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Polymorphic Gene Regulation and Interindividual Variation of UDP-glucuronosyltransferase Activity in Human Small Intestine*

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UDP-glucuronosyltransferases (UGTs) convert dietary constituents, drugs, and environmental mutagens to inactive hydrophilic glucuronides. Recent studies have shown that the expression of the *UGT1* and *UGT2* gene families is regulated in a tissue-specific fashion. Human small intestine represents a major site of resorption of dietary constituents and orally administered drugs and plays an important role in extrahepatic UGT directed metabolism. Expression of 13 *UGT1A* and *UGT2B* genes coupled with functional and catalytic analyses were studied using 18 small intestinal and 16 hepatic human tissue samples. Hepatic expression of *UGT* gene transcripts was without interindividual variation. In contrast, a polymorphic expression pattern of all the *UGT* genes was demonstrated in duodenal, jejunal, and ileal mucosa, with the exception of *UGT1A10*. To complement these studies, interindividual expression of UGT proteins and catalytic activities were also demonstrated. Hyodeoxycholic acid glucuronidation, catalyzed primarily by UGT2B4 and UGT2B7, showed a 7-fold interindividual variation in small intestinal duodenal samples, in contrast to limited variation in the presence of 4-methylumbelliferone, a substrate glucuronidated by most *UGT1A* and *UGT2B* gene products. Linkage of RNA expression patterns to protein abundance were also made with several mono-specific antibodies to the UGTs. These results are in contrast to a total absence of polymorphic variation in gene expression, protein abundance, and catalytic activity in liver. In addition, the small intestine exhibits considerable catalytic activity toward most of the different classes of substrates accepted for glucuronidation by the UGTs, which is supported by immunofluorescence analysis of UGT1A protein in the mucosal cell layer of the small intestine. Thus, tissue-specific and interindividual polymorphic regulation of *UGT1A* and *UGT2B* genes in small intestine is identified and implicated as molecular biological determinant contributing to interindividual prehepatic drug and xenobiotic metabolism in humans.

Glucuronidation is an important process of metabolism and detoxification performed by the UDP-glucuronosyltransferase (UGT)[1] Supergene family (1). UGTs are resident in the endoplasmic reticulum and catalyze the conversion of hydrophobic substrates to usually inactive hydrophilic glucuronides, which subsequently undergo renal and biliary elimination. Compounds targeted for glucuronidation include dietary constituents, therapeutic drugs, endogenous metabolites, hormones, and environmental carcinogens. The human *UGT* genes are differentially regulated in a tissue-specific fashion in hepatic and extrahepatic tissues of the gastrointestinal tract (2–4).

Human UGTs have been divided into the *UGT1* and *UGT2* multigene families (5). The human *UGT1A* gene locus is located on chromosome 2, which encodes at least nine functional UGT1A proteins and three pseudogenes (6). Four exons are located at the 3′ end of the *UGT1A* locus, which are combined with one of a consecutively numbered array of first exon cassettes toward the 5′ end of the gene locus to form individual UGT gene products. Therefore, the amino-terminal 280 amino acids of UGT1A proteins consist of unique exon 1 encoded sequences and the carboxyl-terminal 245 amino acids encoded by exons 2–5 are identical. The tissue-specific expression of the *UGT1A* gene locus has been well characterized and has been suggested to define tissue-specific glucuronidation activity in the human digestive system (2). An analysis of liver tissue led to the characterization of UGT1A1 (7), UGT1A3 (8), UGT1A4 (7), UGT1A6 (9), and UGT1A9 (10) cDNAs. Studies examining the human extrahepatic gastrointestinal tract have led to the identification of three extrahepatic UGT1A transcripts: UGT1A7, which is expressed in stomach and esophagus (3, 4); UGT1A8, which is expressed in colon and esophagus (2, 11, 12); and UGT1A10, which is expressed in gastric, esophageal, biliary, and colonic tissue (2, 4, 13, 14).

