Expression of P-glycoprotein in Human Placenta: Relation to Genetic Polymorphism of the Multidrug Resistance (MDR)-1 Gene

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ABSTRACT

To evaluate whether mutations in the human multidrug resistance (MDR)-1 gene correlate with placental P-glycoprotein (PGP) expression, we sequenced the MDR-1 cDNA and measured PGP expression by Western blotting in 100 placentas obtained from Japanese women. Nine single nucleotide polymorphisms (SNPs) were observed with an allelic frequency of 0.005 to 0.420. Of these SNPs, G2677A (allelic frequency 0.18) and G2677T (0.39) in exon 21 were associated with an amino acid conversion from Ala to Thr and to Ser, respectively. Sixty-one of 65 samples (93.8%), which had a C3435T allele, also had a mutant G2677(A,T) allele, suggesting an association between the two SNPs. Correlations of mutations with expression levels were observed; individuals having the G2677(A,T) and/or T-129C (p < 0.05) allele had less placental PGP. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)-based genotyping tests were developed for the detection of these SNPs. The PCR, in which genomic DNAs obtained from healthy subjects (n = 48) are used as samples, was successful. The frequency of mutations in placental cDNA was identical with that in genomic DNA. When genotype results were compared between Caucasians and Japanese, ethnic differences in the frequency of polymorphism in the MDR-1 gene were suspected. Although it remains to be determined whether these SNPs influence the pharmacokinetic and dynamic properties of clinically useful drugs that are substrates of PGP, the polymorphism of the MDR-1 gene presented here may provide useful information in in vivo study of these issues.

The human multidrug resistance (MDR)-1 gene encodes a 170-kDa transmembrane glycoprotein (P-glycoprotein; PGP), which confers energy-dependent resistance to a number of structurally unrelated types of clinically useful drugs. MDR-1 belongs to the superfamily of ATP binding cassette transporters, present from bacteria to man (Higgins, 1992). The molecular architecture of MDR-1 shows a four-domain arrangement, with two membrane-spanning domains and two nucleotide binding domains. A number of mutational analytical approaches have been used to help elucidate the mechanism of action of human PGP and have indicated that mutations in membrane-spanning domains or nucleotide binding domains are involved in the binding and transport of PGP (reviewed by Ambudkar et al., 1999).

PGP is reportedly expressed in various normal human tissues, such as small and large intestine, adrenal, kidney, liver, and capillary endothelial cells of brain and testes (Fojo et al., 1987; Thiebaut et al., 1987; Sugawara et al., 1988; Cordon-Cardo et al., 1989). Tissue distribution suggests that PGP may play a role in the protection of the organism against toxic xenobiotics. Shinkel et al. (1994) generated mice with a homozygous disruption of the mdr1a gene and found that PGP plays an important role in the blood-brain barrier and that its absence results in elevated drug levels in the brain and many other tissues. PGP was highly expressed in trophoblasts but not in endothelial cells of human placenta. It was suggested that the barrier in the placenta has the ability to block the transfer of hydrophobic xenobiotics across the human placenta and that the PGP in trophoblasts contributes to the function of the barrier (Nakamura et al., 1997).

Whereas the physiologic role of PGP in the human body is not completely understood, the importance of PGP for drug absorption from the gastrointestinal tract and drug elimination via the bile and urine is clear. Recently, Hoffmeyer et al.

