
<td>C1236T</td>
<td>exon 12</td>
<td>silent</td>
</tr>
<tr>
<td>C12/-44T</td>
<td>intron</td>
<td>silent</td>
</tr>
<tr>
<td>C1474T</td>
<td>exon 13</td>
<td>silent</td>
</tr>
<tr>
<td>T17/-76A</td>
<td>intron</td>
<td>silent</td>
</tr>
<tr>
<td>A17/+137G</td>
<td>intron</td>
<td>silent</td>
</tr>
<tr>
<td>C2650T</td>
<td>exon 21</td>
<td>silent</td>
</tr>
<tr>
<td>G2677A,T</td>
<td>exon 21</td>
<td>Ala893Thr (G2677A)</td>
</tr>
<tr>
<td>A2956G</td>
<td>exon 24</td>
<td>Met986Val</td>
</tr>
<tr>
<td>G2995A</td>
<td>exon 24</td>
<td>Ala999Thr</td>
</tr>
<tr>
<td>A3320C</td>
<td>exon 26</td>
<td>Gln1107Pro</td>
</tr>
<tr>
<td>C3396T</td>
<td>exon 26</td>
<td>silent</td>
</tr>
<tr>
<td>T3421A</td>
<td>exon 26</td>
<td>Ser1141Thr</td>
</tr>
<tr>
<td>C3435T</td>
<td>exon 26</td>
<td>silent</td>
</tr>
<tr>
<td>G4030C</td>
<td>exon 28</td>
<td>silent</td>
</tr>
<tr>
<td>A4036G</td>
<td>exon 28</td>
<td>silent</td>
</tr>
</tbody>
</table>

See references 27, 32–36.

C3435T is in linkage disequilibrium with C1236T and G2677T.

were amplified by PCR, covering the coding exons and sequences at the exon-intron boundaries that are important for mRNA splicing. By analyzing 188 Cauca-

sian individuals, they detected 15 single nucleotide polymorphisms (SNPs).

The report by Hoffmeyer et al. had a tremendous impact, since they indicated that a polymorphism, C3435T in exon 26, which caused no amino acid change, was associated with the duodenal expression of MDR1, and thereby intestinal absorption of digoxin, a typical substrate of MDR1.37 Immediately after, further extensive examinations were performed using a larger population around the world to find other polymorphisms,32–38 resulting in the identification of several important SNPs including G2677A,T in exon 21, in parallel with searches on inter-ethnic differences in the frequencies of C3435T.34,39–50 At present, more than 40 SNPs have been found, and 29 representatives identified in pioneering investigations are listed in Table 1. Eight SNPs are located in introns, and 11 change the amino acid. Interethnic differences in genotype frequencies of C3435T are summarized in Table 2. Marked differences in genotype and allele frequencies were apparent between African populations and Caucasian or Asian populations. As pointed out by Ostrovsky et al., the frequencies depend on the origin, even in the same ethnic group living in the same country.49] C3435T was found to be in significant linkage disequilibrium with C1236T and G2677T, the latter resulting in Ala893Ser35,36,43,50–55] Johne et al. defined 4 haplotypes of 11, 12, 21 and 22 based on G2677T and C3435T, where the coding is as follows; 1: identical to the reference sequence, i.e., G-allele at 2677 and C-allele at 3435; 2: different from the reference sequence, i.e., T-allele at 2677 and T-allele at 3435.56] Subsequently, they defined 9 genotypes of 00, 01, 02, 10, 11, 12, 20, 21 and 22, where the coding is as follows; 0: homozygous for nucleotides identical to the reference sequence for the position at both chromosomes; 1: heterozygous; 2: homozygous for nucleotides different from the reference sequence for the position at both chromosomes, that is, the genotypes corresponded to haplotype pair of 11/11, 11/12, 12/12, 11/21, 11/22, 12/22, 21/21, 21/22 and 22/22, respectively.56] A haplotype pair of 12/21 is also possibly defined for genotype 11, but 11/22 is selected based on frequency in Caucasians.56] This assignment is often used to check the importance of haplotype analyses (see below; referred to as “the G2677T/C3435T haplotype” hereafter for convenience). But it is noted that, at position 2677, a variant A-allele was preferentially found in Asians, and has been shown to be important for the pharmacokinetics of the H1-antihistamine fexofenadine,51,57] and thus an analysis based on the G2677T/C3435T haplotype is insufficient to cover the majority of Asians. Some investigators defined 11 haplotypes, MDR1*1 to *11, and their subtypes based on C1236T, G2677A,T and C3435T.36,58,59] We are now considering a definition based on T-129C, C1236T, G2677A,T and C3435T.47,60] Recently, several additional SNPs were found by Kroetz et al. or Allabi et al.38] Kroetz et al. defined 32 haplotypes, MDR1*1 to *32, and their subtypes (a total of 64 haplotypes).27] These assignments should be justified by genotype-phenotype correlation studies.

MDRI Genotype-related MDR1 Expression and Function

1. Update

Hoffmeyer et al. reported that a silent polymorphism, C3435T, was associated with a lower level of MDR1 in the duodenum, and resulted in a higher plasma concentration of digoxin.27] For the past 5 years, clinical investigations on MDR1 genotype-related MDR1 activity have been performed mainly focusing on C3435T and from the viewpoint of the expression of MDR1 or its mRNA rather than the function of MDR1.35,61–80] The results on the effects of C3435T on the expression are summarized in Table 3.
Table 2. Allele and genotype frequencies of a polymorphism of MDR1 C3435T