In contrast to the *UGT1A* gene locus, the *UGT2B* and *UGT2A* genes have been mapped to chromosome 4 are individually encoded and comprise six exons (15–17). Transcripts have been identified for UGT2B4 (18), UGT2B7 (19), UGT2B10 (20), UGT2B11 (21), UGT2B15 (22), UGT2B17 (23, 24), and UGT2A1 (17). Except for UGT2B17 and UGT2A1, hepatic expression was detected for all UGT2B transcripts. Extrahepatic UGT2B expression has been shown for UGT2B7 in intestine, kidney, and brain (25, 26), UGT2B10 and UGT2B15 expression has been shown for esophagus (3), and UGT2B10, UGT2B11, UGT2B15, and UGT2B17 expression has been shown in steroid sensitive tissues such as the mammary gland and the prostate

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[1] The abbreviations used are: UGT, UDP-glucuronosyltransferase; RT, reverse transcription; DRT, duplex RT; PCR, polymerase chain reaction; HDCA, hyodeoxycholic acid; 4-MU, 4-methylumbelliferone; PhIP, 2-amino-1-methyl-6-phenylimidazo-(4, 5-β)-pyridine; bp, base pair(s); PBS, phosphate-buffered saline.

TABLE I
Patient and tissue samples analyzed

Tissue	Number	Mean age (range)	Female/male	Diagnosis
		years		
Duodenum	5	55.75 (51–62)	3/2	Pancreatic carcinoma
Jejunum	5	54.4 (25–77)	2/3	Pancreatic carcinoma (n = 2), gastric ulcer, gastric carcinoma, blind loop syndrome
Ileum	8	62.14 (35–86)	4/4	Colon carcinoma (n = 5), cholangiocellular carcinoma, inflammatory bowel disease, neuroendocrine tumor

(20–24). One report indicates that UGT2B4 is not expressed in the gastrointestinal tract (26).

The genetic multiplicity of the UGTs and their wide range of substrate specificities suggests that UGTs play an important role in human homeostasis and metabolism. Although hepatic glucuronidation is considered to play a central role in drug metabolism, direct contact with xenobiotic compounds is first established in the gastrointestinal tract prior to resorption (27). The small intestine, which extends to a length of 300–400 cm in adults, forms the largest metabolically active external surface of the human digestive system and represents a significant localization for extrahepatic metabolism. The considerable degree of immediate xenobiotic contact in the small intestine including dietary components, drugs, and environmental mutagens would indicate that enzymes located in the mucosal layer are capable of influencing first pass metabolism and may function as a metabolic intestinal barrier. The presented study was undertaken to analyze the regulatory patterns of *UGT1A* and *UGT2B* genes in small intestine as a biochemical basis for defining human extrahepatic xenobiotic glucuronidation.

EXPERIMENTAL PROCEDURES

Tissue Samples

Tissue samples were obtained from the Department of Abdominal and Transplant Surgery, Hannover Medical School, Hannover, Germany. Informed written consent was obtained, and the project was approved by the ethics committee of Hannover Medical School. Macroscopically and histologically normal intestinal tissue was obtained from 18 German patients undergoing surgery for diagnoses summarized in Table I. None of the patients received chemotherapy, steroids, diuretics, or antibiotic therapy prior to sample collection. The records indicated the absence of smoking during 6 months prior to surgery. A high degree of sample normalization results from the additional fact that all patients were fasting at least 12 h prior to the surgical procedures and tissue collection. The collected tissues showed no macroscopic signs of deterioration such as necrosis and were microscopically examined to document normal histology. One patient with ulcerative colitis received a colectomy and had no histological signs of ileal disease. Hepatic tissue RNA and microsomes used for comparisons have been described previously (27).

Intestinal mucosa was dissected immediately on ice after surgical removal, and specimens free of muscularis and most of the submucosa were used in all subsequent experiments except for indirect immunofluorescence. All tissue samples were frozen in liquid nitrogen within 10 min of surgical removal and stored at −80 °C until use.

