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Gene and Protein Expression of P-Glycoprotein, MRP1, MRP2, and CYP3A4 in the Small and Large Human Intestine

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Abstract: The cytochrome P450 3A4 enzyme and the ABC-transporters may affect the first-pass extraction and bioavailability of drugs and metabolites. Conflicting reports can be found in the literature on the expression levels of efflux transporters in human intestine and how they vary along the intestine. The relative levels of mRNA and protein of CYP3A4 and the ABC tranporters Pgp (ABCB1), MRP1 (ABCC1), and MRP2 (ABCC2) were determined using RT-PCR and Western blot for human intestinal tissues ($n = 14$) from jejunum, ileum and colon. The expression of mRNA for CYP3A4, Pgp, and MRP2 was highest in jejunum and decreased toward more distal regions, whereas MRP1 was equally distributed in all intestinal regions. For CYP3A4, a more significant correlation could be established between mRNA and protein expression than for the ABC transporters. The samples showed considerable interindividual variability, especially at the protein level. The apically located Pgp and MRP2 showed a similar expression pattern along the human intestine as for CYP3A4. The gene expression of MRP1 exhibited a more uniform distribution.

Keywords: P-glycoprotein; CYP3A4; MRP; human; intestine; RT-PCR; Western blot

Introduction

Cytochrome P450 (CYP)-mediated metabolism and active intestinal transport (efflux) are important factors that affect intestinal drug absorption and bioavailability as they constitute the first defense against xenobiotic uptake.[1] P-glycoprotein (Pgp), the MDR1 gene product (ABCB1), was the first multidrug resistance ATP-binding cassette (ABC)-transporter to be identified. Pgp is expressed in human tissues including the apical membrane of the small and large intestine.[2,3] It has been reported that intestinal Pgp can affect the absorption and bioavailability of drugs such as digoxin and talinolol by transporting them from the apical membrane back into the lumen.[4,5] MRP1 (ABCC1) and MRP2 (ABCC2) are multidrug resistance transporters of the ABC superfamily, primarily transporting intracellularly formed drug conjugates.

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MRP1 and MRP2 are located in the intestine at the basolateral and apical membrane, respectively, but they are also found in other tissues.[6] CYP3A4 plays an important role in drug metabolism in human intestine and liver. Although the enteric content of CYP3A4 is much lower than in the liver, approximately 1% of liver content, it has been established that the intestine contributes equally to the metabolic first-pass effect for CYP3A4 substrates.[7] In addition, it has been suggested that CYP3A4 cooperates with Pgp leading to an efficient first-pass drug extraction in the intestine.[8,9] However, functional *in vivo* evidence to support this hypothesis is still lacking.

Successful development of controlled release products relies on knowledge of any involvement of metabolism and efflux transport proteins along the human intestine. However, there are some conflicting results in the literature concerning the expression of efflux transporters such as Pgp and MRP in different regions of the human intestine.[2,10−13] The first studies of mRNA levels of Pgp in various human tissues reported an increase of Pgp expression from the small to the large intestine.[2] More recent reports indicate the opposite expression pattern for both Pgp and MRP2 at the mRNA level,[12,13] indicating that these transporters share the same distribution pattern as CYP3A4, with higher levels being expressed in the jejunum than in the ileum and colon.[14] This would be in agreement with the expression in rat and dog for which mRNA of mrp2 has been reported to be higher in the small intestine compared to colon.[15,16] It is of clinical interest to improve the understanding of the regional distribution of ABC-transporters as they might be involved in the development of intestinal inflammatory bowel disease as well as the response to treatment with anti-inflammatory drugs.[17]

The primary aim of this study was to characterize the mRNA and protein expression of Pgp, MRP1, MRP2, and CYP3A4 in human intestinal segments from human jejunum, ileum, and colon, to evaluate the regional expression difference and variability. A secondary aim was to study the correlation of mRNA and protein levels of Pgp, MRP1, MRP2, and CYP3A4 to investigate their value as expression parameters.

Materials and Methods

Intestinal Tissues. Intestinal tissues were obtained from 14 patients during the years 2000 and 2001. Jejunal segments ($n = 3$) were obtained from patients undergoing surgery for gastric bypass or treatment of pancreatic cancer. Segments from the ileum ($n = 4$), proximal colon ($n = 1$), and sigmoideum colon ($n = 6$) were obtained from patients undergoing surgery for intestinal cancer. Immediately after resection the intestinal segments were snap frozen using liquid nitrogen and stored at −80 °C. The segments did not show any signs of inflammation or cancer infiltration. Prior to surgery the patients were informed about the study and they gave their informed consent to their participation. All tissue samples were destroyed after the study. The study was approved by the Ethics Committees of Lund University and Uppsala University.