<table>
<thead>
<tr>
<th>Subjects</th>
<th>N</th>
<th>Allele</th>
<th>Genotype</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caucasian in UK</td>
<td>190</td>
<td>0.48</td>
<td>0.52</td>
<td>0.24 0.48 0.28</td>
</tr>
<tr>
<td>Caucasian in Germany</td>
<td>188</td>
<td>0.52</td>
<td>0.48</td>
<td>0.28 0.48 0.24</td>
</tr>
<tr>
<td>Caucasian in Germany</td>
<td>461</td>
<td>0.46</td>
<td>0.54</td>
<td>0.21 0.50 0.29</td>
</tr>
<tr>
<td>Caucasian in Poland</td>
<td>122</td>
<td>0.62</td>
<td>0.38</td>
<td>0.42 0.41 0.17</td>
</tr>
<tr>
<td>Itarian</td>
<td>106</td>
<td>0.54</td>
<td>0.46</td>
<td>0.26 0.55 0.19</td>
</tr>
<tr>
<td>Portuguese</td>
<td>100</td>
<td>0.43</td>
<td>0.57</td>
<td>0.22 0.42 0.36</td>
</tr>
<tr>
<td>Portuguese in Southern Portugal</td>
<td>100</td>
<td>0.36</td>
<td>0.65</td>
<td>0.12 0.47 0.41</td>
</tr>
<tr>
<td>Russian</td>
<td>290</td>
<td>0.46</td>
<td>0.54</td>
<td>0.21 0.49 0.30</td>
</tr>
<tr>
<td>Spanish</td>
<td>408</td>
<td>0.52</td>
<td>0.48</td>
<td>0.26 0.52 0.22</td>
</tr>
<tr>
<td>African American</td>
<td>88</td>
<td>0.84</td>
<td>0.16</td>
<td>0.68 0.31 0.01</td>
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<tr>
<td>African in KwaZulu-Natal, South Africa</td>
<td>110</td>
<td>0.86</td>
<td>0.14</td>
<td>0.75 0.21 0.04</td>
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<tr>
<td>Ghanaian</td>
<td>206</td>
<td>0.83</td>
<td>0.17</td>
<td>0.67 0.34 0.00</td>
</tr>
<tr>
<td>Kenyan</td>
<td>80</td>
<td>0.83</td>
<td>0.17</td>
<td>0.70 0.26 0.04</td>
</tr>
<tr>
<td>Sudanese</td>
<td>51</td>
<td>0.73</td>
<td>0.27</td>
<td>0.52 0.43 0.06</td>
</tr>
<tr>
<td>Chinese</td>
<td>132</td>
<td>0.53</td>
<td>0.47</td>
<td>0.32 0.42 0.26</td>
</tr>
<tr>
<td>Chinese</td>
<td>98</td>
<td>0.46</td>
<td>0.54</td>
<td>0.24 0.44 0.32</td>
</tr>
<tr>
<td>Filipino</td>
<td>60</td>
<td>0.59</td>
<td>0.41</td>
<td>0.38 0.42 0.20</td>
</tr>
<tr>
<td>Indian in KwaZulu-Natal, South Africa</td>
<td>103</td>
<td>0.42</td>
<td>0.58</td>
<td>0.17 0.50 0.33</td>
</tr>
<tr>
<td>Indians</td>
<td>93</td>
<td>0.38</td>
<td>0.62</td>
<td>0.18 0.39 0.43</td>
</tr>
<tr>
<td>Japanese</td>
<td>114</td>
<td>0.61</td>
<td>0.39</td>
<td>0.35 0.53 0.12</td>
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<tr>
<td>Jewish in Israel, Ashkenazi</td>
<td>100</td>
<td>0.65</td>
<td>0.35</td>
<td>0.42 0.46 0.12</td>
</tr>
<tr>
<td>Malays</td>
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<td>0.48</td>
<td>0.52</td>
<td>0.25 0.46 0.28</td>
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<tr>
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<td>96</td>
<td>0.55</td>
<td>0.45</td>
<td>0.37 0.38 0.26</td>
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<tr>
<td>South-west Asians</td>
<td>89</td>
<td>0.34</td>
<td>0.66</td>
<td>0.15 0.38 0.47</td>
</tr>
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</table>

Table 3. MDR1 C3435T genotype-related expression of MDR1 or its mRNA

<table>
<thead>
<tr>
<th>Subjects</th>
<th>N</th>
<th>Expression of</th>
<th>Tissue</th>
<th>Results</th>
<th>Reference     Note</th>
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<td>Healthy &amp; patients, Caucasian</td>
<td>21</td>
<td>Protein</td>
<td>Duodenum</td>
<td>CC &gt; CT &gt; TT</td>
<td>Hoffmeyer et al.</td>
</tr>
<tr>
<td>Healthy, Japanese</td>
<td>13</td>
<td>mRNA</td>
<td>Duodenum</td>
<td>CC &lt; CT &lt; TT</td>
<td>Nakamura et al.</td>
</tr>
<tr>
<td>Healthy, Caucasian</td>
<td>37,32</td>
<td>Protein, mRNA</td>
<td>Duodenum</td>
<td>CC = CT = TT</td>
<td>Siegmund et al.</td>
</tr>
<tr>
<td>Patients</td>
<td>69</td>
<td>mRNA</td>
<td>Intestine</td>
<td>CC = CT = TT</td>
<td>Goto et al.</td>
</tr>
<tr>
<td>Healthy, Caucasian</td>
<td>26</td>
<td>Protein, mRNA</td>
<td>Liver</td>
<td>large variation</td>
<td>Owen et al.</td>
</tr>
<tr>
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<td>112</td>
<td>mRNA</td>
<td>Liver</td>
<td>large variation</td>
<td>Goto et al.</td>
</tr>
<tr>
<td>Patients, Caucasian</td>
<td>85</td>
<td>Protein</td>
<td>Kidneys</td>
<td>CC &gt; TT</td>
<td>Siegmund et al.</td>
</tr>
<tr>
<td>Healthy, Japanese</td>
<td>89</td>
<td>Protein</td>
<td>Placenta</td>
<td>CC = CT = TT</td>
<td>Tanabe et al.</td>
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<tr>
<td>Healthy</td>
<td>39</td>
<td>Protein</td>
<td>Placenta</td>
<td>CC = CT = TT</td>
<td>Hitzl et al.</td>
</tr>
<tr>
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<td>mRNA</td>
<td>Placenta</td>
<td>CC = CT = TT</td>
<td>Hitzl et al.</td>
</tr>
<tr>
<td>Patients, Caucasian</td>
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<td>mRNA</td>
<td>Heart</td>
<td>CC = CT = TT</td>
<td>Meissner et al.</td>
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<tr>
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<td>mRNA</td>
<td>CD56+ NK cells</td>
<td>CC = CT = TT</td>
<td>Hitzl et al.</td>
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<tr>
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<td>Lymphocytes</td>
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<tr>
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<td>mRNA</td>
<td>Blood</td>
<td>large variation</td>
<td>Effert et al.</td>
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<tr>
<td>Healthy, Caucasian</td>
<td>21</td>
<td>Protein, mRNA</td>
<td>Peripheral blood mononuclear cells</td>
<td>CC &gt; CT &gt; TT</td>
<td>Owen et al.</td>
</tr>
<tr>
<td>Patients</td>
<td>405</td>
<td>mRNA</td>
<td>Mononuclear blood cells from bone marrow</td>
<td>CC &lt; CT &lt; TT</td>
<td>Illmer et al.</td>
</tr>
</tbody>
</table>

1) A study with a subpopulation of n=8 suggested the MDR1 expression after rifampicin treatment also gave the same result.
2) MDR1 mRNA expression was significantly correlated with CYP3A mRNA in 51 duodenal biopsy specimens.
3) Intestinal samples were from recipients of living-donor liver transplantation.
4) Noncancerous renal tissues were obtained from patients with renal epithelial tumors.
5) MDR1 mRNA expression tended to be higher for TT3435 than CC3435, and was higher for TT2677 than GG2677.
6) In CD56+ NK cells, rhodamine 123 fluorescence was higher, that is, MDR1 protein function was lower in TT3435.
Hitzl et al. found no effect on MDR1 mRNA expression in CD56+ NK cells of 31 healthy Caucasians, however, they suggested a greater increase in the fluorescence of rhodamine 123 in the cells of the homozygote for the T-allele, TT3435 than the homozygote for the C-allele at position 3435, CC3435 or the heterozygote, CT3435, which means weaker activity of MDR1 in the subjects with TT3435.61) Oselin et al. also suggested that the expression of MDR1 mRNA in lymphocytes was independent of C3435T in 45 healthy volunteers, but they showed that the efflux of rhodamine 123 from the cells was not related to the polymorphism either.62,63) Efferth et al. did not find a correlation between C3435T and MDR1 mRNA expression in blood obtained from patients with acute lymphoblastic leukemia (ALL).64) Recently, Brenner and Klotz suggested that the age of patients could be important for the C3435T-dependent efflux of rhodamine 123 from CD56+ NK cells.65) In contrast, Owen et al. reported a statistically more significant reduction of MDR1 or its mRNA levels in peripheral blood mononuclear cells obtained from 21 healthy Caucasian subjects with TT3435 or CT3435 than CC3435.66) However, Illner et al. reported that MDR1 mRNA levels in mononuclear blood cells isolated from bone marrow samples were lowest in acute myeloid leukemia (AML) patients with CC3435.67)

