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Title Page

Closing the gaps - A full-scan of the intestinal expression of Pgp, Bcrp and Mrp2 in male and female rats

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Running Title Page

Protein expression of Pgp, Bcrp and Mrp2 along the complete rat intestine

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Abbreviations:

ABC, ATP binding cassette;

Bcrp, breast cancer resistance protein;

cMOAT, canalicular multispecific organic anion transporter;

CYP P450, Cytochrome P450;

GI tract, gastrointestinal tract;

Mrp2, multidrug resistance associated protein 2;

Pgp, p-glycoprotein;

Abstract:

Intestinal ABC transporters may affect the bioavailability and effectiveness of orally administered drugs. Available studies on regional expression of intestinal efflux transporters were done with selected intestinal segments only and inconsistent with regard to the variability of transporter expression and the course of expression along the intestine. For an evaluation of the consistency between mRNA and protein expression, relative expression levels of Pgp (ABCB1), Bcrp (ABCG2) and Mrp2 (ABCC2) were determined using qRT-PCR and Western blot in rat intestinal segments from duodenum, jejunum, ileum and colon. In addition, the protein expression of Pgp, Bcrp and Mrp2 from the entire rat intestine was studied by a complete 3 cm segmentation in order to evaluate the predictive power of expression analyses from selected intestinal segments. Pgp showed an increase from proximal to distal regions, and Bcrp an arcuate pattern with highest expression towards the end of small intestine, and Mrp2 decreased along the intestinal axis from proximal to distal parts. No gender specific differences could be observed. Regarding the concordance of mRNA and protein expression Pgp and Bcrp mRNA samples allow good estimations about the corresponding protein expression (for Pgp limited to the mdr1a isoform) but for Mrp2 pronounced deviation could be observed. All transporters showed considerable intra- and interindividual variability, especially at the protein level making it problematic to take transporter expressions of small sections exemplary for general assumptions on intestinal abundances.

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Introduction:

The intestinal tract is not only the main entry for food and liquids to the body but also for orally administered drugs. The absorption across mucosal tissue is an important factor for the effectiveness of drugs. Emerging knowledge of absorption processes has shown that drug transporters may significantly influence the bioavailability of a given drug (Oude Elferink and Waart, 2007). It became evident that a large number of drugs enter or exit a cell via distinct uptake and efflux transporters. Especially ABC efflux transporters are in the focus of interest because they can dramatically limit the permeation of an intrinsically well permeable compound. A variety of these export proteins is located in the apical membrane of enterocytes (Takano et al., 2006), but they are also found in other organ tissues like liver, kidney, and brain.

ABC transporters belong to a large superfamily consisting of functionally highly diverse members. The most important transporters for clinically relevant drugs are P-glycoprotein (Pgp, ABCB1), multidrug resistance associated protein 2 (Mrp2, ABCC2) and breast cancer resistance protein (Bcrp, ABCG2) (Schinkel and Jonker, 2003). They are responsible for the extrusion of a number of structurally diverse drugs, drug conjugates and metabolites out of cells. Pgp is the first discovered and best studied of these three export pumps. It recognizes a wide range of structurally and pharmacologically unrelated neutral and positively charged hydrophobic compounds (Takano et al., 2006). Mrp2, also named cMOAT (canalicular multispecific organic anion transporter) due to its action in hepatocytes, has a relatively hydrophilic substrate spectrum, including glucuronide, glutathione, and sulfate conjugates of endogenous and exogenous compounds (Suzuki and Sugiyama, 2002; Chan et al., 2004). Bcrp is the most recently discovered of these efflux transporters and recognizes mainly hydrophilic anticancer agents (Doyle and Ross, 2003). All three transport proteins exhibit a considerable overlap in their substrate recognition.

For a successful development of orally administered drugs it is essential to gain information about the expression pattern of these transporters in intestinal tissue with respect to adjusted dosage or controlled compound release (Stephens et al., 2002). The rat has been suggested as a model to predict human oral drug absorption since rat and human appear to show similar drug absorption and transporter expression profiles in the small intestine (Cao et al., 2006). Recently, first data on regional expression - most notably of Pgp and Mrp2 and few of Bcrp - in humans and rodents have been published (Mottino et al., 2000; Englund et al., 2006; Berggren et al., 2007). However, these data are inconsistent and give only information about small and selected parts of the intestine. In addition, most of these expression studies have been made on mRNA level only. But it becomes not fully clear whether these results resemble the actual protein expression of the intestinal ABC transporters as it is known that many of them exhibit posttranslational modifications.

