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Cytochromes P450 and *MDR1* mRNA expression along the human gastrointestinal tract

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Aim
The aim of this study was to quantify the mRNA expression of three cytochromes P450 (CYP) and P-glycoprotein (P-gp) in the human gastrointestinal (GI) tract.

Method
Biopsies were obtained from gastric, duodenal, colonic and rectal mucosa during routine gastro-colonoscopy in 27 patients. The biopsies were snap-frozen in liquid nitrogen. Real-time reverse transcriptase-polymerase chain reaction (RT-PCR) was used for the quantitative analyses of mRNA expressed by the *CYP2E1, CYP3A4* and *CYP3A5* genes, and the *MDR1* gene coding for P-gp protein. The mRNA expression of b-actin was used as an internal standard for comparisons between samples.

Results
All CYP genes were expressed at all locations throughout the GI tract, although all showed substantial interindividual variation. *CYP2E1* had the highest expression at all locations ($P < 0.05$ to $P < 0.0001$), except in the right colon. *CYP3A4* and *CYP3A5* had their highest mRNA expression in the duodenum ($P < 0.001$ and $P < 0.000\,001$, respectively) and *CYP2E1* in the stomach ($P < 0.01$). *MDR1* mRNA concentrations increased along the GI tract with the highest expression being in the left colon ($P < 0.000001$).

Conclusion
Multiple sampling within the same individual enabled us to study the intraindividual variation in expression of CYP and *MDR1* genes along the GI tract. We find that *CYP2E1* mRNA expression is higher than that of the other *CYPs*. *CYP3A* expression is highest in the duodenum and that of *MDR1* increases from stomach and duodenum to colon.

Introduction

Over the past decade the important role of the gastrointestinal (GI) tract and especially the small intestine in the presystemic metabolism of orally administered drugs has become apparent [1–3]. It has been shown that the metabolism of some orally administered drugs is unaffected in patients with impaired liver function [4]. Kolars *et al.* [5] showed that metabolites of cyclosporine were detected in portal venous blood during the anhepatic phase of liver transplantation when the drug was administered directly to the small bowel. Furthermore, Paine *et al.* [6] have shown that midazolam is metabolized in the gut wall.

The cytochromes P450 (CYP) enzymes belong to a superfamily of membrane-bound haeme-containing proteins responsible for the metabolism of several endoge-

nous compounds such as steroids and fatty acids, as well as drugs and other xenobiotics [7, 8]. Three CYP gene families are predominantly involved in drug metabolism, namely CYP1, CYP2 and CYP3, many members of which are induced or inhibited by a range of compounds [9].

Another determinant of the oral bioavailability of drugs is P-glycoprotein (P-gp) which is encoded for by the multidrug resistance gene *(MDR1).* P-gp is a membrane-bound transporter protein, which was first identified as being responsible for the development of multidrug resistance [10]. P-gp acts as a cellular efflux pump in an ATP-dependent process [11]. P-gp is present in mucosal cells of the GI tract, in the biliary canaliculi of the liver, and in the brush border of the proximal tubules of the kidney, where it contributes to the excretion of substances into the gut, bile and urine, respectively [12]. Schwab *et al.* [13] suggested that one of the polymorphisms of the *MDR1* gene, C3435T, which leads to decreased P-gp expression, is associated with increased susceptibility to ulcerative colitis. Furthermore, it has been shown that *mdr1a* knockout mice develop a form of this disease, which can be prevented by antibiotics [14]. These findings suggest that P-gp plays a protective role against intestinal bacteria and toxins.

The use of molecular techniques and especially the real-time reverse transcription-polymerase chain reaction (RT-PCR) makes it possible to detect and quantify RNA in very small samples [15].

The aim of this study was to study the expression of *CYP2E1*, *CYP3A4*, *CYP3A5* and *MDR1* mRNA along the GI tract within the same individual.