MDR1 in the intestinal wall is believed to play an important role in drug absorption after its oral intake, but to date, there have been few reports on the effects of C3435T on MDR1 or its mRNA expression in the intestines. We determined MDR1 mRNA levels in biopsy specimens of the duodenum obtained from healthy Japanese subjects and found them to be higher in individuals with TT3435.68) A significant correlation was found between the mRNA expression of MDR1 and CYP3A4 in individual biopsy specimens, suggesting a lower intestinal absorption of the substrates for CYP3A4 in the subjects harboring C3435T.68) This is consistent with the recent report that the orphan nuclear receptor, SXR/PXR, is involved in the induction of both MDR1 and CYP3A4 expression.63) However, Goto et al. found that C3435T had no effect on enteroctye expression of MDR1 mRNA, but correlated with reduced enteroctye expression of CYP3A4 mRNA in 69 Japanese recipients of a living-donor liver transplantation.69) This can not be explained by SXR/PXR-mediated process, and they hypothesized the linkage disequilibrium between C3435T and an unknown genetic variation of CYP3A4 gene.69) Siegmund et al. also indicated that duodenal expression of MDR1 and its mRNA was not influenced by C3435T, and therefore the disposition of the β1-adrenergic receptor blocker talinolol was also independent of C3435T in healthy Caucasians.70)

MDR1 expresses throughout the body, and defines tissue distribution and subsequent excretion via the kidneys or into bile. Owen et al. reported that substantial variability of MDR1 or its mRNA expression in the liver, and effects of C3435T could not be elucidated.71) Goto et al. reported no effects of C3435T on mRNA expression in liver obtained as biopsy specimens for pathological testing from 112 donors for living-donor liver transplantations.72) Siegmund et al. reported that the C3435T polymorphism was associated with reduced MDR1 expression in noncancerous renal tissue obtained from 85 patients with renal epithelial tumors.73) Tanabe et al. suggested that C3435T had no effect on placental MDR1 expression based on a study in 89 Japanese subjects,13) but recently, Hitzl et al. elucidated that the expression of MDR1, not of MDR1 mRNA, in placenta was significantly lower when both mother and infant had CT3435 or TT3435 than CC3435.74) Meissner et al. reported that the expression of MDR1 mRNA in heart tissue samples obtained from 51 Caucasian patients undergoing coronary artery bypass graft surgery was independent of C3435T (tended to be higher for TT3435), but was higher for TT2677 than GG2677.75)

To date, there is no rational explanation for the association between C3435T and MDR1 expression. The association might reflect linkage disequilibrium between C3435T and a SNP in the promoter region and/or in the exon-intron boundaries that are important for mRNA splicing, although there is no evidence of this. Soranzo et al. have tried to identify candidate causal variants responsible for the altered activity of MDR1.82) Compared with C3435T, there are few reports on the effects of other SNPs. Taniguchi et al. have suggested the importance of analyzing the 5′ regulatory region of the gene, and found that the expression in the colon was associated with T-2410C, T-1910C, and others.76) In the duodenum of healthy subjects, mononuclear blood cells of AML patients, and heart tissues of patients, MDR1 mRNA expression was found to be lower in those with GG2677,67,75,77) however, no relation between G2677A,T and expression of MDR1 or its mRNA was reported in the duodenum.70) Tanabe et al. suggested that G2677A,T resulted in lower expression levels of MDR1 in the placenta.35) The G2677T/C3435T haplotype analysis might be useful to detect the effects of MDR1 genotypes,70) but the effects were contradictory, similarly to T-129C.35,77) These results also imply that C3435T may not itself be causal but rather may be linked with the causal polymorphisms.

Compared with the MDR1 genotype-related expression of MDR1, little information is available on the effects on function. Kim et al. indicated that cells transduced with a variant with G2677T showed a reduced intracellular accumulation of [H]-digoxin compared with cells with the wild-type, suggesting enhanced efflux
when accompanied with G2677T. Recently, Woodahl \textit{et al.} has reported that the intracellular accumulation of rhodamine 123 was about 5-fold higher in G1199A-transfected cells than wild type cells. However, Kimchi-Sarfaty \textit{et al.} indicated that the cell surface expression and function of double mutants of either two of A61G, G1199A and G2677T showed no difference from the wild-type. Morita \textit{et al.} also indicated that G2677T and C3435T had no effect on MDR1 transport activities using the LLC-PK1 cell line as a host. It is noted that we still do not know the co-factors necessary for MDR1 to act, or have a technology to control the expression of MDR1 in variants at levels the same as those in non-variants, so we have to pay attention to the data obtained with transfectants.

2. \textbf{Various factors affect MDR1 expression: no consensus on C3435T-related MDR1 expression}

As shown in Table 3, there seems to be no consensus in reports on the association of C3435T with MDR1 expression, but the association pattern might differ among tissues, and be altered depending on pathological conditions or ethnicity, even in the same tissue. Gene sequencing and molecular cloning of MDR1 allowed an examination of \textit{MDR1} transcriptional regulation to better understand the effect of \textit{MDR1} genotype on MDR1 expression. The \textit{MDR1} promoter contains a GC box for specificity protein 1 (Sp1) and an inverted CCAAT box for Y-box binding protein-1 (YB-1) and nuclear factor-Y (NF-Y). Recently, Labialle \textit{et al.} identified a new regulatory sequence in the inverted MED-1 promoter region. In addition, various types of transcription factors have been suggested to be involved in MDR1 expression, such as EGR1, NF-IL6 and NF-R2. In rodents, effects of C/EBPβ and NF-κB were also proposed. The expression of these transcriptional factors and their regulation might depend on tissue-type, pathological status and ethnicity.

Various types of biological events involve the activation and inactivation of tumor suppressor p53, including cell growth arrest, apoptosis, DNA repair, angiogenesis and stress responses. Much experimental evidence indicates that both p53 and MDR1 play important roles in chemoresistance. Transcriptional dependence of the \textit{MDR1} gene promoter on p53 has been strongly suggested, however, the relationship between MDR1 and p53 is conditional, that is, dependent on the cellular environment and drug used. Mutation of p53, which is the most frequent genetic alteration detected in human cancers, induces \textit{MDR1} promoter transactivation, resulting in increased resistance to chemotherapy and radiation. In contrast, gene therapy to introduce wild-type p53 results in a reduction of \textit{MDR1} promoter expression.

Ziemann \textit{et al.} suggested that reactive oxygen species (ROS) participated in the overexpression of MDR1 in primary rat hepatocyte cultures. Addition of H$_2$O$_2$ or a catalase inhibitor induced the expression of \textit{mdr1b} mRNA, while antioxidants markedly suppressed this effect. H$_2$O$_2$ plays an important role in gene expression by modulating the activity of transcription factors or regulatory enzymes such as protein kinase, and \textit{MDR1} promoter activity is known to be regulated by protein kinase C. Insulin, EGF, TNF-α and doxorubicin also induce \textit{mdr1b} mRNA expression, and these factors are well-known to produce ROS. Up-regulated \textit{mdr1} via NF-κB activation protects cells from ROS-induced apoptosis. We also found that MDR1 up-regulated by apoptotic stimuli suppressed apoptotic signaling, presumably via the mitochondrial pathway.