Therefore, to evaluate the concordance of transporter mRNA and protein expression with regard to mRNA expression studies as a widely used tool to predict protein expression levels of transporters, we have quantified Pgp, Mrp2 and Bcrp mRNA expression via quantitative real-time PCR and protein expression by means of Western blot analysis along the entire GI tract of rats. In addition, little is known about the amount of intraindividual variation of intestinal transporter expression, because up to now there are no literature data available providing detailed and gapless gender-specific information on Pgp, Mrp2 and Bcrp protein expression along the complete intestine of rats. Our data as well as published in vivo studies in humans reveal high interindividual variation of intestinal ABC transporter protein expression (e.g. Berggren et al., 2007). Whether transporter protein expressions of selected intestinal regions can therefore be used for a reliable assumption of drug absorption needs further validation of the expression pattern. Thus, this analysis was done by complete segmentation of the entire length of the intestine in both genders to get a complete protein expression profile and to evaluate the amount of both inter- and intraindividual variation of ABC transporters.

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Methods:

Material

Unless otherwise noted all chemicals were purchased from Sigma-Aldrich, Germany.

Animals

Male and female Han-Wistar rats (200–340 g for Western blot analysis; 265–310 g for PCR analysis) were purchased from Charles River, Sulzfeld, Germany. Animals were maintained under standard diurnal conditions and were allowed access to food (V1534-000 R/M-H, Fa ssniff, Soest, Germany) and water ad libitum in accordance with animal protection standards. All surgical and experimental procedures were reviewed and approved by legal authorities and were performed around 9 a.m.

RNA isolation

The intestines of male rats were removed and rinsed with physiological saline. Three cm pieces of duodenum, jejunum, ileum and colon were cut, the mucosa was squeezed out and immediately transferred to RNAlater (Sigma, Germany) and stored at -80 °C until further use. Duodenum was defined as the first 3 cm post stomach, jejunum as the middle 3 cm of small intestine, ileum as the last 3 cm before caecum and colon as the first 3 cm of large intestine. Total RNA of each piece was isolated using the RNeasy mini kit (Qiagen, Germany) according to the manufacturers' instructions, eluted with water containing RNasin (Promega, USA) and loaded again on RNeasy mini columns following the "cleanup protocol" (Qiagen, Germany) to get rid of remaining contaminations. Final elution was performed with water containing RNAsin. RNA concentration was spectrophotometrically quantified with a Nanodrop1000 device (Peqlab, Erlangen, Germany). The integrity of RNA samples was checked electrophoretically on agarose gels stained with ethidium bromide.

Preparation of cDNA and real-time RT-PCR

1 µg total RNA of each intestinal region (duodenum, jejunum, ileum and colon) of 4-5 male rats was reverse transcribed using the iScript cDNA Synthesis kit (BioRad, Germany).

To quantify the amount of mRNA of mdr1a, mdr1b, mrp2 and bcrp, real-time PCR was performed on a LightCycler 1.5 instrument (Roche, Mannheim, Germany) using the FastStart DNA Master plus SYBR Green I kit (Roche). Villin was used for normalization purposes. Each 10 µl PCR reaction contained 2 µl of SYBR Green Master Mix, each 0.5 µM forward and reverse primer and 25 ng cDNA (for mdr1a and bcrp) or 100 ng cDNA (for mdr1b and mrp2). Villin was amplified of both 25 and 100 ng cDNA respectively. The sequences of the primers used are given in Tab. 1.

Real-time PCR was carried out in 20 µl glass capillaries (Roche). The amplification program for all genes consisted of 1 preincubation cycle at 95 ºC with a 10 min hold, followed by 45 amplification cycles with denaturation at 95 ºC with a 10 s hold, an annealing temperature of 50 °C with a 10 s hold and a slope of 5 °C/s, and an extension at 72 ºC with a 10 s hold. Amplification was followed by a melting curve analysis, which ran for 1 cycle with denaturation at 95 ºC with a 0 s hold, annealing at 65 ºC with a 15 s hold, and melting at 95 ºC with a 0 s hold. Water was included as a negative control in each run to access specificity of primers and possible contaminations. PCR products were checked on agarose gels and gave single bands of appropriate size (data not shown).

Standard curves for all genes were generated to calculate PCR reaction efficiencies using diluted pooled cDNA of the investigated animals.