Patients and methods

Patients

Patients referred to the endoscopy unit, University Hospital of Uppsala, were asked to participate in the study. Demographic and clinical features of the patients are shown in Table 1. The ethics committee of the University of Uppsala approved the project. After informed consent, biopsies from five different locations in the GI tract (stomach, duodenum, right colon, left colon and rectum) were collected in 27 patients together with biopsies for routine clinical investigation. Samples were obtained from macroscopically normal mucosa where possible, and snap-frozen in liquid nitrogen and stored at −70°C until preparation of mRNA.

Purification of mRNA

mRNA was obtained from the samples (wet weight about 5 mg) using the Quickprep micro mRNA purification kit according to the instructions of the manufacturer (Amersham Biosciences, SE 75184, Uppsala, Sweden).

Subsequentely, mRNA was precipitated in 95% ethanol at −20°C for 1 h. The pellet obtained was then resuspended in 50 µl Rnase-free water. The amount of mRNA was determined by UV spectroscopy at 260 nm, with correction for background at 320 nm.

CDNA synthesis

Purified mRNA (50 ng) was used for cDNA synthesis using a First strand cDNA synthesis kit (Amersham Biosciences). Less than 50 ng mRNA was obtained from 15 out of the 135 samples, which was corrected for in the analysis of the data.

Real-time PCR

The cDNA was diluted 1 : 10 with Millipore filtered water before real-time RT-PCR. Primer pairs and fluorescent Taqman™ probes specific for *CYP2E1, CYP3A4, CYP3A5* and *MDR1* were used, the design of which was described earlier [16, 17]. The house-keeping b-actin gene was used as an internal control to enable comparison between samples. The b-actin probe was labelled with FAM (6-carboxyfluorescein) and purchased from Applied Biosystems.

Ten microlitres of the diluted cDNA mix, containing 3.3 ng mRNA, 300 nM each of the forward and reverse primers, 50 nM of the probe and a ready-made mastermix with heat-activated Taq polymerase, uracil-N-glycosylase (UNG) and nucleotides (Applied Biosystems) were used in the PCR reaction.

Real-time PCR was performed using an ABI PRISM™ SDS 7700 instrument (Perkin-Elmer 549 Albany Street, Boston, MA, USA). UNG inactivation for 2 min at 50°C and a Taq polymerase activation step at 94°C for 10 min were followed by 50 cycles (each at 95°C 15 s; 60°C 30 s). All samples were run in duplicates. At least two nontemplate controls were included in all PCR runs.

The cycles to threshold (Ct) value was determined during the period when the different PCR reactions were in the early logarithmic phase. The limit of detection of mRNA was defined in an earlier study [16] at a Ct value of 38.

The variation in Ct values for duplicates of all samples (n = 135) varied between 0 and 47% with a median of 1.7% and a mean of 3.6%. The mean coefficient of variation (CV) for one sample run on four occasions varied between 0.1 and 4.6% (mean = 2.8%) in five samples from one patient.