Generally, a variety of endogenous and exogenous stimuli could be involved in the molecular regulation of MDR1 expression, including cytotoxic drugs, heat shock, irradiation, inflammation, and various cytokines and growth factors. As for exogenous substances, digoxin results in an up-regulation of MDR1 expression, similar to the antimicrobial agent rifampicin, a well-known MDR1-inducer. Whereas the antihypertensive agent verapamil decreases the expression, Wada \textit{et al.} indicated the possibility of altering MDR1 levels by plant-derived materials. Vos \textit{et al.} suggested the up-regulation of MDR1 expression by endotoxin, and we showed that an anticancer agent cisplatin has similar effects. It is not surprising that these cellular environments depend on tissue-type, pathological status and ethnicity.

In addition, as for the inter-ethnic difference in the results, it is possible that certain haplotypes are more prevalent in one race than another, and in fact, we found a substantial difference in haplotype between Caucasians and Japanese, where the haplotype was assigned based on T-129C, C1236T, G2677A,T and C3435T. As stated, SXR/PXR is involved in the induction of both MDR1 and CYP3A4 expression, and it has been suggested that up-regulation of MDR1 expression negatively accompanies down-regulation of SXR/PXR expression. Recently, genetic polymorphisms have been found for SXR/PXR, and its inter-ethnic difference may shed light on the relationship between \textit{MDR1} genotypes and the expression of MDR1. In addition, Yagüe \textit{et al.} have suggested the importance of mRNA turnover in the cells and translational regulation in the regulation of MDR1 expression. No reports are available concerning the relationship between genotype and mRNA turnover.

Collectively, MDR1 expression is susceptible to a number of endogenous and exogenous stimuli, resulting in accidental up- or down-regulation, and at present, it can not be concluded that there are discrepancies in the association of \textit{MDR1} C3435T with the expression of...
Table 4. MDR1 C3435T genotype-related pharmacokinetics after single or multiple oral administrations

<table>
<thead>
<tr>
<th>Subjects</th>
<th>N</th>
<th>Drugs</th>
<th>Parameters</th>
<th>Results</th>
<th>Reference</th>
<th>Note</th>
</tr>
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<td>Healthy, Caucasian</td>
<td>8</td>
<td>digoxin</td>
<td>single</td>
<td>AUCO&lt;sub&gt;0-144&lt;/sub&gt;, C&lt;sub&gt;max&lt;/sub&gt;</td>
<td>CC&lt;CT&lt;TT</td>
<td>Hoffmeyer et al. (17)</td>
</tr>
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<td>digoxin</td>
<td>single</td>
<td>AUCO&lt;sub&gt;0-4&lt;/sub&gt;, C&lt;sub&gt;max&lt;/sub&gt;</td>
<td>CC&lt;CT&lt;TT</td>
<td>Gerloff et al. (190)</td>
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<td>single</td>
<td>AUC&lt;sub&gt;0-14&lt;/sub&gt;</td>
<td>CC&lt;CT&lt;TT</td>
<td>Sakaeda et al. (143)</td>
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<tr>
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<td>digoxin</td>
<td>single</td>
<td>AUC&lt;sub&gt;0-14&lt;/sub&gt;, C&lt;sub&gt;max&lt;/sub&gt;</td>
<td>CC&lt;CT&lt;TT</td>
<td>Kurata et al. (144)</td>
</tr>
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<td>single</td>
<td>AUC&lt;sub&gt;0-24&lt;/sub&gt;, C&lt;sub&gt;max&lt;/sub&gt;</td>
<td>CC&lt;CT&lt;TT</td>
<td>Becqueu et al. (150)</td>
</tr>
<tr>
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<td>single</td>
<td>AUC&lt;sub&gt;0-4&lt;/sub&gt; or AUC&lt;sub&gt;0-24&lt;/sub&gt;</td>
<td>CC&lt;CT&lt;TT</td>
<td>Versut et al. (151)</td>
</tr>
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<td>CC&lt;CT&lt;TT</td>
<td>Versut et al. (151)</td>
</tr>
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<td>single</td>
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<td>CC&lt;CT&lt;TT</td>
<td>Kim et al. (170)</td>
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<td>CC&lt;CT&lt;TT</td>
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<td>CC&lt;CT&lt;TT</td>
<td>Min &amp; Ellingrod (177)</td>
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<td>CC&lt;CT&lt;TT</td>
<td>Frohlich et al. (148)</td>
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<td>CC&lt;CT&lt;TT</td>
<td>Hoffmeyer et al. (17)</td>
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<td>steady-state</td>
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<td>CC&lt;CT&lt;TT</td>
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<td>steady-state</td>
<td>maintenance dose, dose-adjusted C&lt;sub&gt;min,s&lt;/sub&gt;</td>
<td>CC&lt;CT&lt;TT</td>
<td>von Ahsen et al. (180)</td>
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<td>steady-state</td>
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<td>CC&lt;CT&lt;TT</td>
<td>Mai et al. (177)</td>
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<td>steady-state</td>
<td>maintenance dose, dose-adjusted C&lt;sub&gt;min,s&lt;/sub&gt;</td>
<td>CC&lt;CT&lt;TT</td>
<td>Hauflord et al. (181)</td>
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<td>AUC&lt;sub&gt;0-4,ss&lt;/sub&gt;, AUC&lt;sub&gt;0-12,ss&lt;/sub&gt;, C&lt;sub&gt;max,ss&lt;/sub&gt;</td>
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<td>CC&lt;CT&lt;TT</td>
<td>Hesselink et al. (184)</td>
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<td>steady-state</td>
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<td>CC&lt;CT&lt;TT</td>
<td>Bonhomme-Fraie et al. (186)</td>
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<td>Patients</td>
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<td>steady-state</td>
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<td>Hauflord et al. (181)</td>
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<td>steady-state</td>
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<td>CC&lt;CT&lt;TT</td>
<td>Tsuchiya et al. (188)</td>
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<td>CC&lt;CT&lt;TT</td>
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<td>single or steady-state</td>
<td>AUC&lt;sub&gt;0&lt;/sub&gt;, or AUC&lt;sub&gt;0-24&lt;/sub&gt;</td>
<td>CC&lt;CT&lt;TT</td>
<td>Siegmund et al. (191)</td>
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<td>nefinavir, efavirenz</td>
<td>steady-state</td>
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<td>CC&lt;CT&lt;TT</td>
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<td>steady-state</td>
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<td>CC&lt;CT&lt;TT</td>
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<td>Patients</td>
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<td>risperidone</td>
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<td>C&lt;sub&gt;min,ss&lt;/sub&gt;</td>
<td>CC&lt;CT&lt;TT</td>
<td>Yashi-Furukori et al. (194)</td>
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</table>

CC<CT<TT: comparison among 3 groups; CC<CT+TT: comparison between T-noncarriers and T-carriers.

1) Data were under rifampin induction.
2) Subjects with the genotype CC<sup>3435</sup>, CT<sup>3435</sup> and TT<sup>3435</sup> accompanied with GG<sup>2677</sup>, GT<sup>2677</sup> and TT<sup>2677</sup>, respectively.
3) The authors conducted the investigation again (Versut et al., 2003, 150,151).
4) Study was done in 25 Caucasians, 6 Africans and 1 Asian.
5) Study was done in 11 African Americans and 3 Caucasians.
6) Cyclosporine pharmacokinetics was independent also of CYP3A4 genotype.
7) Pharmacokinetics depended on CYP3A5 genotype.
8) Subjects with the genotype CC<sup>3435</sup>, CT<sup>3435</sup> and TT<sup>3435</sup> accompanied with CC<sup>1236</sup>/GG<sup>2677</sup>, CT<sup>1236</sup>/GT<sup>2677</sup> and TT<sup>1236</sup>/TT<sup>2677</sup>, respectively.
9) Study was done in 12 Asians, 11 Blacks and 87 Caucasians for cyclosporine A, and 6 Asians, 9 Blacks and 49 Caucasians for tacrolimus.
10) Tacrolimus pharmacokinetics depended on CYP3A4 or CYP3A5 genotypes.
11) Tacrolimus pharmacokinetics depended on CYP3A5 genotype.
12) Data were either of those after single oral administration or those at steady-state. G2677A,T had an effect on pharmacokinetics.
MDR1. Further studies should be conducted to obtain a conclusion.