Relative quantification of ABC transporter mRNA

Relative expression of mdr1a, mdr1b, mrp2 and bcrp mRNA in different intestinal regions was calculated using REST© (Relative Expression Software Tool; Pfaffl et al., 2002). ABC transporter expression was normalized to villin and for each transporter the normalized

expression in jejunum, ileum and colon respectively was set relative to the expression in duodenum. Differences of transporter expression between duodenum and each of the other three intestinal regions were tested for significance by a Pair Wise Fixed Reallocation Randomisation Test© (Pfaffl et al., 2002).

Preparation of mucosa homogenates for the study of transporter protein expression in selected intestinal regions

To get a first macropicture of the intestinal protein expression of ABC transporters and for the comparison of protein and mRNA expression, crude mucosal homogenates of corresponding intestinal regions as used for mRNA quantification were prepared and the protein expression of Pgp, Bcrp and Mrp2 was quantified.

The whole intestines were dissected from pylorus up to colon without caecum and intestinal homogenates were prepared by a method described by Mohri and Uesawa (2001) with some modifications. Briefly, the intestines were rinsed with ice cold "rinsing buffer" (1.5 mM KCl, 96 mM NaCl, 27 mM sodium citrate, 8 mM KH_2PO_4, 5.6 mM Na_2HPO_4, pH 7.3 and protease inhibitor cocktail). Three cm pieces of each duodenum, jejunum, ileum and colon (for segment definitions refer to RNA isolation) were cut and rinsed with "separation buffer" (137 mM NaCl, 2.7 mM KCl, 1.5 mM KH_2PO_4, 8 mM Na_2HPO_4, 1.5 mM EDTA, 0.5 mM dithiothreitol, pH 7.4 and protease inhibitor cocktail). To get the mucosal tissue, pieces were placed on an ice cold glass plate and the mucosa gently squeezed out. The separation procedure was repeated three times. The separated cells clumps were washed with "homogenizing buffer" (5 mM histidine, 0.25 M sucrose, 0.5 mM EDTA, pH 7 and protease inhibitor cocktail) and centrifuged at 800 g for 10 min at 4 °C. The cells were homogenized in a small Elvehjem potter at 500 rpm in 3 ml "homogenizing buffer" and centrifuged at 850 g for 5 min at 4 °C. The resulting supernatants were carefully removed and stored in aliquots at -80 °C until use.

Protein concentration of each homogenate was determined with the BCA Assay Protein kit (Pierce, Rockford, USA) according to the manufacturers' instructions.

Preparation of mucosal homogenates for the detailed study of transporter protein expression along the complete intestine

To enravel the gapless spatial resolution of transporter protein expression along the intestinal tract Pgp, Mrp2 and Bcrp were quantified in 3 cm successive spacing along the complete small intestine and colon.

Therefore, the complete intestines of male and female rats from pylorus up to the colon except caecum were cut into 3 cm successive pieces and homogenates were prepared and processed from each intestinal piece as described above.

Western blot analysis

For gel electrophoresis, 100 µg of total protein of each sample were precipitated with ice-cold acetone, resuspended in sample loading buffer (equal mixture of 8 M urea and LDS sample buffer containing DTT (Invitrogen, Karlsruhe, Germany)) and denatured for 30 min at 56 °C. As a positive control 100 µg of Caco-2 lysate were loaded on each gel.

Samples were separated by electrophoresis in a NuPAGE® Novex® 7-12 % Bis-Tris gel (Invitrogen, Karlsruhe, Germany) and transferred to a nitrocellulose membrane according to the manufacturers' instructions. Membranes were blocked with 5 % non-fat milk in TBS-T. For detection of ABC transporter proteins, blots were incubated with the respective primary antibodies diluted in 1 % BSA in TBS-T: mouse monoclonal anti-Pgp (C-219 1:100, Alexis Biochemicals, Germany), mouse monoclonal anti-Mrp2 (M_2 III-6 1:100, Alexis Biochemicals, Grünberg, Germany) and rat monoclonal anti-Bcrp (BXP-53 1:50, Alexis Biochemicals). Villin was used as a reference protein (rabbit monoclonal H-60 1:2000, Santa Cruz Biotechnology Inc., USA). Detection of bound antibodies was done with affinity-purified rabbit anti-mouse

IgG (for transporter antibodies) and goat anti-rabbit IgG (for villin antibody) coupled to peroxidase (secondary antibody, Sigma, München, Germany) diluted 1:20000 in 1 % BSA in TBS-T.