The standard curves for *CYP2E1, CYP3A4, CYP3A5*,

Table 1
Clinical features of the patients

Patient	Age (years)	Sex	Smoking, cigarettes/day	Alcohol last week	Medication (most important)	Clinical diagnoses
1	74	M	No	32 g	None	HP[1]-gastritis, duodenitis
2	69	M	No	110 g	None	Anaemia HP-gastritis
3	46	F	No	No	Steroids, metronidazole	Gastric erosions, proctitis
4	34	M	No	58 g	None	Anaemia—normal PAD[2]
5	36	F	No	No	Steroids, mesalazine	IBD[3]—normal PAD
6	30	F	No	82 g	None	Microscopic colitis
7	37	F	20	45 g	Paracetamol, salicylic acid	Focal intestinal gastric metaplasia
8	52	M	20-30	10 g	Omeprazole	Erosive gastritis, duodenitis
9	24	F	No	No	Sulphasalazine, citalopram cetirizin	Inactive proctitis
10	58	F	No	No	None	IBS[4]
11	82	M	Pipe	No	None	Anaemia, gastric ulcer, HP-neg.
12	59	F	1-2	20 g	Paracetamol, calcium	IBS
13	36	F	5-10	148 g	None	IBS
14	31	F	No	6 g	Oral contraceptives	Caeliac disease—villous atrophy
15	41	M	10-20	40 g	Paracetamol, fluconazole, theophylline	Chronic gastritis, lymphocytic colitis
16	47	F	No	No	Metoprolol	Atrophic gastritis
17	43	F	No	50 g	Promethazine	HP-gastritis, IBS
18	25	F	No	No	Salicylic acid, NSAID oral contraceptives	IBS
19	79	F	No	No	Digoxin, furosemide, clopidogrel, lanzoprazole	Collagenous colitis
20	60	M	No	35 g	Metoprolol, felodipine	HP-gastritis, IBS
21	20	F	20	No	Oral contraceptives	Suspected Crohn's disease
22	47	F	10-15	No	Ventoline	Chronic gastritis, IBS
23	30	M	No	80 g	None	IBS
24	22	M	No	No	None	IBS
25	59	M	No	72 g	Data missing	IBS
26	65	M	No	Data missing	Allopurinol, digoxin, spironolactone	Caecal adenoma
27	70	M	No	No	Hydroxyurea, omeprazole	Data missing

[1]HP, Helicobacter pylori. [2]PAD, Peripheral arterial disease. [3]IBD, inflammatory bowel disease. [4]IBS, irritable bowel syndrome.

and *MDR1* cDNA were generated from three or four samples containing different known concentrations of cDNA. The standards were run at the same time as the samples.

It has previously been shown by Bowen *et al.* [18] that different enzymes give rise to the same standard curve in real-time RT-PCR, given that all reactions are optimized. Therefore, the concentration of b-actin mRNA was calculated from a calibration curve that was a composite of 10 different standard curves from four different CYP enzymes [16].

The amplicons of each reaction have previously been sequenced for confirmation of identity [17].

Statistical analysis

For comparison between paired samples the Friedman ANOVA with Kendall coefficient of variation and Wilcoxon matched pair test were used, and for comparison between unpaired samples the Mann-Whitney *U*-test was used (Statistica®] (StatSoft Inc., 2300 East 14th Street, Tulsa, OK, USA).

Results

The percentage of biopsies with measurable amounts of each mRNA is shown in Figure 1. *CYP2E1* mRNA expression was detected in 81–96% of the samples at the different sites along the GI tract. *CYP3A5* mRNA was detected in all samples at all locations, whereas *CYP3A4* mRNA detection varied between 41% and 96% and P-gp mRNA between 59% and 96%. However, the data from all samples, and also those with a Ct value above 38, are included in Figure 2, since they all expressed measurable concentrations of b-actin-specific mRNA, that is, a Ct value below 38. Because of the small size of the forceps used, biopsies contained only mucosal tissue. The majority of the patients were diag-

nosed with irritable bowel disease (Table 1) and as expected there was no inflammation in these biopsies. When all samples were considered, no difference in the median b-actin mRNA expression was observed in those that showed inflammation compared with those that did not.

The expression of *CYP2E1* mRNA was highest at all sites ($P < 0.05$ to $P < 0.0001$) except in right colon, where it was equal to the expression of *CYP3A5* mRNA (Figure 2 and Table 2). The highest expression of *CYP2E1* mRNA was observed in the stomach and duodenum ($P < 0.01$), but there was a substantial inter-individual variation (Kendall coefficient of concordance = 0.16). In the duodenum the expression of *CYP2E1* mRNA exceeded that of *CYP3A4*, *CYP3A5* and P-gp by approximately seven-, six- and 300-fold, respectively.

The expression of *CYP3A4* mRNA was highest in the duodenum ($P < 0.001$), with no or low expression in the stomach and colon (Figure 2 and Table 2), and showed great interindividual variability (Kendall coefficient of concordance = 0.25).