**MDR1 Genotype-related Pharmacokinetics**

1. **Update**

In the report by Hoffmeyer et al., plasma concentrations of digoxin after treatment with rifampin or maximal concentrations of digoxin after multiple oral administrations were used for the discussion of intestinal absorption. Unfortunately, the correlation with the plasma concentrations after a single administration without rifampin was not presented, requiring further investigation into the relationship between **MDR1** genotype and the pharmacokinetics. Additional clinical studies have been performed mainly on the effects of C3435T, and the results after single or multiple oral administrations are summarized in Table 4. Most of the research was done using digoxin, CsA, FK506, fexofenadine and HIV protease inhibitors, since they are or are expected to be typical MDR1 substrates.

**a) Single oral dosing:** MDR1 expressed in the intestinal wall is believed to affect absorption, and clinical studies on the effects of C3435T have been performed focusing on the association with the pharmacokinetics after a single oral administration. The plasma/serum/blood concentrations, and their maximum values, C_{max} as well as the area under the concentration-time profile, AUC, as an index of systemic exposure, are often used for evaluations.

Becquemont et al. indicated that C_{max} or AUC values of digoxin after a single oral administration with or without grapefruit juice were independent of C3435T in 12 healthy subjects. However, in their recent report, it has been shown that the plasma concentrations were higher for TT^{3435} than CT^{3435} or CC^{3435}, and they concluded that the absence of statistical significance in the effects of C3435T in the previous study was due to insufficient statistical power. The first extensive examination concerning **MDR1** genotype-related pharmacokinetics with a larger number of subjects was performed by Kim et al., who concluded that the plasma concentrations of fexofenadine after a single oral administration were lower for TT^{3435} than CT^{3435} or CC^{3435}. They also suggested that the concentrations tended to be lower for TT^{3435} than CC^{3435}. Recently, it has been found that fexofenadine is typical substrate for a member of organic anion transporting polypeptides (OATPs), and it might be inappropriate as a probe. We too have indicated that on systemic exposure to digoxin after a single oral administration, concentrations were lower for CT^{3435} than TT^{3435} consistent with our results on MDRI mRNA levels in biopsy specimens of the duodenum. However, Drescher et al., Gerloff et al., Min and Ellingrod, Pauli-Magnus et al., Putnam et al., and Frohlich et al. have suggested that the plasma or blood concentrations of fexofenadine, digoxin, CsA, an antidiarrheal agent loperamide, an antimicrobial agent dicloxacillin and an antiviral agent saquinavir after a single oral administration were independent of C3435T, respectively. The importance of haplotype analysis to detect the effects of **MDR1** genotype has been suggested for single oral dosing. Kurata et al. have reported that serum concentrations of digoxin were lower in subjects with GG^{2677} and CC^{3435} than those with GT^{2677} and CT^{3435} or with TT^{2677} and TT^{3435}, and this type of analysis may contribute to the statistical power. Skarke et al. suggested that the plasma concentrations of loperamide tended to increase in the subjects with TT^{3435} compared to non-carriers, and also that an analysis based on the G2677T/C3435T haplotype possibly resulted in a significant difference. Yi et al. have suggested the importance of G2677A, more frequently found in Asians, in plasma concentration-time profiles of fexofenadine after a single oral administration.

**b) Steady-state after multiple oral dosing:** The effects of C3435T on steady-state pharmacokinetics after multiple oral administrations were also extensively investigated. The trough concentration, C_{0,ss} or C_{min,ss}, dose needed to maintain a similar C_{min,ss} (maintenance dose) or dose-adjusted C_{min,ss} are often used for evaluations, which are defined by oral bioavailability and systemic clearance, and additionally the following 2 parameters are sometimes used; AUC value assessed at a steady-state, AUC_{ss}, and the concentration at 2 hr after oral administration, C_{2,ss}, as a surrogate marker of UAC_{ss}, due to its correlation with AUC_{ss}. It is noted that the subscript “ss” is used in this text, to distinguish the data after a single administration and at a steady-state. The maximum concentration at a steady-state, C_{max,ss}, is sometimes used for discussion, but it is confusing, since it is a complicated function of absorption, distribution, metabolism and excretion.

Fellay et al. have reported that C_{min,ss} values of the antiviral agent nelfinavir or efavirenz, were lower in subjects with TT^{3435} suggesting lower oral bioavailability and/or higher systemic clearance, whereas no influence on the C_{min,ss} of lopinavir was reported by Winzer et al. However, Johne et al. indicated that the C_{min,ss} of digoxin was lower in healthy subjects with CC^{3435} and suggested the importance of analyzing G2677T/C3435T haplotype. Yasui-Furukori et al. reported that steady-state plasma concentrations of risperidone and 9-hydroxyrisperidone were independent of G2677A,T and C3435T in 85 schizophrenic patients, and moreover they were similar for the patients with GG^{2677} and CC^{3435}, and those with WW^{2677} (W: A or T) and TT^{3435}. Siegmund et al. also reported that the pharmacokinetics of talinolol after
single or multiple oral administrations was independent of C3435T, but G2677A,T had an effect.\textsuperscript{70}

Two major immunosuppressants, CsA and FK506, are potent and toxic drugs with narrow therapeutic ranges, but with substantial pharmacokinetic inter-individual variability, and pharmacogenomic analysis has the potential to improve dosing strategies, although therapeutic drug monitoring currently provides a surrogate marker for adjusting the maintenance dose.\textsuperscript{167–171} Their biological fates are understood to be defined by both MDR1 and drug metabolizing enzymes, CYP3A family, and a number of clinical studies have been performed on the effects of their genotyping.\textsuperscript{53,146–160}

Recently, it has been found that CYP3A4\textsuperscript{*1B} is associated with higher levels of CYP3A4,\textsuperscript{172} and that CYP3A5\textsuperscript{*3} results in a deficiency of protein,\textsuperscript{173,174} and the subjects are often stratified as CYP3A4\textsuperscript{*1B}*1/*1B- or *1B/*1B or non-carriers, *1/*1I, and CYP3A5\textsuperscript{*1}*1*/*1* or non-carriers, *3/*3. The first preliminary report on the effects of their genotypes was presented by von Ahsen et al., who indicated that the maintenance dose or dose-adjusted C\textsubscript{min,ss} of CsA was independent either of MDR1 C3435T or CYP3A4 genotype, after an analysis of 124 Caucasian renal transplant recipients.\textsuperscript{146} Mai et al. indicated no effects of MDR1 C2677T or C3435T on the dose-adjusted AUC\textsubscript{0–12,ss}, C\textsubscript{min,ss} and C\textsubscript{2,ss} of CsA in 95 renal transplant recipients, and an analysis based on the G2677T/C3435T haplotype\textsuperscript{56} also failed to predict the pharmacokinetics of CsA.\textsuperscript{147} Kuzuya et al. also reported no effects of MDR1 T-129C, T1236C, G2677A,T and C3435T on the dose-adjusted AUC\textsubscript{0-2,ss} of CsA in 57 renal transplant recipients.\textsuperscript{148} In 2002, the Consensus on Neoral C2.\textsuperscript{50} Expert Review in Transplantation (CONCERT) conference was convened to undertake a multidisciplinary review of pharmacokinetic and clinical data on CsA microemulsions, and an international consensus on a patient management strategy with the oral administration of a CsA microemulsion has come to be presented based on the monitoring of C\textsubscript{2,ss}, as a surrogate marker of AUC\textsubscript{0-4,ss}, AUC\textsubscript{ss}, up to 4 hr after oral administration, which was recently found to be predictive of immunosuppressive effects and toxicity, when compared with conventional C\textsubscript{ss} monitoring.\textsuperscript{175–178} One important issue to be resolved is the existence of “slow absorbers”, who show a C\textsubscript{max,ss} at 4 hr or later, and are susceptible to over-dosing of CsA based on C\textsubscript{2,ss} monitoring. We analyzed the MDR1 genotypes in slow absorbers among Japanese pediatric patients, but found that they could not be characterized by the genotypes.\textsuperscript{149}