Isolation of RNA and Synthesis of Complementary DNA

Tissue—Approximately 200 mg of tissue was pulverized under liquid nitrogen and immediately lysed in acidic phenol/guanidinium-isothiocyanate solution (Tripure; Roche Molecular Biochemicals) as described previously (4). RNA concentrations were determined by spectrophotometry at 260 nm, and the purity was verified by 260/280-nm ratios. Intact RNA was isolated from hepatic, duodenal, jejunal, and ileal tissue samples and stored in water at −80 °C until further analysis.

cDNA Synthesis—Three μg of total RNA were denatured for 10 min at 70 °C in the presence of 0.5 μg of oligo(dT) primer and quick chilled on ice. The volume of RNA was adjusted to 19 μl containing 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 2.5 mM $MgCl_2$, 10 mM dithiothreitol, and 0.5 mM of each dNTP incubated at 42 °C for 5 min prior to the addition of 1 μl (200 U) of reverse transcriptase (SuperscriptII; Life Technologies, Inc.). The final volume (20 μl) was incubated at 42 °C for 50 min and then 70 °C for 15 min and chilled on ice. Contamination of total RNA with genomic DNA was excluded by RT-PCR using primers for human β-actin. The sense primer 5′-ggcggcaccaccatgtaccct-3′ and the antisense primer 5′-aggggccggactcgtcatact-3′ span the exon 4/intron 5/exon 5 junction of the β-actin gene. PCR with cDNA leads to a 202-bp product, but contamination with genomic DNA template would lead to a 312-bp PCR product, which can be clearly distinguished from the 202-bp cDNA amplification product.

Isolation of Microsomal Protein from Intestinal Tissue

Approximately 300 mg of intestinal tissue was pulverized under liquid nitrogen, resuspended in 1 ml of 50 mM Tris-HCl (pH 7.4) and 10 mM $MgCl_2$, and homogenized with a Potter-Elvehjam tissue grinder. The tissue homogenate was centrifuged at 10,000 × g for 5 min at 4 °C in an Eppendorf (Hamburg, Germany) microcentrifuge, and the supernatant was collected. The pellet was resuspended in 0.5 ml of 50 mM Tris-HCl (pH 7.4) and 10 mM $MgCl_2$ and centrifuged, and the supernatant collected. The combined supernatants were centrifuged at 150,000 × g for 60 min at 4 °C in a Beckman (Palo Alto, CA) TL100 ultracentrifuge, and the pellet was resuspended in 0.2 ml of Tris-HCl (pH 7.4) and 10 mM $MgCl_2$. Protein concentration was determined by the method of Bradford. Microsomal protein was stored at −80 °C.

Catalytic Activity Assay of Human Intestinal and Liver Microsomes

Glucuronidation substrates (all 18 tested substrates are listed in the legend to Fig. 3) were solubilized in methanol with the exception of 7-hydroxy-benzo(α)pyrene, which was resuspended in acetone. 7-Hydroxy-benzo(α)pyrene, 3-hydroxy-acetylaminofluorene, and 2-amino-1-methyl-6-phenylimidazo-(4,5-β)-pyridine (PhIP) were obtained from the National Cancer Institute Chemical Carcinogen Repository (Midwest Research Institute, Kansas City, MO); 2-hydroxyamino-1-methyl-6-phenylimidazo-(4,5-β)-pyridine (*N*-hydroxy-PhIP) was purchased from Toronto Research Chemicals Inc. (Toronto, Canada); and all other substrates were from Sigma-Aldrich. Catalytic activities of 25 μg of microsomal protein isolated from intestinal or hepatic tissue were assayed in duplicate as described previously in detail (3). Protein was precipitated, and supernatants were lyophilized and resuspended in methanol prior to separation by thin layer liquid chromatography in *n*-butanol/acetone/acidic acid/water (35:35:10:20%). The production of ^{14}C-labeled glucuronides was detected by autoradiography. To determine specific catalytic activities, the ^{14}C-labeled glucuronides were quantitated using a Fujifilm BAS-1000 phosphoimager (Raytest GmbH, Straubenhardt, Germany) and TINA 2.0 software (Raytest GmbH) and expressed as pmol glucuronide formed/min/mg of microsomal or recombinant protein. As a control, autoradiography hard copies were additionally analyzed with a GS-710 calibrated imaging densitometer (Bio-Rad) using the Quantity One software package.