ABBREVIATIONS: PGP, P-glycoprotein; MDR-1, multidrug resistance-1; SNP, single nucleotide polymorphism; SSCP, single-strand conformation polymorphism; RT-PCR, reverse transcriptase-polymerase chain reaction; PCR-RFLP, PCR-restriction fragment length polymorphism; TPBS, 1× phosphate-buffered saline, 0.1% Tween 20; bp, base pair(s).
polymorphisms in the human MDR-1 gene and described their distribution in a Caucasian population. They also reported a significant correlation of a polymorphism in exon 26 (C3435T) of MDR-1 with the expression level and function of PGP. Individuals homozygous for the C3435T allele had significantly reduced duodenal MDR-1 and increased digoxin (a substrate of PGP) plasma levels. However, because the C3435T allele is not associated with an amino acid substitution and because of its location at a non-coding nonpromoter position in the MDR-1 gene, it is unlikely that this SNP directly influences PGP expression. Therefore, it is of interest whether other SNPs exist in regions of the MDR-1 gene that control expression, and whether known and unidentified SNPs correlate with the MDR-1 expression in human tissues. The discovery and characterization of variations in the MDR-1 gene and diagnostic tests for the discrimination of different MDR-1 alleles in human individuals may provide a potent tool for improving the therapy of diseases with drugs that are substrates of PGP.

In this study, we had three aims. First, we intended to assess the genetic structure of the MDR-1 gene using 100 placentas and 48 genome DNAs obtained from Japanese subjects and to compare the allelic frequency between Caucasian and Japanese populations. Second, we intended to assess the correlation of MDR-1 gene polymorphism with placental PGP expression. Finally, we developed PCR-RFLP-based methods to diagnose the mutations in the MDR-1 gene using genomic DNA.

Materials and Methods

Placentas and DNA Samples. One hundred human full-term placentas were obtained from patients at Tottori University Hospital. Almost all the patients experienced a normal pregnancy, but five had intra-uterine growth retardation (IUGR), and three had toxemia of pregnancy (TP). Because the expression levels of PGP were not different from other samples, these eight samples were included in the present study. Highly enriched human placental trophoblast populations were prepared (Nakamura et al., 1997). Human placental samples for the RNA extraction were immediately frozen in liquid nitrogen after delivery and stored at −80°C until preparation. To obtain genomic DNA, 48 unrelated healthy subjects were enrolled. Each patient and healthy subject gave written informed consent to participate in the study, which was approved by the Tottori University Ethics Committee and the Institutional Review Board of the Clinical Pharmacology Center, Medical Co., Ltd.

RNA Extraction and Reverse Transcription-PCR (RT-PCR). Total RNA from whole human placenta was isolated by use of ISOGEN (Nippongene, Tokyo, Japan), and the same placental section was fixed in 10% neutral formalin overnight at 4°C and embedded in paraffin for subsequent immunohistochemistry to confirm the PGP expression. First-strand synthesis from total RNA was performed by use of random hexamers (Promega, Madison, WI) and Moloney murine leukemia virus reverse transcriptase (Life Technologies, Rockville, MD). As a negative control, template RNA was processed without reverse transcriptase. The resulting cDNA was amplified by PCR with 27 sets of primers specific for the human MDR-1 nucleotide sequence. The primer design was based on published sequences of the mRNA of MDR-1 (GenBank accession number M14758 for the whole mRNA) to avoid amplification of sequences from homologous genes. These primers were designed to divide the cDNA of the MDR-1 sequence into 27 fragments of ~300 bp, for the screening of mutations by subsequent single-strand conformation analysis (SSCP) (Fig. 1). PCR was carried out in a total volume of 50 μl in the presence of 100 ng of cDNA, 0.25 μM each primer, 10X PCR buffer II, 1.5 mM MgCl2, 0.2 mM each deoxynucleoside-5’-triphosphate, and 1.25 to 2.5 U of AmpliTaq Gold DNA polymerase (Applied Biosystems, Foster City, CA). After an initial denaturation at 94°C for 5 min, 30 to 45 cycles of 0.5 to 1 min at 94°C, 0.5 to 1 min at 50 to 60°C and 1 to 2 min at 72°C, as well as a final extension period of 5 min at 72°C, were carried out. PCR products were analyzed on 3% agarose gels to check both size and specificity of the products.