Tsuchiya et al. reported no effects of C3435T on the maintenance dose or dose-adjusted C\textsubscript{min,ss} of FK506 in 30 renal transplant recipients, but found that they were defined by CYP3A5 genotype.\textsuperscript{150} The maintenance dose was higher, whereas the dose-adjusted C\textsubscript{min,ss} was lower, for CYP3A5*1/*1 + *1/*3 than CYP3A5*3/*3.\textsuperscript{150} The importance of CYP3A5 or CYP3A1P1 genotyping, the latter linked to CYP3A5, rather than MDR1 C3435T for the prediction of FK506 pharmacokinetics was suggested by MacPhee et al.\textsuperscript{151,179} Tada et al. reported that MDR1 C3435T had no effect on FK506 even after stratification of 39 patients with renal transplantation based on CYP3A5 genotype.\textsuperscript{152} Hesselink et al. reported that the dose-adjusted C\textsubscript{min,ss} of both CsA and FK506 in renal transplant recipients was independent of C3435T due to extensive variability, but that carriers of CYP3A4*1B and CYP3A5*1 showed a lower dose-adjusted C\textsubscript{min,ss} for FK506, but not CsA.\textsuperscript{153} No effects of MDR1 C1236T, G2677T and C3435T on the dose-adjusted C\textsubscript{min,ss} of CsA or FK506 in renal transplant recipients were also reported by Hauflroid et al.\textsuperscript{154} Additionally, they suggested that there was no difference in the dose-adjusted C\textsubscript{min,ss} of CsA or FK506 among patients with CC1236-GG2677-CC3435, those with CT1236-GT2677-CT3435 and those with TT1236-TT2677-TT3435, but that the values were higher for CYP3A5*3/*3 than *1/*3.\textsuperscript{154}

In contrast, Yates et al. reported that the AUC\textsubscript{0-12,ss} value of CsA was higher in renal transplant recipients with CC3435 than TT\textsuperscript{143} or TT\textsuperscript{145}. Chowbay et al. reported that concentrations of CsA were higher in heart transplant recipients with TT1236, TT2677 and TT3435 than those with CC1236, GG2677 and CC3435, and subjects with CT1236, GT2677 and CT3435 showed intermediate values.\textsuperscript{53} Bonhomme-Faivre et al. also suggested that the dose-adjusted C\textsubscript{2,ss} of CsA was higher, whereas a lower maintenance dose was needed in T3435-allele carriers among liver-transplant recipients.\textsuperscript{156} Angelicheau et al. suggested that dose-adjusted concentrations of CsA were independent of C3435T, but were higher in T1236-allele carriers in renal transplant recipients.\textsuperscript{157} As for FK506, Zheng et al. suggested that the dose-adjusted C\textsubscript{min,ss} of FK506 was lower in pediatric heart transplant patients or adult lung transplant recipients with CC3435 than CT\textsuperscript{143} or TT\textsuperscript{145,159,159}. They also suggested the importance of CYP3A5 genotyping,\textsuperscript{158,159} or analyses based on the G2677T/C3435T haplotype\textsuperscript{50} for MDR1.\textsuperscript{160}

c) Others: There are a few reports on the changes in pharmacokinetics after a single intravenous dosing, where systemic clearance was shown to be independent of C3435T.\textsuperscript{141,161–163} Kurata et al. reported no difference in the pharmacokinetics of digoxin after infusion among subjects with GG2677 and CC3435, those with GT2677 and CT3435, and those with TT\textsuperscript{144} and TT\textsuperscript{144}. Goh et al. indicated that the systemic clearance of midazolam after a bolus injection and docetaxel after a 1-hr infusion was independent of C3435T,\textsuperscript{161} and Eap et al. reported that CYP3A activity measured by the midazolam test was too.\textsuperscript{164} Plasschaert et al. also indi-
cated no effect of G2677T or C3435T on the systemic clearance of vincristine after a bolus injection into childhood ALL patients. In contrast, very recently, Nakajima et al. suggested that the systemic pharmacokinetics of the paclitaxel metabolite depended on C3435T. Very recently, Wong et al. reported that hepatic elimination of 99mTc-labeled sestamibi (hexakis-2-methoxyisobutyl isonitrile) was reduced in cancer patients with TT2677 or TT3435.166) van Geel and his co-workers proposed that hepatic elimination of 99mTc-labeled sestamibi (hexakis-2-methoxyisobutyl isonitrile) was reduced in cancer patients with TT2677 or TT3435.166)

MDR1 is expressed in the intestinal wall, liver, brain and kidneys and plays an important role in membrane transport, but little information is available on the effects of C3435T on these processes. We elucidated that the duodenal absorption of digoxin, as well as the plasma concentration after the oral intake of a conventional digoxin tablet, was suppressed more in subjects with TT3435 than CC3435 and only for the latter, was an increase in bioavailability on the co-administration of clarithromycin observed.163) Kurata et al. suggested that subjects with TT2677 or TT3435 showed almost 32% less renal clearance of digoxin than those with GG2677 and CC3435, and only for the latter, was an increase in bioavailability on the co-administration of clarithromycin observed.163)

2. C3435T-related pharmacokinetics: fact or fiction?

Similar to the effects of C3435T on the expression and function of MDR1, there is considerable disagreement regarding the effects of this SNP on the concentration-time profiles of drugs after single or multiple oral administrations. To make a long story short, one can say that MDR1 located in the intestinal wall does not restrict the intestinal absorption of substrates in the case of commercially available oral drugs, including digoxin, CsA and FK506, and presumably fexofenadine and HIV protease inhibitors, and therefore MDR1 genotypes are little associated with the pharmacokinetics after oral administration. The details are explained below.