The expression of *CYP3A5* mRNA was highest in the duodenum and stomach ($P < 0.000\,001$) and was less in the colon (Kendall coefficient of concordance = 0.34) (Figure 2 and Table 2).

To validate our finding that the expression of *CYP3A5* mRNA was generally higher than that of *CYP3A4*, we analysed expression of these *CYP* genes in four different liver samples and one additional intestinal sample, obtained at surgery. In addition, another laboratory analysed the same livers and intestine using a similar method and found comparable results.

For P-gp, mRNA expression increased from stomach and duodenum to left colon ($P < 0.000\,001$] a Kendall coefficient of concordance = 0.34) (Figure 2 and Table 2).

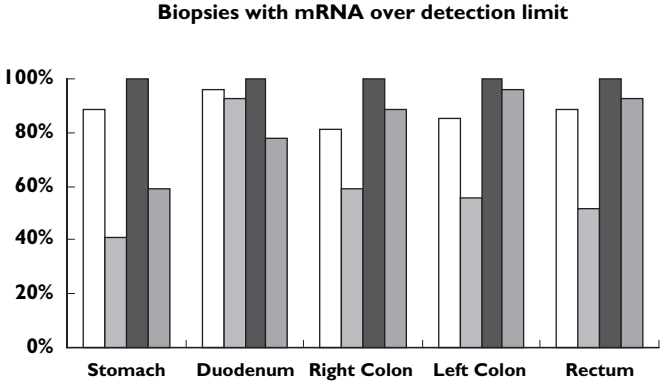

Figure 1

The number of biopsies containing mRNA above the detection limit (Ct over 38) expressed as a percent of all biopsies. CYP2E1 (□), CYP3A4 (▨), CYP3A5 (■), P-gp (▩)

Figure 2

Box plots of the CYP-specific/b-actin mRNA ratio for each enzyme at different locations of the human gastrointestinal tract (data from all samples included). Arrows indicate the location with the highest mRNA expression for each enzyme. Median (□), 25%–75% (▭), Min-Max (⊥)

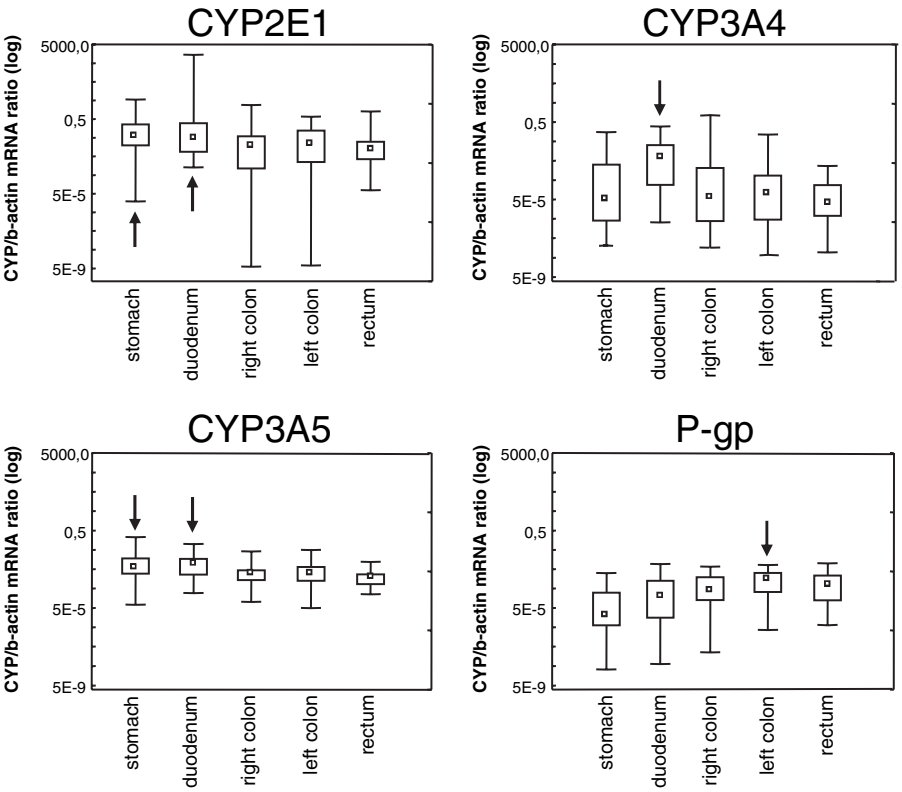

Table 2
Median (25-75% quartile) mRNA/â-actin ratios in different parts of the gastrointestinal tract