PCR-SSCP. To screen mutations of the MDR-1 gene, SSCP analysis was performed using the GenePhor system (Amersham Pharmacia Biotech AB, Uppsala, Sweden) as recommended by the manufacturer. The RT-PCR product (6 μl) was mixed with 3 μl of 20 mM EDTA, 95% formamide, and 0.05% bromophenol blue, and this mixture was heated at 95°C for 5 min and then quick-chilled in an ice-water bath. The resulting single stranded DNA (5 μl) was then loaded on a 12.5% polyacrylamide gel (GeneGel excel 12.5/24 kit; Amersham Pharmacia Biotech AB). Electrophoresis was carried out at 450 V of constant power at 20°C for 2 to 5 h, depending on the

![Fig. 1. Polymorphism in the human MDR-1 gene. The positions of primers for SSCP analysis and the locations of the identified polymorphisms (arrows) are indicated in relation to the exon structure of the human MDR-1 gene and the predicted topology of PGP. Sequence numbering is from Chen et al. (1990).](image-url)
fragment size. After electrophoresis, gels were stained using an automated gel stainer with PlusOne (Amersham Pharmacia Biotech AB).

**DNA Sequence.** All PCR products were sequenced either directly or after subcloning on an ABI 377 automatic sequencer (Applied Biosystems) using a Big-Dye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems). If the direct sequencing was incomplete, each amplified PCR product was subcloned into the pGEM vector (Promega) and transformed into JM109 competent cells (Promega). Prior to the sequencing, reaction mixtures were purified with a DyeEx Spin kit (QIAGEN GmbH, Hilden, Germany). The sequencing primers were those used in the PCR amplifications. The sequencing of both strands was analyzed for products from at least two independent PCR amplifications to ensure that the identified mutations were not PCR-induced artifacts.

**PCR-RFLP.** PCR-RFLP-based genotyping tests were developed for the detection of the new and known mutations using genomic DNA. Venous blood (10 ml) was obtained from each healthy subject, and genomic DNA was isolated from peripheral lymphocytes using GENOMIX (Talent srl, Trieste, Italy). The PCR conditions were the same as for RT-PCR, but different primer sets were developed. The PCR product was digested with an appropriate restriction enzyme under standard conditions without purification (Table 2). Digested PCR products were analyzed on 3% agarose gels and stained with ethidium bromide. As shown in Table 1, A2956G and G4030C mutations were not observed in the examined genomic DNA samples; thus, placental cDNAs with these mutations were used as templates to establish the PCR-RFLP-based genotyping tests.

**Immunohistochemistry.** A 4-μm section was cut from the paraffin blocks of human placentas, deparaffinized in xylene, and rehydrated. Endogenous peroxidase activity was blocked with 0.3% (v/v) H2O2 in methanol for 30 min. A mouse monoclonal anti-P-glycoprotein (Kamiya Biomedical Company, Seattle, WA), was applied for 2 h at 37°C. The primary antibody was visualized using the Histofine Simple Stain PO (Nichirei, Tokyo, Japan) according to the instruction manual. The slide was counterstained with hematoxylin.

**Western Blot Analysis.** Human placental trophoblast cultures were homogenized in a lysis buffer containing 50 mM Tris-HCl (pH 7.6), 150 mM NaCl, 0.1% SDS, 1 mM dithiothreitol, and 1× Complete Protease Inhibitor Cocktail (Roche Molecular Biochemicals, Indianapolis, IN). The lysate was centrifuged at 15,000g for 30 min at 4°C, and the supernatant was separated. Protein concentrations of the supernatant were determined by the Bio-Rad Protein Assay (Bio-Rad, Hercules, CA) using bovine serum albumin as a standard. The supernatants (80 μg of protein) were loaded onto SDS-4 to 20% (w/v) gradient polyacrylamide gels (Tefco, Tokyo, Japan) and transferred to Sequi-Blot polyvinylidene difluoride membranes (Bio-Rad, Hercules, CA) at 180 mA for 2 h. Thereafter, the membranes were blocked with 5% skim milk in TBPS (1× phosphate-buffered saline, 0.1% Tween 20) for 2 h at room temperature, then incubated overnight at 4°C with Clone F4 at a final concentration of 10 μg/ml in 5% skim milk in TBPS. The membrane was washed three times with TBPS, then incubated for 1 h at room temperature with 1000-fold diluted horseradish peroxidase-conjugated secondary antibody, peroxidase-conjugated goat IgG fraction to mouse IgG (ICN Pharmaceuticals, Aurora, OH). Polyvinylidene difluoride membranes were rinsed four times for 10 min with TBPS, then evenly coated using the ECL Western blotting detection system (Amersham Pharmacia Biotech AB) for 1 min. The membrane was immediately exposed to Kodak X-OMAT AR film (Kodak, Tokyo, Japan) at room temperature. To assure the quantitative expression of PGP, an additional marker protein expressed in placenta, alkaline phosphatase, was measured according to the same protocol except that different primary (polyclonal rabbit anti-human placental alkaline phosphatase) and secondary (peroxidase-conjugated goat IgG fraction to rabbit IgG) antibodies were used. Multiple drug-resistant KB cell lines, which were selected from human epidermoid KB-3-1 carcinoma cells