In 1995, Amidon and his co-workers proposed the Biopharmaceutical Classification System (BCS), in which drugs were divided into 4 groups based on in vivo permeability and in vitro solubility: Class 1: high permeability & high solubility; Class 2: high permeability & low solubility; Class 3: low permeability & high solubility; and Class 4: low permeability & low solubility.180) The objective of the BCS is to predict the in vivo oral pharmacokinetic performance of drug products from the data on in vivo permeability (determined as the extent of oral absorption) and in vitro solubility, and the BCS has provided significant insight for the pharmaceutical scientific community, although both properties are dependent.181) Immediately thereafter, the BCS was exploited by the FDA for the development of a new regulatory guidance for bioequivalence studies182) and simultaneously this concept served as an important strategy during the early stage of drug development, since it clearly indicated the effects of both efflux and absorptive transporters on the in vivo pharmacokinetics of a number of drug candidates after oral administration.183) Briefly, the effects will be minimal for candidates belonging to Class 1, since such candidates give high concentrations in the gut to saturate them.183) In contrast, efflux transporters will predominate for Class 2 candidates, since the high permeability will allow ready access into gut membranes and uptake transporters will have no effect on absorption, but the low solubility will limit the concentrations coming into enterocytes, thereby preventing saturation of the efflux transporters.183) As for Class 3, the concentration in the gut will be high, but absorptive transporters will be necessary to overcome the poor permeability.183) Intestinal apical efflux transporters may also be important when sufficient enterocyte penetration is achieved by absorptive transporters.183) For Class 4 candidates, both transporters will affect the in vivo intestinal absorption.183)

We tabulated the molecular and pharmacokinetic properties of 222 commercially available oral drugs and analyzed their correlations.184) It is noted that the data on fexofenadine and HIV protease inhibitors are not included in this examination. As shown in Table 5, the drugs analyzed have a mean (±SD) molecular weight (Mw) of 332±140 daltons and ClogP of 1.73±2.21, where ClogP is the calculated logarithmic value of the n-octanol/water partition coefficient, and the substrates for carrier-mediated absorption are characterized by lower lipophilicity, while those of MDR1 are character-

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<th>Table 5. Molecular and pharmacokinetic properties of 222 commercially available oral drugs</th>
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<td>Molecular weight (daltons)</td>
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<td>Bioavailability (%)</td>
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The values are the mean ± SD. See reference 184.
ized by higher molecular weight and higher lipophilicity.\textsuperscript{184} Most of the drugs (207/222; 93\%) have molecular weights of less than 500, and 15 exceptions are characterized by a sugar moiety and/or large cyclic structure, and 7 of 15 exceptions are the substrate for MDR1, including digoxin (Mw: 781), CsA (Mw: 1201) and FK506 (Mw: 804).\textsuperscript{184} Most of the drugs (213/222; 96\%) showed ClogP values of less than 5, and 4 of 9 exceptions are those for MDR1.\textsuperscript{184} Although “Rule of 5” by Lipinski et al.\textsuperscript{185} was confirmed even for commercially available oral drugs, surprisingly, both exceptions suggest that intestinal MDR1 does not restrict the intestinal absorption of the substrates. The MDR1 substrates show a mean bioavailability of 47.2±21.7\%, and indeed this is lower than that of non-substrates, 67.0±28.9\%. However, the mean values of molecular weight are 479±240 and 311±115 daltons, respectively (Table 5). The bioavailability is negatively correlated with molecular weight,\textsuperscript{184} and MDR1 substrates are expected to be more effectively received the first-pass effect than non-substrates, since it has been serendipitously noted that MDR1 and CYP3A4 share significant overlap in substrate specificity.\textsuperscript{186–190} To cancel out the first-pass effect, the fraction absorbed (\%) was calculated using the urinary excretion ratio (\%) to be 80.4±26.5\% and 85.2±24.8\% for substrates and non-substrates, respectively (Table 5), also clearly rising the question of the role of intestinal MDR1. Fexofenadine (Mw: 502) and HIV protease inhibitors including saquinavir (Mw: 671), indinavir (Mw: 614), ritonavir (Mw: 721) and nelfinavir (Mw: 568), also often used for the investigations on the effects of C3435T, have the molecular weights of more than 500. Although the values are less than those for digoxin, CsA and FK506, the intestinal MDR1 might not act as barrier even for fexofenadine and HIV protease inhibitors. In the BCS panel proposed by Amidon and his co-workers, these commercially available substrates must be members of Class 1, since the effects of MDR1 are minimal.

To confirm that the effects of intestinal MDR1 are minimal for the intestinal absorption of the substrates after oral administration in the case of commercially available oral drugs, we have conducted a cross-over clinical study in which a digoxin solution was sprinkled directly over the surface of the duodenum using an endoscope or a conventional digoxin tablet was administered orally.\textsuperscript{165} The rate of absorption was evaluated by an analysis of serum concentrations for the initial 15 min after the intra-duodenal administration of the solution, and we concluded that C3435T is associated with suppression of the duodenal absorption of digoxin. It is noted that, simultaneously, we could confirm that digoxin was absorbed very rapidly from the duodenum, and thus serum/plasma concentration-time profiles after conventional oral administration are significantly affected by disintegration, dissolution and gastric emptying. Consequently, oral absorption would be extensively affected by the experimental conditions, including the volume of water used for the intake of a tablet or a capsule, and the position taken during the investigation, i.e., standing or sitting. This might contribute in part to the discrepancies in the reports on the effects of C3435T on the pharmacokinetics after oral administration. The minimal effects of the intestinal MDR1 on intestinal absorption of commercially available oral drugs are consistent with a recently conducted experiment with knock-out mice, showing the absorption is not altered extensively, when compared with wild-type mice, although the distribution into brain is enhanced in the former (Dr. T. Fujita, personal communication.).

Besides the role of intestinal MDR1, it is noted that the concentration-time profiles after oral administration are defined not only by absorption, but also by distribution and excretion. Since little information is available on the effects of C3435T on the distribution and excretion of the substrates, to date, it is not surprising that we have no consensus in the results of pharmacokinetic data after single or multiple oral administrations. As for the latter, additionally we have to understand that multiple administrations sometimes resulted in up-regulation of MDR1. Recently, digoxin has been reported to up-regulate the expression of MDR1 after 24 hr exposure at 1 \( \mu \)M using Caco-2 in vitro (Fig. 2).\textsuperscript{119,120} This concentration of 1 \( \mu \)M is equivalent to 0.25 mg, contained in a tablet, per 320 mL, usually retained in the stomach. Moreover, a certain class of NSAIDs and antiepileptic drugs were also found to have altered MDR1 expression (Dr. K. Takara, personal communication.). To date, we do not have any data on the effects of MDR1 genotypes on the magnitude of the up- or down-regulation of MDR1, and it is not a question that the effects of MDR1 genotypes after multiple oral dosing are inconsistent with those after single oral dosing.