	CYP2E1	CYP3A4	CYP3A5	P-gp
Stomach	0.061 (0.017-0.29)	0.000053 (0.0000040-0.0036)	0.0058 (0.0026-0.020)	0.00002 (0.0000060-0.00034)
Duodenum	0.053 (0.0078-0.33)	0.0072 (0.00026-0.036)	0.0095 (0.0024-0.019)	0.00019 (0.000014-0.0014)
Right colon	0.017 (0.00010-0.06)	0.00006 (0.0000030-0.0023)	0.0028 (0.0012-0.0048)	0.00042 (0.00011-0.0022)
Left colon	0.023 (0.0022-0.13)	0.0001 (0.0000040-0.00095)	0.0029 (0.0011-0.0074)	0.0015 (0.00030-0.0036)
Rectum	0.012 (0.0032-0.034)	0.000032 (0.0000060-0.00031)	0.0018 (0.00076-0.0030)	0.00073 (0.00011-0.0026)

The interindividual variability in expression at each site in the GI tract was again very high. Each individual showed a similar expression pattern for the investigated CYP enzymes and P-gp throughout the GI tract.

We did not find any difference in mRNA expression between smokers ($n = 8$) and nonsmokers ($n = 19$), or between those who had an alcohol intake of less than 50 g compared with those who consumed more than that ($n = 7$) during the week before investigation, for any of the CYPs studied. Furthermore, there were no sex differences in expression (F = 15, M = 12), or differences between patients on regular medication ($n = 16$) compared with patients not taking medication ($n = 11$).

Discussion

To our knowledge, this is the first study to characterize the expression of CYP enzymes and P-gp along the length of the GI tract of the same individual in a large patient population. We determined the mRNA expression of three important *CYP* enzymes and the *MDR1* gene at five different sites in 27 patients. The methods used were sensitive enough for several analyses of the very small amount of biopsy material (about 5 mg wet weight) that can be obtained during the endoscopic investigation. However, there was insufficient to study these CYPs or P-gp at a functional or protein level.

We found that *CYP2E1* had the highest mRNA expression of the CYPs studied in all parts of the GI tract. Our findings are consistent with those of other investigators who have demonstrated the presence of CYP2E1 in the terminal ileum and colon of patients with irritable bowel disease, Crohn's disease and ulcerative colitis, respectively [19]. However, because of post-transcriptional regulation [20], different authors have failed to find a relationship between CYP2E1 mRNA and protein concentration [21, 22]. This suggests that there is no clear association between CYP2E1 mRNA expression and metabolic activity.

It has been claimed that there is a relationship between mRNA and protein expression for CYP3A4 and CYP1A2 [21, 22], although the data are conflicting, and our group has not been able to confirm this assertion (unpublished data).

Watkins and coworkers [23] have published data on the relative abundance of CYP3A4 in the intestinal mucosa, and protein expression and catalytic activity have been reported to decrease longitudinally along the small intestine [24]. We found higher *CYP3A4* mRNA expression in the duodenum than in the stomach and the colon, which is in agreement with earlier reports based on human transplant donors [25, 26].

It has been shown that CYP3A4 in the gut is an important determinant of the bioavailability of cyclosporine, because approximately one-half to two-thirds of its metabolism occurs presystemically when the drug is orally administered [1, 27]. Furthermore, other drugs such as midazolam are metabolized to a varying degree by CYP enzymes in the gut [28]. It has also been shown that plasma concentrations of digoxin (with which two of the patients were treated) decrease with concomitant administration of rifampicin. This may be explained by an increase in intestinal P-gp during treatment with this inducing agent [3].