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**Table 1**

<table>
<thead>
<tr>
<th>Exon/Position</th>
<th>Nucleotide Sequence</th>
<th>Wild Type</th>
<th>Mutation (WT)</th>
<th>Allele Frequency</th>
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<tbody>
<tr>
<td>5′-flanking</td>
<td>cccGAGTGAT</td>
<td>Noncoding</td>
<td>G-41aG</td>
<td>50 31 19 65.5 34.5 21 20 7 64.6 35.4</td>
</tr>
<tr>
<td>1a/145</td>
<td>agggCCTGA</td>
<td>Gly412Gly</td>
<td>50 31 19 65.5 34.5 21 20 7 64.6 35.4</td>
<td></td>
</tr>
<tr>
<td>21a/145</td>
<td>agggtTCTGG</td>
<td>Ile1145Ile</td>
<td>35 46 19 58.0 42.0 14 21 13 51.0 49.0</td>
<td></td>
</tr>
<tr>
<td>21a/145</td>
<td>agggTCTGA</td>
<td>Ala893Ser</td>
<td>14 (G/G) 35 (G/T) 16 (T/T) 43.0 39.0 (T) 9 (G/G) 8 (G/T) 10 (T/T) 36.5 41.7 (T)</td>
<td></td>
</tr>
<tr>
<td>5′-flanking</td>
<td>cccGAGTGAT</td>
<td>Noncoding</td>
<td>G-41aG</td>
<td>50 31 19 65.5 34.5 21 20 7 64.6 35.4</td>
</tr>
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<td>1a/145</td>
<td>agggCCTGA</td>
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<td>50 31 19 65.5 34.5 21 20 7 64.6 35.4</td>
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<tr>
<td>21a/145</td>
<td>agggtTCTGG</td>
<td>Ile1145Ile</td>
<td>35 46 19 58.0 42.0 14 21 13 51.0 49.0</td>
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<tr>
<td>21a/145</td>
<td>agggTCTGA</td>
<td>Ala893Ser</td>
<td>14 (G/G) 35 (G/T) 16 (T/T) 43.0 39.0 (T) 9 (G/G) 8 (G/T) 10 (T/T) 36.5 41.7 (T)</td>
<td></td>
</tr>
</tbody>
</table>

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**Note:** Positions, sequences, and frequencies of MDR-1 variants in human placentas (cDNA) and genomic DNAs.
by increasing the concentration of colchicine, were used as positive controls (Akiyama et al., 1985). The immunoblots were quantitated using a public domain NIH image program (written by Wayne Rasband at the U.S. National Institutes of Health and available from the Internet by anonymous ftp from zippy.nimh.nih.gov or on floppy disk from NTIS, 5285 Port Royal Rd., Springfield, VA 22161, part number PB93-504648).

**Statistical Analysis.** Data were shown as the mean ± S.D. Results of PGP expression versus mutation [G2677(A,T) and C3435T in Fig. 5] were analyzed by a Kruskal-Wallis one-way analysis of variance followed by Dunn’s test. Wilcoxon’s signed-ranks test was used when comparing only two groups (T-129C, in Fig. 5). Significance was defined as p < 0.05.