Western Blot. The frozen intestinal samples were cut into pieces on dry ice and homogenized with 0.1 M TRIS (tris-(hydroxymethyl)-aminomethane)/HCl (pH 7.6), supplemented with 1 mM EDTA and "Complete, EDTA-free protease inhibitor cocktail" tablets (Roche, Mannheim). The homogenate was centrifuged at 4 °C, 6500*g* for 15 min, and the supernatant was centrifuged at 4 °C, 100000*g* for 30 min. The pellet containing membrane proteins was dried and resuspended in homogenizing buffer, and aliquots were frozen using liquid nitrogen and were stored at −80 °C.

Membrane proteins (25 μg) were separated with SDS−PAGE and electroblotted on Immobilon-P membranes (Millipore, Neu-Isenburg, Germany). Blots were blocked with 10% (w/v) nonfat milk in Tris-buffered saline (TBS)/0.05% (v/v) Tween for 1 h and then probed with the primary antibody in TBS/Tween overnight at 4 °C. The immunoreaction was visualized using a horseradish peroxidase-conjugated goat anti-mouse antibody diluted in TBS/Tween, followed by use of the chemiluminescence (ECL)-plus technique (Amersham, Braunschweig, Germany).

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Table 1. Primers Used for Reverse Transcription Polymerase Chain Reaction of Villin, CYP3A4, MRP2, Pgp, and MRP1

	primer sequence		base pairs	GenBank accession number human cDNA
villin	sense	5′-CAG CTA GTG AAC AAG CCT GTA GAG GAG C-3′	2311−2329	NM_007127
	antisense	5′-CCA CAG AAG TTT GTG CTC ATA GGC AC-3′	2611−2587	NM_007127
CYP3A4	sense	5′-CCT CAC AAA CCG GAG GCC TTT TGG T-3′	407−430	NM_017460
	antisense	5′-ATC AGG GTG AGT GGC CAG TTC ATA C-3′	1132−1108	NM_017460
Pgp	sense	5′-CCC ATC ATT GCA ATA GCA GG-3′	3020−3040	M 14758
	antisense	5′-GTT CAA ACT TCT GCT CCT GA-3′	3176−3156	M 14758
MRP2	sense	5′-ACT TGT GAC ATC GGT AGT ATG-3′	4062−4082	U 49248
	antisense	5′- GTG GGC GAA CTC GTT TTG-3′	4556−4539	U 49248
MRP1	sense	5′-G AAG ACC AAG ACG TAT CAG GT-3′	1651−1671	L 05628
	antisense	5′-CAA TGG TCA CGT AGA CGG CAA-3′	1910−1890	L 05628

Densitometric analysis of blots was performed to obtain optical densities (OD).[18]

Antibodies. The monoclonal antibody C494 (0.1 mg/mL; Alexis Biochemicals, Grünberg, Germany) was used for the detection of Pgp in a dilution of 1:1000. This murine anti-human MDR1 antibody recognizes a gene-specific, intracellular epitope exclusively found in MDR1/Pgp. Immunopure goat anti-mouse IgG (Pierce, Rockford) was used as the secondary antibody in a dilution of 1:25000. For CYP3A4, the murine monoclonal antibody WB-MAB-3A1°Ab (Gentest Corp. BD Biosciences, Woburn) was used in a 1:500 dilution. This antibody is aimed at several C-terminal domains in the human CYP3A4. It also reacts with CYP3A5 and CYP3A7, which are not expressed in the human intestine to a noteworthy extent.[19,20] As secondary antibody anti-mouse IgG HRP2°Ab (Gentest Corp. BD Biosciences, Woburn) was used in a 1:20000 dilution. For MRP1 and MRP2 the monoclonal antibodies MRPm6 and M_2III-6 (Alexis Biochemicals, Grünberg, Germany), were used, respectively, in a dilution of 1:100. The secondary antibodies for MRP1 and MRP2 were the same as for Pgp.