Protein bands were visualized by enhanced chemiluminescence (Western lightning plus, Perkin-Elmer, Überlingen, Germany) and blots were photographed with a ChemiDoc XRS camera (Bio-Rad, München, Germany). Optical densities for bands of interest were determined using the Quantity One software (Bio-Rad) and normalized to villin. For a relative expression profile of ABC transporters in selected intestinal regions the villin normalized intensity of duodenum was individually set to 1 and the intensities of jejunum, ileum and colon were set relative to it.

For a calculation of the relative transporter expression along the complete intestine the villin normalized intensity of the piece showing the weakest signal intensity was set to 1 and the intensities of the other segments were set relative to it.

Results:

ABC transporter profile in selected intestinal regions – comparison of mRNA and protein expression

To get a general picture of the expression profile of ABC transporters in the intestinal tract, transporter protein and mRNA expression were quantified in duodenum, jejunum, ileum and colon of male rats.

As is becomes apparent from Figure 1, there is a constant increase of about 5 fold for Pgp protein and the corresponding mdr1a mRNA expression along the intestine from proximal to distal parts. Mdr1b mRNA, however, was relatively evenly expressed in different intestinal parts and at a lower level as compared to the mdr1a isoform. This is consistent with previously published data on intestinal Pgp expression (Fricker et al., 1996; Li et al., 1999; Brady et al., 2002; Mouly and Paine, 2003).

In contrast to the distally oriented expression gradient of Pgp, Bcrp was arcuately expressed along the rat intestine both on the mRNA and protein level, i.e. increasing within the small intestine from proximal to distal and decreasing thereafter in the colon (Fig. 1) resembling the same expression pattern as shown for mice (Enokizono et al., 2007). The maximum increase of Bcrp expression was similar for mRNA and protein (~ 2.5 fold) but weaker than that of Pgp. Expression of Mrp2 - as opposed to the other two export pumps - decreased continuously along the intestine (Fig. 1) as was also shown previously by Mottino et al., 2000. This decrease however, was much stronger on the mRNA than on the protein level with no detectable amounts of mRNA in colonal samples in contrast to 60 % remaining protein in the colon.

For all three transporters a considerable amount of interindividual variation could be observed concerning both mRNA and protein expression all over the intestine. For Pgp, the variability appeared to be higher in distal parts, whereas for Bcrp and Mrp2, no similar trend for protein and mRNA expression could be observed. Bcrp showed a higher variability of mRNA expression in distal parts of the intestine but a lower variability of protein expression. For Mrp2 a converse trend could be shown.

Micropicture of ABC transporter protein expression along the whole length of the rat intestine

Due to the pronounced variability of transporter expression in distinct parts of the intestine it seemed advisable to verify the patterns of protein expression of Pgp, Mrp2 and Bcrp in a gapless full-scan of the intestinal tract in order to 1) investigate the extent and course of expression within the missing, not yet studied parts of the GI tract, 2) to estimate the amount of intraindividual variation and 3) to explore gender-specific differences of transporter expression. Therefore, the protein expression profile and variation of Pgp, Bcrp and Mrp2 along the whole length of the intestinal tract within successive 3 cm segments of male and female rats were plotted individually (Fig. 2).

Fig. 3 shows the result of a typical full-scan Western blot of intestinal ABC transporters obtained with antibodies against Pgp, Mrp2, Bcrp and villin. Pgp was located at an apparent molecular mass of approx. 140 kDa, Bcrp was detected in its monomeric form with an apparent molecular mass of 72 kDa, and Mrp2 as a band at approx. 190 kDa. The reference protein villin could be visualized in a molecular mass range of about 95 kDa.

The general trend of transporter expression with single samples from distinct locations of the GI tract (Fig. 1) could be roughly confirmed, however, this finding was somehow covered by a very high inter- and also intraindividual variability in the expression of all three transporters along the intestine. The interindividual variability was neither equal within one tranport protein analyzed along the intestine nor between males and females concerning one transporter nor equal for different transporters in the same sex. The intraindividual variation was also intense for all transporters but not equally pronounced between all individuals. Some animals displayed a quite regularly pulsative pattern with distinct spikes and troughs. Single individuals showed extremely high amplitudes of expression for one transporter, but surprisingly constant expression levels for the other transporters within adjacent 3-cm segments (Fig. 2). The extent of intraindividual variation was partly of the same scale than that of the intraindividual variation (e.g. male Pgp expression).