**Results**

**MDR-1 Polymorphisms in Placental cDNA.** All PCR procedures developed and used in the present study to amplify the MDR-1 gene were successful. In all cases, a single PCR product of predicted size was obtained and matched the sequence predicted from the published cDNA. The sequence was inspected for deviations from the original (Chen et al., 1990), which we define as the “wild type”. Nine SNPs were detected by SSCP analysis and were identified by subsequent sequencing (Fig. 2; Table 1). Three of these polymorphisms resulted in protein alterations in exons 21 and 24. G→T and A transversions at position 2677 [G2677(A,T), for position numbering refer to Chen et al., 1990] were associated with an amino acid change from Ala893 to Ser893 and to Thr893, respectively. The variation in exon 21 occurred in 58.0% of samples as heterozygosity and 28.0% as homozygosity for the mutant allele (Table 1). Four mutations were located in non-coding regions with frequencies from 0.005 to 0.305. C3435T in exon 26 did not change an amino acid and occurred in 46.0% of samples as heterozygosity and 19.0% as homozygosity for the mutant allele. Sixty-one of 65 samples (93.8%), which had a C3435T allele, also had a mutant G2677(A,T) allele; fifteen of the 61 samples were homozygous for the mutant allele; the others were heterozygous. Heterozygous samples for the T-129C allele (n = 12) also had a mutant G2677(A,T) allele; however, an association between T-129C and C3435T was not observed. In the present study, all placental samples had at least one mutation in the MDR-1 gene.

**MDR-1 Polymorphisms in Genomic DNA Diagnosed by PCR-RFLP-Based Genotyping.** To diagnose an individual’s genotype using genomic DNA, PCR-RFLP-based genotyping tests were developed (Table 2; Fig. 2). An A→G transition 41 bases upstream from the initial position of exon 1a, (A-41aG), which was observed in our previous study (Ito et al., 2001), was also included. The allele frequencies of A-41aG, C-145G, T-129C, T1236C, G2677T, G2677A, A2956G, C3435T, G4030C, and A4036G in 48 healthy subjects were 9.4, 1.0, 8.3, 35.4, 41.7, 21.8, 0.0, 49.0, 0.0, and 25.0%, respectively (Table 1). A2956G and G4030C mutations, which were detected in placental cDNAs (n = 100), were not observed in 48 genomic DNAs. As shown in Table 1, the frequencies of mutations in genomic DNA were the same as those in placental cDNA, and all subjects had at least one mutation in the MDR-1 gene. Among 10 SNPs, A-41aG in the 5′-flanking region and T-129C in exon 1b appeared to be linked. We consistently observed (with one exception; see Table 1) at these positions the homozygous combinations A/A-T/T and G/G-C/C and the heterozygous combinations A/G-T/C. For other polymorphisms, linkage could not be predicted directly from the sequence data.