Duplex Reverse Transcription Polymerase Chain Reaction for UGT1A and UGT2B Transcripts

The presence of UGT1A and UGT2B transcripts in total tissue RNA was analyzed by PCR amplification performed as a duplex RT-PCR coamplification with β-actin cDNA as a control, as outlined below.

UGT1A DRT-PCR—The *UGT1A* locus predicts the existence of nine proteins termed UGT1A1 and UGT1A3–1A10. UGT1A2, UGT1A11 and UGT1A12 lack an uninterrupted open reading frame and have therefore been identified to be pseudogenes. DRT-PCR detection of all nine UGT1A transcripts predicted by the human *UGT1A* locus was performed using nine exon 1-specific sense primers and two antisense primers located within exons 2–5 or within a common portion of the 3′ end of the first exons (4). As already reported elsewhere (4), exon-specific primers were generated that lead to RT-PCR products of distinct molecular sizes: UGT1A1, 644 bp; UGT1A3, 483 bp; UGT1A4, 572 bp; UGT1A5, 659 bp; UGT1A6, 562 bp; UGT1A7, 754 bp; UGT1A8, 514 bp; UGT1A9, 392 bp; and UGT1A10, 478 bp. Coamplification of UGT1A

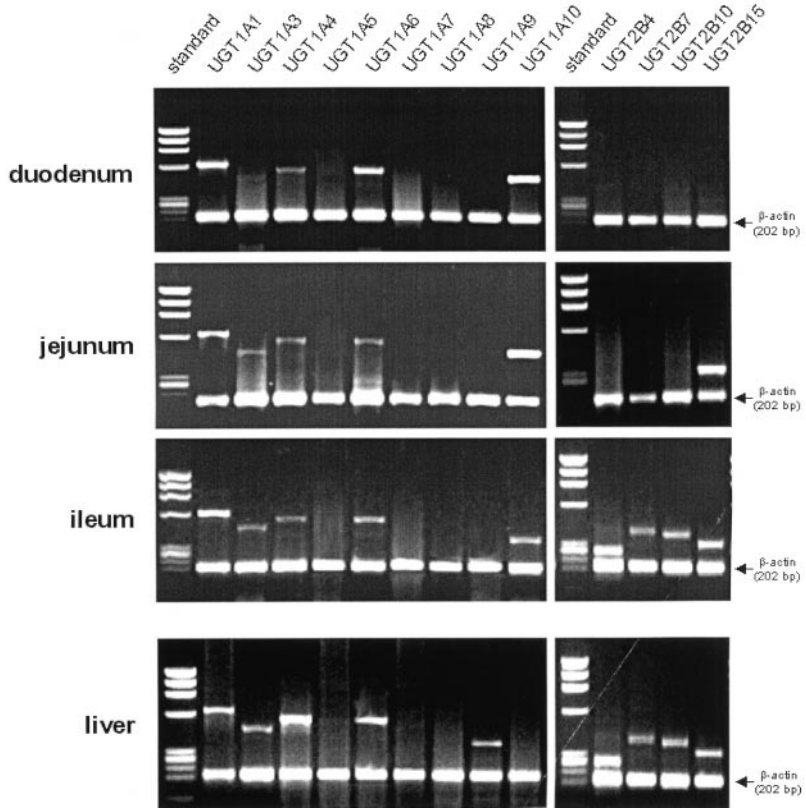

FIG. 1. **Polymorphic regulation of the *UGT1A* gene locus and *UGT2B* genes in human duodenum, jejunum, ileum, and liver.** UGT gene expression in duodenal, jejunal, ileal, and hepatic epithelium was detected by UGT isoform-specific DRT-PCR. The ethidium bromide stained gels show isoform-specific DRT-PCR products coamplified in the presence of β-actin primers as a control. Examples of a single patient are given for each tissue source. In the duodenal example UGT1A3 mRNA is low, and none of the UGT2B transcripts are detectable. The jejunal sample shows a typical intestinal pattern with the absence of UGT1A5, UGT1A7, UGT1A8, UGT1A9, UGT2B4, UGT2B7, and UGT2B10. The *bottom panel* shows the previously reported hepatic UGT expression profile found in all liver samples examined (4).