For Pgp an increase of expression from proximal to distal intestinal parts could be observed in males, joined with a corresponding increase of variability along the intestine. In females the expression was more variable without a clear trend and of a minor relative increase than in male animals (Fig. 2 and 3).

Bcrp showed also a more uniform pattern of expression in males than in females. For both sexes an increase of protein expression along the small intestine could be found followed by a further descent starting around the ileum, resembling the arcuate pattern found already in selected intestinal segments. The heterogeneity of Bcrp expression in females is also mirrored

by the high distal interindividual variability. In contrast to Pgp the maximal increase was higher in females than in males (Fig. 2 and 3).

The decrease of Mrp2 expression along the intestine found in selected segments could be confirmed for both sexes by the full-scan survey. This decrease was more pronounced in females than in males, resembling a much steeper course, although females showed very strong variability at the beginning of the intestine. Some individuals also showed a peak in the upper/middle jejunum (Fig. 2 and 3).

Discussion:

Several investigations on the local distribution of ABC transporters in the human and rodent intestinal tract have been made (for example human: Berggren et al., 2007; Englund et al., 2006; Gutmann et al., 2005; Maliepaard, 2001; Mouly and Paine, 2003; Prime-Chapman, 2004; Taipalensuu, 2001; Zimmermann et al., 2005; rodent: Brady et al., 2002; Cherrington, 2002; Dietrich et al., 2003; Fricker et al., 1996; Gotoh, 2000, Johnson et al., 2006; Mottino et al., 2000; Rost et al., 2002; Tamura et al., 2003; Trezise, 1992; Valenzuela et al., 2004). However, due to the pronounced heterogeneity of already existing studies it is difficult to get a reliable picture of intestinal ABC transporter expression. Altogether, the results of these studies are somewhat contradictory and of limited informative value due to several reasons. Data both in humans and rats were obtained at different levels, i.e. on mRNA and on protein level or in functional assays. But it is known that many proteins, including ABC transporters like Mrp2 and Bcrp, exhibit posttranslational modifications. As is seen in Fig. 1, the relative change of expression did not match well between mRNA and protein level for all transporters being investigated. Regarding Pgp and Bcrp, relative expression data obtained from mRNA samples allow good estimation about the corresponding protein expression (for Pgp this is however limited to the mdr1a isoform). For Mrp2, deducing the relative protein expression from mRNA expression data clearly underestimates the actual relative amount of transporter protein. In

addition, existing data on the relationship between transporter mRNA and protein levels or transporter activity give a rather contradictory picture, as was mentioned for Pgp (Albertioni et al., 1995; Fricker et al., 1996; Greiner et al., 1999). Taipalensuu et al. (2004) have pointed out that the predictive power of transcript analysis has to be investigated on a gene-by-gene, or even case-by-case basis. Therefore all in all, data obtained on the mRNA level will only give hints on a general pattern of expression and no information on the actual level of protein abundance or the activity of ABC transporters.

Another difficulty in studying intestinal transporter expression is that up to date, almost all investigations on regional differences of intestinal ABC transporter abundance were conducted with selected intestinal segments lacking any information about intersubject variability or differences of expression in adjacent segment of the GI tract. However, this approach is only valid under the assumption that transporter abundances in adjacent segments are very similar. In fact, it is unknown if this is true. In addition, the size of the intestinal segments under investigation varied from study to study. Sometimes no precise information about segment size was provided at all. The situation becomes even more complicated by the fact that in the available studies data on transporter abundance both in human and rodent are sometimes normalized to housekeeping genes/proteins and sometimes not, which provides fundamentally different information. In our study optical densities of transporters were normalized to villin, an actin cross-linking structural protein found exclusively in epithelial microvilli. Therefore, villin-normalized data resemble the actual enterocytal transporter abundance of mucosal preparations devoid of contaminations by other cell types and relate the amount of transporter to the number of enterocytes. This is relevant, because it is known, that the number of enterocytes decreases along the intestinal tract. A non-normalization of transporter expression to the number of enterocytes will therefore result in the measurement of higher amounts of transporters in distal parts of the intestine.

In order to evalutate the extent of inter- and intraindividual variation of intestinal transporter expression and to evalutate the reliability of established “pick-and-choose” expression studies we compared the protein expression of Pgp, Bcrp and Mrp2 in selected intestinal segments of males and females to a gapless piece-by-piece survey along the intestine.