**SNP Correlates with Placental MDR-1 Expression.** To assure that quantitative Western blots reflect the specific expression of PGP in placenta, an additional marker enzyme that characterizes the enrichment of trophoblasts in placental microvilli, alkaline phosphatase, was measured in all the examined samples (Booth et al., 1980; St-Pierre et al., 2000). In addition, before the blotting, PGP expression was confirmed by immunohistochemical staining (Fig. 3). Because PGP was not clearly identified in 10 samples (but there were no particular changes in their MDR-1 sequences), and one sample was used as a control (sample 100), 89 total samples were used (Fig. 4). A comparison of the MDR-1 genotyping results and corresponding placental PGP levels of the 89 samples shows a correlation between the level of expression and SNPs in exon 1b (T-129C) and exon 21 [G2677(A,T)] (Fig. 5). T-129C (T/C) was associated with significantly lower levels of PGP than T/T (wild type) (1.07 ± 0.92 versus 1.99 ± 1.48, p = 0.002). Although the difference was not significant, G2677(A,T) was also associated with lower levels of PGP in placentas; the mean expression levels in homozygotes for the wild-type allele, heterozygotes, and homozygotes for the mutant allele were 2.44 ± 1.57, 1.97 ± 1.62, and 1.45 ± 0.87, respectively. Heterozygous individuals displayed an intermediate phenotype. The mean of the PGP expression levels for the C/C, C/T, and T/T genotypes at position 3435 was 2.11 ± 1.84, 1.85 ± 1.28, and 1.51 ± 0.97, respectively. The standard deviation was large, and the mean was comparable between each other group.
MDR-1 Gene Polymorphisms. The positions in the MDR-1 gene of the nine polymorphisms found in the present study, in relation to the predicted structure of the encoded PGP, are presented in Fig. 1. The G\(^{2677}\)T and A transversions at position 2677 in exon 21 were missense mutations located on the intracellular side of PGP after transmembrane region 10. At position 2677, Ala893 is replaced by Thr or Ser, which results in a change from a lipophilic residue to a hydrophilic one. Alanine is a structurally neutral amino acid that does not affect the secondary structure. The G\(^{2677}\)T transversion was initially isolated from a full-length MDR-1 cDNA from human adrenal, where PGP is expressed at a high level (Kioka et al., 1989). The expression of the construct indicated that the amino acid substitution at codon 893 from Ala to Ser did not affect the resistance to colchicine but was associated with changes in the resistance to Adriamycin and vinblastine (Kioka et al., 1989). Subsequently, two genetic polymorphisms in the MDR-1 gene at position 2677 and 2995 in exons 21 and 24 were identified (McKay et al., 1990). These polymorphisms at position 2677 and 2995 in exons 21 and 24 were not identified in healthy volunteers and in refractory lymphomas. In that study, the

### TABLE 2

Genotyping procedures for the MDR-1 polymorphisms using genomic DNA

<table>
<thead>
<tr>
<th>Primer</th>
<th>Length (bp)</th>
<th>Restriction Enzyme</th>
<th>Cutting Position</th>
<th>Wild/Wild</th>
<th>Wild/Mutant</th>
<th>Mutant/Mutant</th>
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<tbody>
<tr>
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<tr>
<td>T-129C</td>
<td>243</td>
<td>HaeIII</td>
<td>68,79</td>
<td>33,35,79</td>
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<tr>
<td>T1236C</td>
<td>147</td>
<td>NcoI</td>
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<td>G2877T</td>
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<td>Alul</td>
<td>83</td>
<td>24,83,107</td>
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<tr>
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<td>AfaI</td>
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<td>81</td>
<td>NdeII</td>
<td>30,81</td>
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</table>

Fig. 3. Immunohistochemical identification of PGP in human placenta.

Fig. 4. Western blot analysis of placental PGP. Lane 1, sample 100 (control for calculations); lanes 2 through 8, placenta. The position of molecular mass markers is indicated on the left.
polymorphism at position 2677 (G→T transversion) was found to be heterozygous in 43% of the samples. Since the G→A transversion was first identified in the present study, its functional effect remains unknown. Another protein alteration that we found changed Met986 in exon 24 to Val. This polymorphism is located in the putative carboxy-terminal half of transmembrane region 12 (residues 974–994). Biochemical and genetic studies with PGP have identified putative transmembrane region 12 as involved in drug interactions with amino acid residues conserved among PGP family members shown to be essential for transport (Loo and Clarke, 1993, 1994). However, unlike in the putative amino-proximal half of transmembrane region 12, the replacement of the nonconserved residue Met at codon 986 with Ala, a small lipophilic residue like Val, had no effect on drug transport except for a partial reduction in bodipy-verapamil extrusion (Hafkemeyer et al., 1998). Thus, the impact of this polymorphism on PGP function in vivo remains to be clarified.