RT-PCR. About 0.5 g of frozen tissue was homogenized in 2 mL of ice-cold guandinium thiocyanate solution containing 1% mercaptoethanol. Homogenates were centrifuged at 4 °C and 2200*g* for 10 min. A 1.6 mL volume of the supernatant was put on top of 2.4 mL of cesium chloride solution in precooled vials, and these were centrifuged at 20 °C and 100000*g* for 20 h. The pellet was then washed with 400 μL of ice-cold 70% ethanol and centrifuged at 4 °C and 17500*g* for 5 min. The pellet was resuspended in 10−20 μL of diethylpyrocarbonate−H_2O and kept at −80 °C. The primer sequences used for the reverse transcription polymerase chain reaction are given in Table 1.

Statistics. Statistical calculations were made using the Kruskal−Wallis one way analysis of variance to compare Wilcoxon rank sum variables between the intestinal sections of the jejunum, ileum, and colon. The relationships between pairs of protein and mRNA expression were studied in plots and by correlation analysis. Owing to the number of variables studied (7), the three intestinal sections, and the number of correlations calculated, the significance level used was $P < 0.01$.

Results

Regional Distribution of Pgp, MRP1, MRP2, and CYP3A4 along the Human Intestine. A comparison of the regional distribution of the expression of mRNA and protein of Pgp, MRP1, MRP2, and CYP3A4 was made in tissues from human jejunum, ileum, and colon. The protein and mRNA expression for each individual tissue sample, $a-n$ ($n = 13$ or 14), are displayed in Figure 1. In all groups, except MRP1 mRNA, the jejunum was ranked with the highest expression level of both mRNA and protein (using Wilcoxon rank sums), although the only significant difference ($p < 0.01$) was observed for the MRP2 mRNA. The MRP2 mRNA levels varied in the order jejunum > ileum > colon, with a significant difference ($p < 0.01$) between jejunum and colon (Table 2). The mRNA level of CYP3A4 in the jejunum was higher than in the colon ($p = 0.0245$), and the CYP3A4 protein level in the jejunum was higher than in both ileum and colon ($p = 0.0397$), although the differences were not statistically significant (i.e., $p < 0.01$). Western blot bands of Pgp, MRP2, CYP3A4, and MRP1 are shown in Figure 2.

The Correlation between mRNA and Protein Levels in Human Intestinal Tissues. The correlation between the mRNA and protein levels was analyzed with the intention of examining their usefulness as expression parameters in human intestinal tissue. The linear correlation between protein and mRNA was high and statistically significant for CYP3A4 ($r = 0.779$, $n = 13$, and $p = 0.0017$). All observations except one were arranged along a straight line (Figure 3). When examined on their own the observations from colon patients alone in this plot also showed a significant correlation ($r = 0.943$, $n = 6$, and $p = 0.0046$), as is evident in the plot, whereas observations of the three jejunum patients were clustered in the upper right corner