first exon and β-actin sequences was performed using three cycling protocols: for UGT1A1 and UGT1A6, 94 °C for 1 min, 59 °C for1 min, and 72 °C for 1 min; for UGT1A3, UGT1A4, and UGT1A5, 94 °C for 1 min, 56 °C for 1 min, and 72 °C for 1 min; and for UGT1A7, UGT1A8, UGT1A9, and UGT1A10, 94 °C for 1 min, 64 °C for 1 min, and 72 °C for 1 min. Each protocol was preceded by a 3-min incubation of the reaction mixture at 94 °C and followed by a 7-min elongation at 72 °C. The specificity and kinetics of this assay have previously been documented in detail (4). Experiments were performed in duplicate, and controls without cDNA, primers, or thermophilic polymerase were included.

UGT2B DRT-PCR—Specific primer pairs were generated for the amplification of UGT2B4, UGT2B7, UGT2B10, and UGT2B15 sequences, respectively, as recently reported elsewhere (3). Cross-reactivity was excluded using sequence alignments and PCGene (Oxford Molecular, Campbell, CA) software, as well as a computerized data bank search using the blastn software (GenBank™). UGT2B cDNA was coamplified with β-actin cDNA in a starting volume of 92 μl containing 10 mM KCl, 20 mM Tris-HCl (pH 8.8), 10 mM ammonium sulfate, 2 mM magnesium sulfate, 1% Triton X-100, 0.2 mM each dNTP, and 2 μM of UGT2B primers and VENT (exo-) DNA polymerase (New England Biolabs, Beverly, MA). After a hot start at 94 °C for 3 min, six cycles of 94 °C for 30 s, 57 °C for 30 s, and 72 °C for 30 s were run on a Perkin-Elmer GeneAmp PCR 2400 system. The same β-actin primers used for UGT1A DRT-PCR were added to 0.4 μM each, and cycling was resumed for a total of 32 cycles. Specificity of this assay was determined by PCR using all four primer pairs on each cloned UGT2B4, UGT2B7, UGT2B10, and UGT2B15 template cDNA to exclude cross-reactivities. PCR products of the expected sizes were generated: UGT2B4, 281 bp; UGT2B7, 407 bp; UGT2B10, 388 bp; and UGT2B15, 330 bp. To confirm the detection of specific UGT1A and UGT2B cDNAs using this assay, the PCR products were partially sequenced to document the identity of the specific gene product.

Western Blot Analysis

Twenty μg of microsomal protein from five human duodenal and five human hepatic tissues samples was boiled for 90 s in loading buffer (2% sodium dodecyl sulfate, 62.5 mmol/liter Tris-HCl (pH 6.8), 10% glycerol, and 0.001% bromphenol blue) with β-mercaptoethanol and resolved by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis prior to electrotransfer onto nitrocellulose membrane. As controls, a 5-μg sample of total Sf9 cell lysate expressing recombinant UGT1A1 and UGT2B7 protein as well as Sf9 cells expressiong no recombinant UGT protein were included (3). Immunodetection was performed following published protocols (28). UGT1A1 and UGT2B7 protein was detected using a monospecific rabbit anti-human UGT1A1 and rabbit anti-human UGT2B7 antibody purchased from NatuTec/Gentest (Frankfurt, Germany) at a dilution of 1:1500. Visualization was achieved with an alkaline phosphatase-conjugated goat anti-rabbit IgG (Sigma) diluted 1:4000.

Indirect Immunofluorescence Analysis

Fresh intestinal resection specimens were subjected to cryostat sectioning following previously published methods (29). Tissue sections were used for indirect immunofluorescence using a previously described rabbit anti-peptide (SSLHKDRPVEPLDLA) anti-human UGT1A antibody, which was generated using branched lysine multiple antigen peptide technology (2). Antibody was diluted 1:20, 1:40, 1:80, and 1:160 in phosphate buffered saline without magnesium or calcium (PBS), and immobilized tissue slices were incubated at room temperature in a humidified chamber for 60 min. Incubation with a normal rabbit serum was included as a control. Following two wash steps with PBS, the slides were incubated at room temperature in a humidified, dark chamber for 60 min with fluorescein (dichlorotriazinyl-aminofluoresceine)-conjugated affinity purified goat anti-rabbit IgG (H+L) (Dianova, Hamburg, Germany) diluted 1:100 in PBS. Following two wash steps with PBS, tissue slices were covered with glycerol containing 10% PBS and were immediately analyzed using an Olympus IMT 2 immunofluorescence microscope (Tokyo, Japan).