Three SNPs (including A-41aG) were identified in the promoter or 5′-flanking region. Several studies have attempted to define the regulatory sequences involved in MDR-1 basal transcription (Ueda et al., 1987; Kioka et al., 1992; Cornwell and Smith, 1993). The three SNPs that we found did not map to known sequence elements such as G box, CAAT box, and heat-shock responsive elements. C-145G and T-129C were located 9 bp upstream (+9) and 7 bp downstream (+7), respectively, from the transcription initiation site (CCTGAGCTCA**+7**TTCGAGTAG). A mutation study showed that nucleotides A and T at position +1 and +3, respectively, were essential, whereas other nucleotides in this region had little effect on promoter activity (van Groenigen et al., 1993). However, interestingly, T-129C was observed with a higher frequency in patients with hematological malignancies than in normal controls (Rund et al., 1999).

In the present study, linkage between SNPs in the 5′-flanking region and exon 1b, and a strong association between C3435T and G2677(A,T), were observed. In addition, all placental cDNAs and genomic DNAs had at least one mutation in the MDR-1 gene. These results were consistent with a recent report by Hoffmeyer et al. (2000) in which 21 healthy Caucasian volunteers had at least one mutation in their MDR-1 gene. Although our attempts to determine allele haplotypes in the MDR-1 gene did not provide clear answers, it is speculated that various haplotypes exist for the MDR-1 gene.

When the frequency of homozygosity for the mutant alleles, calculated on the basis of the Hardy-Weinberg distribution, was compared between Caucasians (Hoffmeyer et al., 2000) and Japanese, it was found that the frequency of T/T at position 3435 in exon 26 did not differ (23.0% in Caucasians versus 24.0% in Japanese); however, that of C/C in exon 1b (0.4 versus 0.7%) and of C/T at position 1236 in exon 12 (38.7 versus 12.5%) did. In addition, in contrast to our results, they did not find the polymorphism at position 2677, even though, for this position, 24 different individuals were screened. Although further study is needed to address the conclusion, these results suggest an ethnic difference in the frequency of polymorphism in the MDR-1 gene.

Placental PGP Expression Levels. Among the nine SNPs found in the present study, T-129C in the promoter region and G2677(A,T) in exon 21 correlated with PGP expression levels in the placenta. The correlation of T-129C with the expression was significant (*p < 0.05*). As mentioned above, a previous study (van Groenigen et al., 1993) found that A at position +1 and T at +3 were essential for initiator function. However, single base substitutions of other nucleotides of the MDR-1 gene initiator resulted in low transcription efficiency. As shown in Fig. 5, the mean PGP ex-
pression level in heterozygous (T/C) samples was 2-fold lower than that in homozygous (T/T) samples; thus, the effect of T-129C on PGP expression cannot be ruled out. Another SNP, G2677(A,T), also correlated with the level of PGP expression but not significantly. Recently, Hoffmeyer et al. (2000) reported that individuals with the C3435T allele had a significantly reduced duodenal PGP expression. Because C3435T does not change the amino acid sequence and is not located at a promoter position in the MDR-1 gene, it is unlikely that this SNP directly influences PGP expression. Interestingly, a strong association between the C3435T and G2677(A,T) allele was observed in our placental samples. Since G2677(A,T) is a missense mutation, it rather than G2677(A,T) allele was observed in our placental samples. Interestingly, a strong association between the C3435T and G2677(A,T) also correlated with the level of PGP expression. Recently, Hoffmeyer et al. (1999) observed a 2-fold lower expression in heterozygous (T/C) samples. It is unlikely that this SNP directly influences PGP expression. Since G2677(A,T) is a missense mutation, it rather than G2677(A,T) allele was observed in our placental samples.

In conclusion, this study identified various SNPs in the MDR-1 gene and characterized their effects on PGP expression in the placenta. Of these SNPs, T-129C and G2677(A,T) are particularly interesting with regard to the level and frequency of PGP expression among Japanese subjects. Although it remains to be determined whether these SNPs influence the pharmacokinetic and pharmacodynamic properties of clinically useful drugs that are substrates of PGP, the PCR-RFLP-based genotyping tests developed here may be useful in the study of these issues.

References


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