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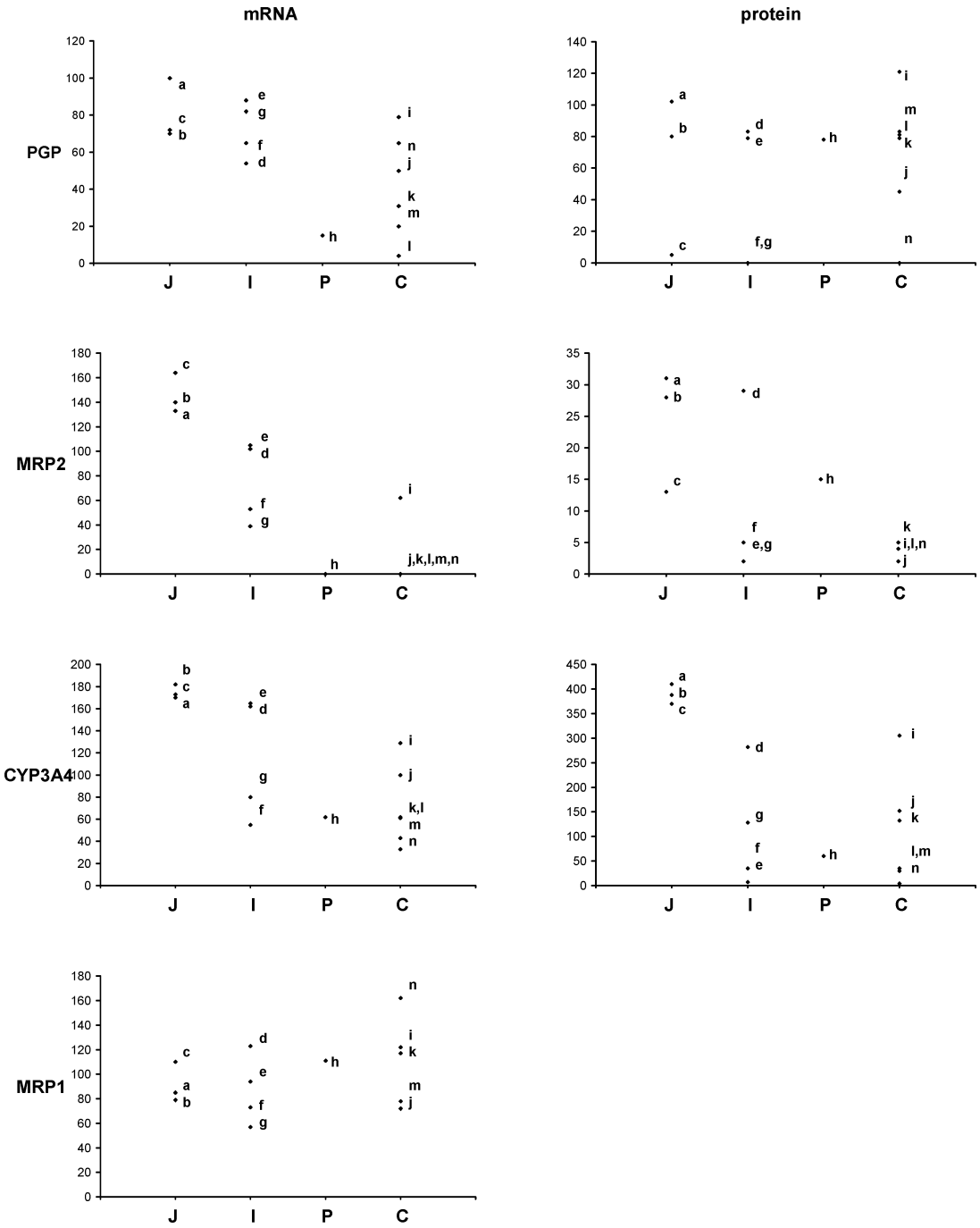

Figure 1. mRNA and protein expression as a percentage of the optical density (OD) of villin in the jejunum (J), ileum (I), proximal colon (P), and distal colon (C) from human intestine. Letters *a*−*n* represent the individual intestinal segments. *d* (ileum) and *h* (proximal colon) are taken from the same patient. *h* is not included in the statistical evaluation.

(Figure 3). For Pgp the correlation between mRNA and protein was not statistically significant ($r = 0.115$, $n = 13$ and $p = 0.708$). For MRP2 there was some correlation for all segments ($r = 0.690$, $n = 12$, $p = 0.0130$), but, when the analysis was conducted for taking the measurements for each region together, the observations from the three different intestinal regions did not show the same common pattern and trend as had the analysis combining all the observations together. The intestinal segment *h* for the proximal colon was not included in the statistical analysis as an ileal tissue sample for this patient was included.

Discussion

Investigations of the regional distribution of cytochrome P450 enzymes and ABC-transporters along the human small and large intestine are crucial if it is to be possible to avoid drug−drug interactions, and also to enable a better understanding to be attained of the extensive interindividual variability in the pharmacokinetics of several drugs after their

Table 2. Wilcoxon Mean Scores for mRNA and Protein for CYP3A4, Pgp, MRP2, and MRP1 in Various Intestinal Regions[a]

	mRNA				protein		
	CYP3A4	Pgp	MRP2	MRP1	CYP3A4	Pgp	MRP2
jejunum	12.00	10.00	12.00	6.33	12.00	8.00	10.33
ileum	7.00	8.63	8.00	5.50	5.38	5.25	5.63
colon	4.50	4.42	3.83	7.40	5.58	7.67	4.90
P-value	0.0245	0.0769	0.0078[b]	0.7314	0.0397	0.5485	0.0925

[a] Average scores were used for ties. *P*-values were calculated using the Kruskal−Wallis test. [b] *P*-value < 0.01.