RESULTS

Polymorphic Regulation of UGT1A and UGT2B Gene Transcripts in Human Duodenum, Jejunum, and Ileum—UGT mRNA expression was analyzed by isoform-specific DRT-PCR (Fig. 1 and Table II, right column). The liver was characterized by the expression of UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A9, UGT2B4, UGT2B7, UGT2B10, and UGT2B15 mRNA, as previously demonstrated (3, 26). In the 16 different liver samples, there was little variation in abundance of the RNA transcripts when each was compared with the levels of expression of actin (not shown but previously demonstrated in Ref. 14). This is in sharp contrast to intestinal tissue, which exhibited dramatic differences in *UGT* gene transcript expres-

TABLE II
Expression of UGT1A and UGT2B mRNA in human small intestine and liver

Table II summarizes the expression of UGT transcripts detected by UGT isoform-specific DRT-PCR. Although a constitutive expression is demonstrated for UGT1A10 (bold type) and UGT2B15 (with the exception of two duodenal samples) in small intestine, the remaining transcripts are expressed in a polymorphic fashion. In contrast, hepatic UGT gene expression is not polymorphic. ND, not detected by DRT-PCR.

UGT Isoform	Duodenum (n = 5)	Jejunum (n = 5)	Ileum (n = 8)	Liver (n = 16)
UGT1A1	3/5	1/5	3/8	16/16
UGT1A3	3/5	5/5	5/8	16/16
UGT1A4	4/5	4/5	7/8	16/16
UGT1A5	ND	ND	ND	ND
UGT1A6	4/5	1/5	6/8	16/16
UGT1A7	ND	ND	ND	ND
UGT1A8	ND	ND	ND	ND
UGT1A9	ND	ND	ND	16/16
UGT1A10	**5/5**	**5/5**	**8/8**	ND
UGT2B4	1/5	2/5	5/8	16/16
UGT2B7	2/5	3/5	2/8	16/16
UGT2B10	ND	ND	1/8	16/16
UGT2B15	3/5	5/5	8/8	16/16

sion. Intestinal expression was characterized by the presence or absence of UGT1A and UGT2B transcripts in the different samples of intestinal tissue.

Analyses of 13 different UGT transcripts demonstrated that UGT1A10 mRNA was expressed in each sample of duodenal, jejunal, and ileal mucosa, whereas UGT2B15 was absent in only two of the duodenal samples. UGT1A3 and UGT1A4 were found to be expressed in the majority of duodenal, jejunal and ileal mucosa samples. In contrast, UGT1A5, UGT1A7, UGT1A8, and UGT1A9 transcripts were not detected, and UGT2B10 was found in only one of the ileum preparations. It is interesting to note that in the ileum sample in which UGT2B10 RNA was expressed, all of the other *UGT2B* gene products were also detected (Fig. 1, *bottom panel*).

The appearance of UGT1A1, UGT1A6, UGT2B4, and UGT2B7 mRNA showed the most dramatic variability between the different intestinal samples. In the duodenum, UGT1A1 was detected in three of the five samples examined, UGT1A6 was in four of the five samples, UGT2B4 was in one of the five samples, and UGT2B7 was in two of the five samples. The ratios of these gene products were found to be similar in the jejunum, although UGT1A6 mRNA was only detected in one sample. In the ileum, UGT2B4 was detected in a greater number of samples than found in the duodenum and jejunum, demonstrating that UGT2B4 is not liver-specific as previously indicated (26). Combined, analysis of *UGT* gene expression as presented by sensitive RT-PCR analysis clearly demonstrate that the expression of RNAs encoding UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT2B4, UGT2B7, UGT2B10, and UGT2B15 is not coordinately regulated in the different tissues of the small intestine. The only exception appears to be UGT1A10, which is expressed in all portions of the small intestine, as well as all other tissues of the gastrointestinal tract including the colon, the esophagus, the stomach, and the biliary tract (2–4, 26, 27).