Collectively, the effects of intestinal MDR1 on the intestinal absorption of substrates is expected to be minimal in the case of commercially available oral drugs, including digoxin, CsA and FK506, and presumably fexofenadine and HIV protease inhibitors, and therefore MDR1 genotypes are little associated with the pharmacokinetics after oral administration. If we will have MDR1 substrates as oral drugs in the future, which show relatively low bioavailability and therefore are expected to be members of Class 2 or 4 in the BCS, MDR1 genotypes will become one of the predictors of pharmacokinetics after oral administration, therefore being a useful tool to establish personalized medicine.
An association of MDR1 genotypes with pharmacodynamics was reported for antiretroviral therapy, steroid therapy, anti-epileptic pharmacotherapy, adverse events with CsA or FK506, and others. Fellay et al. reported that HIV-1-infected patients with TT3435 showed a greater rise in the CD4-cell count after antiretroviral treatment for 6 months than those with CC3435 or CT3435, but Nasi et al.191 and Winzer et al.192 suggested no relationship of G2677A,T or C3435T with virological and immunological responses under antiretroviral therapy, and Haas et al. reported no relationship with phase 1 viral decay.193 Alonso-Villaverde et al. reported an efavirenz-induced increase in HDL-cholesterol was more predominant in patients with the C-allele at 3435.194 Zheng et al. reported that significantly larger numbers of CC3435 patients remain on steroids for 1 year after pediatric heart transplantation,52,195 and Asano et al. found that the risk of steroid-induced osteonecrosis of the femoral head was decreased in patients with WW2677 or TT3435 after renal transplantation, suggesting increased activity of MDR1 in these patients.196 Siddiqui et al. demonstrated that patients with drug-resistant epilepsy were more likely to have CC3435 than TT3435, when compared with patients with drug-responsive epilepsy,54 but very recently Tan et al.197 and Sillis et al.198 failed to find an association of C3435T with responders or non-responders to pharmacotherapy for epilepsy. Zheng et al. found that C3435T was a major predictor of acute persistent rejection in the first postoperative year in adult lung transplant patients.199 Yamauchi et al. suggested that MDR1 G2677A,T might be a predictor of FK506-induced neurotoxicity in Japanese liver transplant recipients.200 Hebert et al. reported that G2677T was associated with CsA- or FK506-induced nephrotoxicity in patients with post-liver transplantation and that the G2677T/C3435T haplotype might be useful.201 Hauser et al. also reported an association of C3435T with CsA-induced nephrotoxicity in patients with post renal transplantation.202 In contrast, Kotrych et al. reported no association of MDR1 C3435T with tremors found in allogenic renal transplant patients treated with CsA.203 Drozdzik et al. reported that gingival overgrowth in renal transplant recipients treated with CsA and others was not associated with MDR1 C3435T.204 Singh et al. reported that C3435T was independent of inhibition of lymphocyte proliferation by CsA.205 Kajinami et al. suggested that C3435T was significantly and independently associated with a reduction in LDL-cholesterol and with an increase in HDL-cholesterol during therapy with the HMG-CoA reductase inhibitor atorvastatin, in a gender-specific manner.206 Gawronska-Szklarz et al. reported that TT3435 was more frequently found in patients cured after a first cycle of triple anti-H.pylori therapy than in those in which eradication failed.207 Multidrug resistance is one of the most serious causes of the failure of chemotherapy, and biological factors in multidrug resistance have been investigated, including the down-regulation of uptake or induction of efflux systems such as MDR1, induction of inactivation enzymes, alteration of targeted enzymes, changes in DNA repair processes, and alteration of apoptotic signaling.208–216 MDR1 expression is the best characterized of these mechanisms, and the magnitude of resistance depends on the expression of MDR1 up-regulated by chemotherapy, suggesting personalization of chemotherapy based on MDR1 genotyping.217–224 Isla et al. suggested that outcome in docetaxel/cisplatin-treated advanced non small cell lung cancer was independent of C3435T,225 but Goreva et al. reported that G2677T and C3435T could be a predictor of the efficiency of chemotherapy for lymphoproliferative diseases.55 We suggested that the chemosensitivity of colorectal adenocarcinoma was independent of MDR1 genotype, presumably due to substantial variability in the expression.226,227 At present, only a few reports have been published, but clinical studies are underway.

**MDR1 Genotype-related Susceptibility to Diseases**

Potential roles for MDR1 in detoxification systems of normal tissues have been suggested by a number of non-clinical and clinical reports in which expression levels...
were found to be associated with susceptibility to a certain class of diseases, and MDRI genotypes have been investigated in terms of risk factors for such diseases, e.g., inflammatory bowel diseases (IBDs) and Parkinson’s disease. In 2003, Schwab et al. have indicated a significantly increased frequency of the T-allele at position 3435 in patients with ulcerative colitis (UC), not with Crohn’s disease, when compared with healthy subjects. The etiology of these IBDs is not understood completely, although a hypersensitive immunological reaction is assumed to be involved in chronic inflammation. Panwala et al. indicated that mdr1a-/-knock-out mice were susceptible to a spontaneous UC-like intestinal inflammation under specific pathogen-free conditions. Since mdr1a-/-knock-out mice are immunologically normal, the development of spontaneous colitis is presumably due to a defect in the intestinal epithelial barrier. They also indicated that infection with Helicobacter bilis induced diarrhea, weight loss, and IBDs in mdr1a-/-knock-out mice. These observations strongly suggest that the susceptibility to IBDs depends on the expression of MDR1, and therefore on MDRI genotype. Immediately thereafter, some investigators attempted to replicate the findings of Schwab et al. Although studies with independent northern European cohorts and with Greek populations failed to obtain the same results, Glas et al. obtained partially consistent results, and subsequently Brant et al. and Ho et al. suggested that G2677T or C3435T was associated with IBDs. It is noted that MDRI genotype might be important also from the viewpoint of pharmacotherapy for IBDs. Furuno et al. reported that the frequency of TT3435 was relatively higher in an early-onset Parkinson’s disease group. Drozdzik et al. reported a significant association between parkinsonism on exposure to pesticides and C3435T. Kimura et al., Pawlik et al., and Kivisto et al. suggested that there was no association of C3435T with primary biliary cirrhosis, rheumatoid arthritis and hypertension, respectively.

There are several reports on the relationship between MDRI genotype and susceptibility to cancer. Siegsmund et al. and Jamroziak et al. have suggested that the T-allele at position 3435 is a risk factor for renal epithelial tumors and childhood ALL, respectively. Kurzawski et al. have indicated the association with colon cancer, based on the pioneering studies by Potocnik et al. and Humeny et al. On the other hand, Stanulla et al. suggested a significant reduction in the risk of relapse in the central nervous system in childhood ALL for patients with the T-allele at 3435, and Miller et al. reported no association with adult glioma. Illmer et al. have reported MDRI genotype-related susceptibility to acute myeloid leukemia, where the heterozygote for C1236T, G2677T and C3435T was more frequently found among patients.

Recent investigations have challenged the notion that MDRI has evolved merely to facilitate the eflux of xenobiotics and raised the possibility that MDRI plays a fundamental role in regulating apoptosis and immunology. Although little information is available concerning the role of MDRI in the system, Smyth and Johnstone and their co-workers suggested that MDRI protected cells against caspase-dependent apoptosis induced by cytotoxic drugs, Fas ligation, tumor necrosis factor, and ultraviolet irradiation. We also found that MDRI expression was up-regulated by apoptotic stimuli and suppressed caspase-dependent apoptotic signaling, presumably via a mitochondrial pathway. The role of MDRI in apoptosis and immunological reactions has been sometimes discussed from viewpoints of the sphingomyelin-ceramide pathway, acidification of the intracellular space, cholesterol esterification and cytokine release from lymphocytes. These observations suggest that pharmacodynamics are related to the MDRI genotype, independently of the effects on pharmacokinetics, and the polymorphisms of the MDRI gene might be a risk factor for a certain class of disease, especially in cases where a MDRI-related etiology is demonstrated.

Conclusions
For the past 5 years, a number of clinical studies have been conducted on the association of MDRI genotype, especially of a silent C3435T in exon 26, with the MDRI expression and function in tissues and with the pharmacokinetics and pharmacodynamics of drugs; however, there are still considerable discrepancies in the results. According to a recently proposed concept, the Biopharmaceutical Classification System, to predict in vivo oral pharmacokinetic performance, it can be concluded that MDRI genotypes are little associated with the pharmacokinetics of drugs after oral administration, since the effects of intestinal MDRI on the intestinal absorption of substrates is expected to be minimal in the case of commercially available oral drugs, including digoxin, CsA and FK506, and presumably fexofenadine and HIV protease inhibitors.

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