Taken data from selected intestinal regions (Fig. 1), Pgp abundance showed an increase from proximal to distal regions of the intestine with no gender-specific differences being observed. This finding is in accordance with previous investigations in the rat, which also found an increase in Pgp protein expression from upper to lower small intestine but a decrease in large intestine (Trezise et al., 1992; Valenzuela et al., 2004; Johnson et al., 2006). This pattern of expression appears to be concordant with observations of Pgp expression in humans, where an increase of Pgp abundance from upper to lower intestinal parts has been observed (Mouly and Paine, 2003; Englund, 2006; Zimmermann et al., 2005).

Bcrp displayed an arcuate course of expression in both sexes with highest abundance in ileal samples (Fig. 1 and 2). To our knowledge there is only one other study of rat intestinal expression of Bcrp available (Tanaka et al., 2005), which showed a similar pattern of abundance both in males and females on the mRNA level. Han and Sugiyama (2006) also found an increase of Bcrp mRNA expression within the small intestine of mice. In humans Englund et al. (2006) also confirmed an arcuate course of mRNA expression, whereas Maliepaard et al. (2001) showed highest mRNA expression in the colon.

Mrp2 protein expression showed a pronounced decrease along the intestinal axis from proximal to distal parts both in males and females (Fig. 1 and 2). This finding is parallel to recent studies (Johnson et al., 2006; Mottino et al., 2000; Rost et al., 2002). However, the latter two found highest protein expression in the jejunum, which could be confirmed in some (mostly female) individuals of the present study (Fig. 2). Human studies on intestinal MRP2 expression were mainly conducted on the mRNA level but confirmed a decrease of expression along the

intestine (Prime-Chapman, 2004; Berggren et al., 2007; Englund et al., 2006; Zimmermann et al., 2005).

Considering the abundance of Pgp, Bcrp and Mrp2 along the entire intestine piece-by-piece, it appears to be obvious that the expression of these proteins shows a large intra- and interindividual divergency (Fig. 2). This is surprising, due to the use of a genetically homogenous pool of investigated individuals as well as controlled housing conditions for the animals and a timed sample preparation one could have expected a lower variability.

Although following a general trend of in- or decrease, the course within a single individual often seemed to be zigzagged including dramatic up- and downward deflections. It needs further investigation if this is somehow related to the peristaltic movement of the intestinal muscle layer and the resulting trafficking of the food and an altering of the efficacy of substrate/transporter contact, hence not all villi may have the same exposure to food containing transporter substrates. In this context it is interesting to know, whether these oscillations are somewhat related to patterns of intestinal CYP P450 expression. Several CYP P450s are expressed in the rat intestine. Although general patterns of expression of CYP P450s along the intestinal tract have been recognized in the rat, the intra- and interindividual variation of CYP P450 protein expression is also remarkable (Lindell et al., 2003; Mitschke et al. in press). A recent study in brain capillaries (Bauer et al., 2004), showed a very rapid upregulation of Pgp upon exposure to ligands of the orphan receptor PXR (pregnane X receptor), which initiates a signaling cascade of Pgp expression.This receptor could be involved in the variability of export pump expression in closely located intestinal segments as it is also present in the GI tract (Kullak-Ublik et al., 2004).

All in all, it appears to be problematic to take transporter expressions of small sections exemplary for general assumptions on intestinal abundances, especially if levels of mRNA expression are used for reliable assumptions on the respective protein expression. This limitation is supported by the fact that the scale of in- or decrease of a specific transporter was

highly enlarged in some individuals, which makes it difficult to estimate mean regional differences of transporter abundance. Taking these inponderabilites together the present study procures the most detailed data on gender-specific regional intestinal transporter differences available.

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Legends for Figures:

FIG. 1: Comparison of intestinal mRNA and protein expression in selected intestinal regions. Bars represent mean ± SD of 4-6 male rats. For sample preparation and region definition see Methods. The levels of mRNA and protein were normalized to villin. Expression intensities of duodenum were individually set to 1 and the intensities of jejunum, ileum and colon relative to it.

FIG. 2: Densitometrical analysis of Western blots of Pgp, Bcrp and Mrp2 along the entire intestinal tract of male and female rats. Samples were prepared as described in Methods. The first three samples after the stomach were defined as duodenum, the last sample before the caecum as ileum and all samples inbetween as jejunum (Sharp and LaRegina, 1998). Transporter intensities were normalized to villin. For each transporter the course of expression from 3 – 5 animals is shown in parallel.