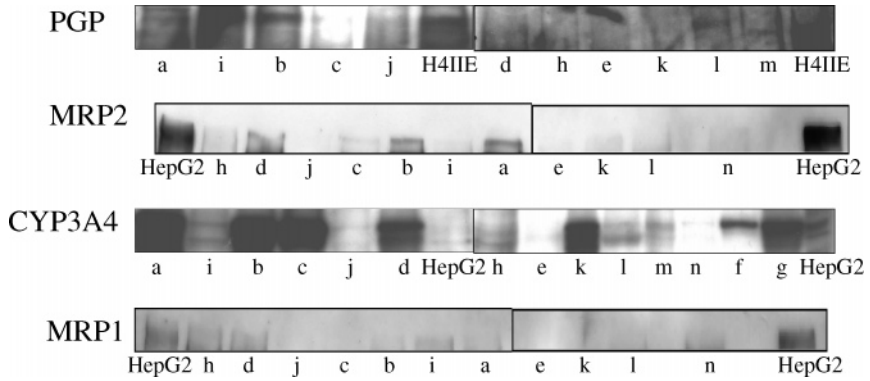

Figure 2. Western blots of Pgp, MRP2, CYP3A4, and MRP1 for jejunum (a, b, c), ileum (d, e, f, g), proximal colon (h), and distal colon (i, j, k, l, m, n). Rat H4IIE cells was used as control for Pgp, and human HepG2 cells were used as controls for CYP3A4, MRP2, and MRP1.

oral administration. In this exploratory study we investigated the regional distribution of CYP3A4, Pgp, MRP2, and MRP1 at the mRNA and protein level in 14 human intestinal samples derived from the jejunum, ileum, and colon using RT-PCR and the Western blot technique. The correlation between the expression of mRNA and the corresponding protein was also examined to evaluate the usefulness of both of these parameters for the analysis of the expression of the genes being investigated and of gene products in human small and large intestinal tissue.

Among the three ABC-transporters analyzed, MRP2 exhibited the most distinct variance in distribution along the human intestine. As mentioned above, the mRNA level varied in the order jejunum > ileum > colon, with a statistically significant difference between the jejunum and colon ($p < 0.01$) (Figure 1). This is in accordance with recent reports comparing MRP2 mRNA in the human duodenum and colon.[12,21] Furthermore, a similar regional distribution has also been found in rat and dog intestine, which suggests that these species are good preclinical models in this respect.[15,16]

The same regional pattern could be seen for the MRP2 protein (Figure 1) as for the mRNA, although no statistically significant differences could be found (Table 2). The mRNA and protein correlation analysis showed some discrepancy when evaluating the intestinal regions individually, but revealed a fairly good correlation when all samples were combined. The mRNA and protein levels of MRP2 exhibited a pattern similar to that of CYP3A4, i.e., they significantly decreased from the jejunum to the colon. This finding is in agreement with a previous report regarding the activity of CYP3A4 in various intestinal regions in humans.[7,22] However, it has been reported that MRP2-related efflux activity is higher in the ileum than the jejunum in rat and human intestine.[23,24]

For Pgp, the mRNA levels appear to be slightly lower toward the colon, but there was a considerable variability between individuals, and no statistically significant difference was observed between any of the intestinal regions. These results were in line with recent reports showing significantly higher Pgp mRNA levels in human duodenum and jejunum compared to the levels in the colon.[12,25] However, the results were in contrast to earlier data suggesting an increase in MDR1 expression from the stomach toward the colon.[2,26−28]