CYP3A5 mRNA expression was found in all the samples studied. Earlier studies [29, 30] demonstrated the presence of *CYP3A5* mRNA in the small intestine, but CYP3A5 protein was not detected. In contrast, Lown *et al.* [31] found CYP3A5 protein in the small bowel of 70% of the patients studied, and they suggested that this enzyme may be expressed more in the intestine than in the liver. Kolars *et al.* [26] detected *CYP3A5* and *CYP3A4* mRNA in all regions of the GI tract using microsomes from a human organ donor.

The median concentration of *CYP3A5* mRNA was highest in duodenal mucosa but the difference was not as pronounced as for *CYP3A4* mRNA. Although CYP3A4 is considered to be the major CYP enzyme in

the small intestine [26]. Lown *et al.* [32] described similar concentrations of CYP3A4 and CYP3A5 protein in enterocytes. In the present study we found similar expression of *CYP3A4* and *CYP3A5* mRNA in the duodenum. The expression of CYP3A5 protein is polymorphic, and individuals with at least one *CYP3A5*1* allele express greater amounts of the enzyme. Based on our data, the expression of *CYP3A5* mRNA is monomorphic, since the *CYP3A5*3* allelic variant also expresses mRNA to a varying degree [33].

In the colon there was greater expression of *CYP3A5* mRNA compared with that of *CYP3A4*, which is in agreement with findings of McKinnon *et al.* [25]. Other data also indicate that CYP3A5 is the predominant CYP3A enzyme in colonic mucosa [26, 32]. Furthermore, there appears to be greater expression of CYP3A5 in the stomach than CYP3A4 [26], which is consistent with the findings of the present study.

It seems that there is a relatively low CYP3A phenotypic variability among healthy adults compared with that seen in archived intestinal and liver tissue samples [34]. One possible explanation for this is the different rates of CYP3A4 and CYP3A5 degradation during the harvesting and cold storage of the tissues [35], which could explain the discrepancies between the relative proportion of *CYP3A4* and *CYP3A5* mRNA expression in our study and previous reports [29, 30]. It is unclear whether the rates of degradation of CYP3A4 and CYP3A5 are different in the GI tract compared with the liver.

Lown *et al.* [2] found that variation in intestinal P-gp accounted for about 30% of the variability in peak blood concentration of orally administered cyclosporine. We found a continuous increase in *MDR1* expression along the GI tract to distal descending colon. Our findings are in agreement with those of Fojo *et al.* [36], although this report was based on colon samples from different patients and only one jejunum and rectum sample. Mouly and Paine [37] showed an increase in P-gp expression from proximal to distal small intestine. It has been suggested by Stephens *et al.* [38] that the ileum and distal colon are regions with high permeability for xenobiotics and that this is compensated for by enhanced expression of P-gp. The distribution pattern of *MDR1* mRNA is opposite to that of *CYP3A4* mRNA. These two proteins have overlapping substrate specificity and tissue distribution [24]. High expression of P-gp in the more distal part of the GI tract may be important in expelling xenobiotics not yet metabolized by CYP3A.

We found no influence of smoking, alcohol intake or sex on mRNA concentrations. However, the groups were quite small, and hence these findings must be interpreted with caution. There was no detectable relationship between the medication taken by patients and mRNA expression. However, the reported drug consumption was very heterogeneous among the patients.

We conclude that among the CYP enzymes investigated *CYP2E1* mRNA expression was highest throughout the GI tract, with that of *CYP3A* being greatest in the duodenum. This finding is relevant because of the emerging role of the gut in the metabolism of a large number of clinically important drugs. *MDR1* mRNA expression increased continuously from duodenum to colon, a finding that may be linked to a natural role for P-gp in the protection against xenobiotics produced by the intestinal microflora.

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