FIG. 3: Representative Western blots of mucosal samples of rat intestinal pieces of Pgp, Bcrp, Mrp2 and villin. The figure resembles samples of successive pieces from a complete intestine from pyloric valve up to colon without caecum. Blots were prepared as described in Material and Methods and lanes were loaded with 100 µg of total protein.

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Tables:

Table 1. Primers used for the analysis of ABC transporter gene expression by qRT-PCR

Primers	Sense and Antisense	PCR Product (bp)	Reference
mdr1a	5´-AGCGGTCAGTGTGCTCACA-3´	202	this paper
	5´- CTTGGCATATATGTCTGTAGCA-3´		
mdr1b	5´-GAAATAATGCTTATGAATCCCAAAG-3´	325	Zhang et al. (1996)
	5´-GGTTTCATGGTCGTCGTCTCTTGA-3´		
bcrp	5´-TCCAAGGTTGGAACTCAGTTTA-3´	224	this paper
	5´-AAGATGGAATATCGAGGCTG-3´		
mrp2	5´-GAAGGCATTGACCCTATCT-3´	318	this paper
	5´-CCACTGAGAATCTCATTCATG-3´		
villin	5´-AGAGATCCGAGACCAGGA-3´	201	this paper
	5´-TCGGAGTCAGACACATGG-3´		

Fig. 1

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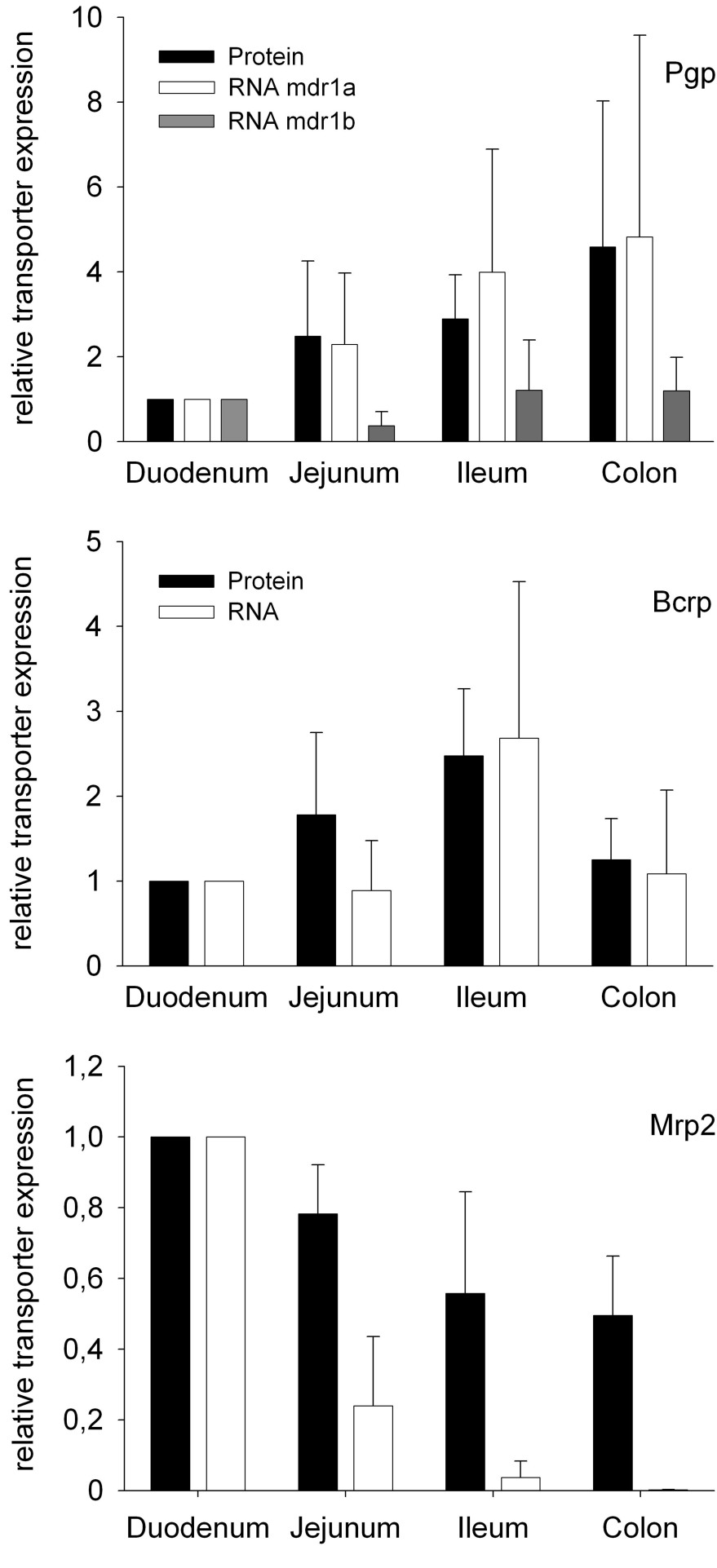