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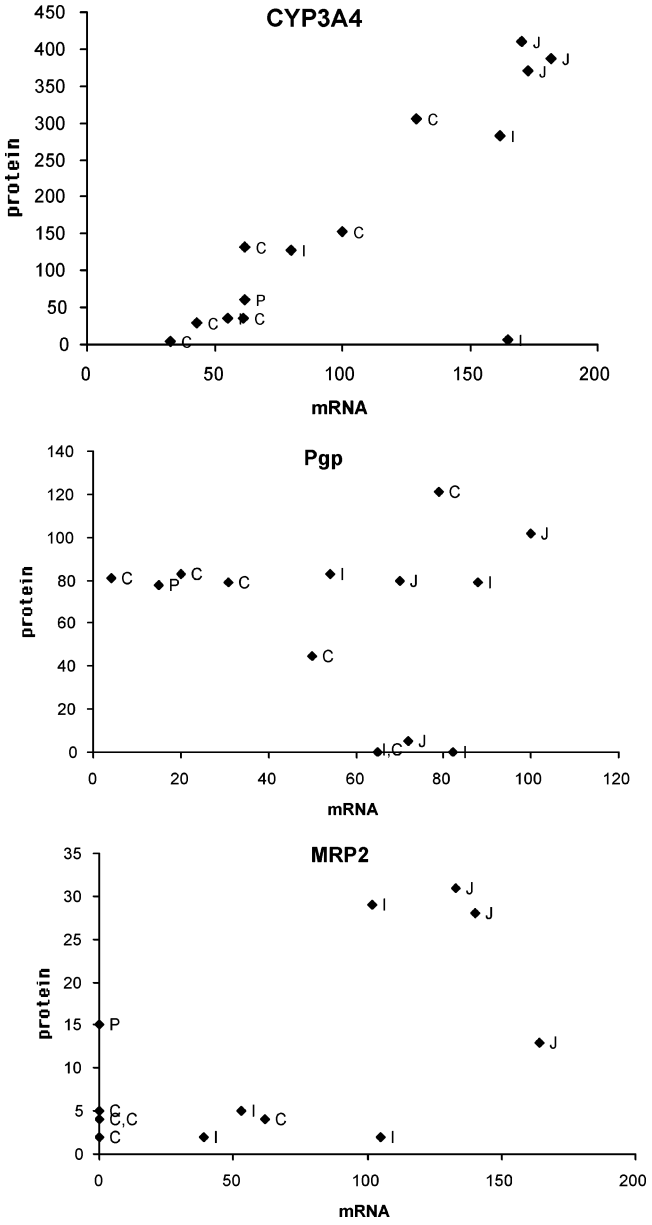

Figure 3. Correlation between mRNA and protein of CYP3A4, Pgp, and MRP2 including jejunum (J), ileum (I), proximal colon (P), and distal colon (C). mRNA levels are given as a percentage of the optical density (OD) of villin and the protein levels are given as relative to a standard sample. P is not included in the statistical evaluation.

The discrepancy between the results in different reports may arise from the study design, the patient population, the sampling techniques, or the analytical methods, or any combination of these. For Pgp protein, the interindividual variability was even more extensive than for mRNA, and, consequently, no region-dependent expression pattern could be established (Figure 1). It was also shown that the mRNA and protein levels were not correlated for Pgp (Figure 3).

The gene expression of MRP1, the only basolaterally located transporter in this study, did not vary regionally along the gastrointestinal tract at the mRNA level. The MRP1 protein level was not determined becaues the expression was too low for Western blot analysis to give a reliable measure. MRP1 is known to be present in most tissues from previous investigations, but the physiological function of this transporter in the intestinal membrane is not fully understood. It has been suggested that the role of MRP1 is to protect the membrane itself rather than the overall organism, as has been suggested to be the case for the other, apically located transporters,[6] and, therefore, a more uniform distribution pattern of MRP1 along the human intestine would be anticipated.

CYP3A4, Pgp, MRP2, and MRP1 exhibited considerable variability between the tissue samples studied. In addition to experimental variability, this could be due to inherent differences in gene and/or protein expression. Individual variation can also be caused by ligands (hormones, other drugs, herbs, and nutrients) to orphan receptors such as the pregnane X receptor (PXR) which is known to regulate the expression of CYP3A4, Pgp, and MRP2.[29,30] The existence of a common regulatory mechanism would explain the similarities in the distribution of the expression of the genes investigated in the intestine. Likewise, the tissue for individual *i* in this study had the highest expression level for CYP3A4, Pgp, and MRP2 (Figure 1). However, no plausible inducing factors such as prior medication or common diseases could be identified.

The mRNA and protein levels were significantly correlated for CYP3A4 and to a lesser extent for MRP2 but not at all for Pgp. The absence of correlations of mRNA, protein, and enzyme activity has previously been described for enzymes in the liver and intestine, and may be related to different pathways of mRNA translation, protein modification, and processing.[31] It is important to be aware of this when extrapolating gene and protein expression to enzymatic activity, and as shown in this study, also for the expression and activity of ABC transporters.

In conclusion, the mRNA and protein levels of MRP2 and Pgp exhibited a pattern similar to that of CYP3A4, i.e., they significantly decreased from the jejunum to the colon. In contrast, MRP1 mRNA expression levels did not exhibit any marked regional differences. The mRNA and protein levels were significantly correlated for CYP3A4 and correlated to a lesser extent for MRP2, but they were not correlated at all for Pgp. This confirms the notion that in many instances mRNA quantification cannot predict protein levels in intestinal tissue samples.

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