Fig. 2

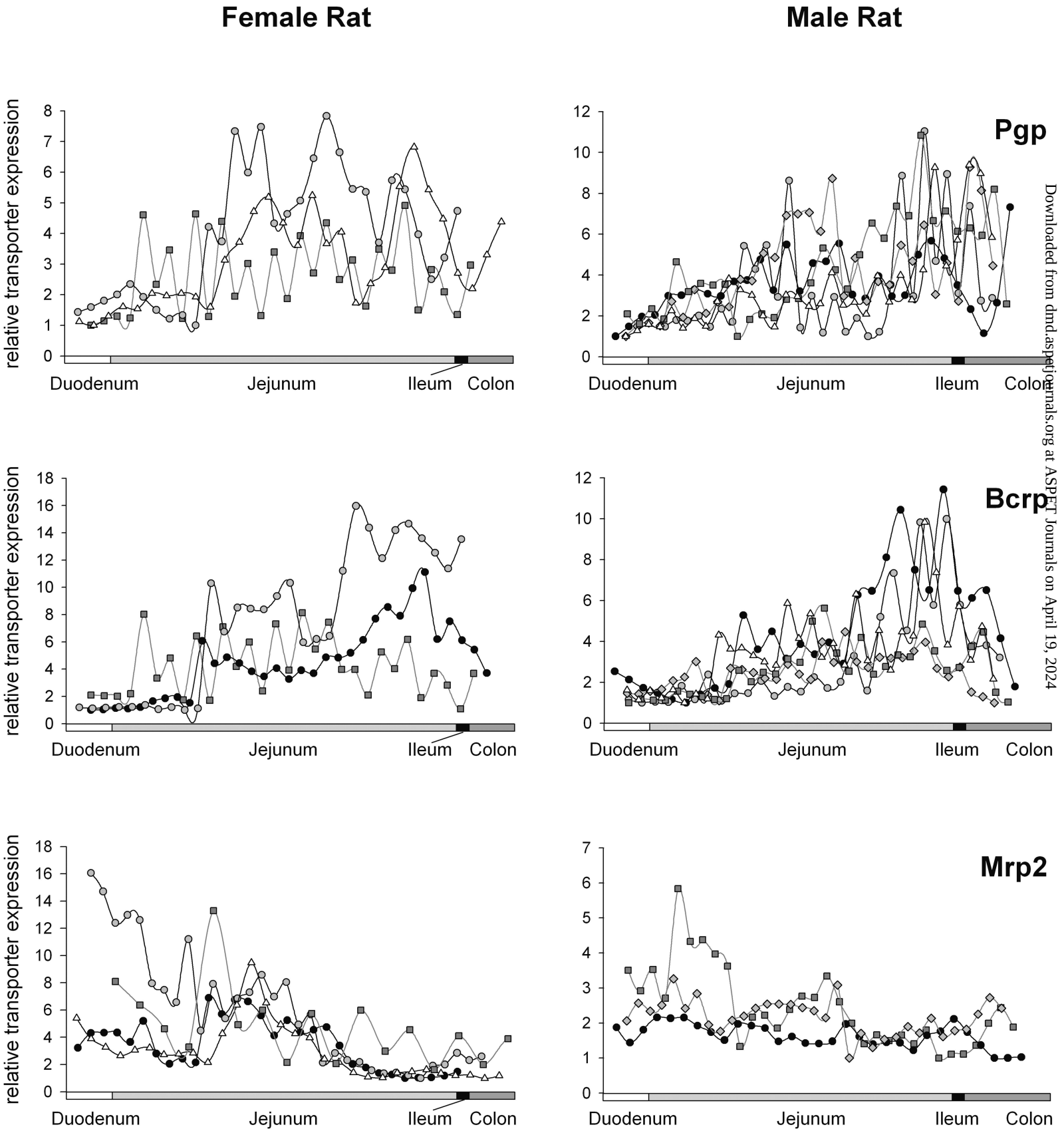

Fig. 3