Interindividual Differences in UGT Activities in Small Intestine—Most of the UGTs possess the ability to glucuronidate many of the same substrates, making it a challenge to use functional studies to follow the expression patterns of any single UGT (5). However, several substrates can be employed to monitor the catalytic activities of a limited number of the UGTs. For example, HDCA has been identified to be glucuronidated primarily by UGT2B7 (30), with detectable activity observed with expressed UGT2B4 (31) and UGT1A3 (5, 32). Because the gene expression pattern demonstrated considerable interindividual differences in UGT2B4 and UGT2B7 expression, experiments were undertaken to examine the functional properties of these proteins in microsomal preparations from small intestinal tissue samples using HDCA as a substrate. In contrast, 4-methylumbelliferone (4-MU) was chosen as a more general substrate because it is glucuronidated by most of the UGT1A as well as some of the UGT2 proteins (34). Using five duodenal, jejunal, and hepatic microsomal preparations, UGT activities confirmed that there existed considerable interindividual variation of HDCA glucuronidation in the small intestinal samples when compared with liver microsomal preparations. In jejunum tissues, this variation was seen to be 7-fold. It is therefore likely that the differences in HDCA UGT activity reflect the variation observed in UGT2B4 and UGT2B7 RNA transcript expression in these samples. In addition, up to a 2.3-fold variation of 4-MU glucuronidation (Fig. 2*A*, *top panel*) was observed with both intestinal tissues, which might be reflected in the differences seen with UGT1A1, UGT1A3, UGT1A4, and UGT1A6 RNA expression (Table II). As predicted from the mRNA expression data, there was very little variation of HDCA and 4-MU glucuronidation activity in the different liver samples. Although the duodenum, jejunum, and liver tissue samples were taken during surgery from different individuals, the differences observed in catalytic activity in the intestinal tissue strongly implicates that the polymorphic regulation of UGT mRNA leads to interindividual variation in UGT expression and activity.

Additional support for the findings observed with RNA as well as functional analysis that the UGTs are differentially expressed in intestinal tissue could be verified by Western blot analysis. In Fig. 2*B* the analysis of UGT1A1 and UGT2B7 protein expression in five samples of human duodenum and liver are shown and correlated with the detection of transcripts of these genes. The expression of UGT1A1 mRNA in three of the five samples and UGT2B7 mRNA in two of the five samples (Table II) is confirmed at the protein level by Western blot analysis, which detected UGT1A1 and UGT2B7 in the same samples. Duodenal sample 5 in Fig. 2*B* was used in the RT-PCR analysis and is shown in Fig. 1, demonstrating the expression of UGT1A1 RNA but not of UGT2B7 RNA. This finding convincingly links the expression of RNA to protein. Interestingly, this same sample does not express UGT2B4 RNA, whereas UGT1A3 RNA is barely detectable. UGT2B4, UGT2B7, and UGT1A3 are capable of catalyzing the glucuronidation of HDCA, and this sample of duodenum elicited the lowest HDCA UGT glucuronidation activity of the small intestinal samples that were collected. Combined, these findings demonstrate that the polymorphic interindividual regulation of UGT1A1 and UGT2B7 gene expression results in the detectable presence or absence of these specific UGT proteins. Thus, polymorphic regulation of UGT genes in small intestine leads to variations of catalytically active UGT, which determine microsomal glucuronidation activity between individuals.

Differences of Hepatic and Small Intestinal Glucuronidation—A panel of 18 substrates was used to characterize the UGT activity profile of the small intestine and liver. The consistent expression of UGT1A3, UGT1A4, UGT1A10, and different UGT2B forms would suggest activities toward steroid hormone and tertiary amine substrates in addition to phenolics. The putative tobacco carcinogens PhIP, *N*-hydroxy-PhIP, and 7-hydroxy benzo(α)pyrene, as well as 3-hydroxy-acetylaminofluorene were also included. Microsomal protein from two samples each of duodenal, jejunal, and ileal mucosa, were analyzed in the presence of the substrates, and their activities are shown

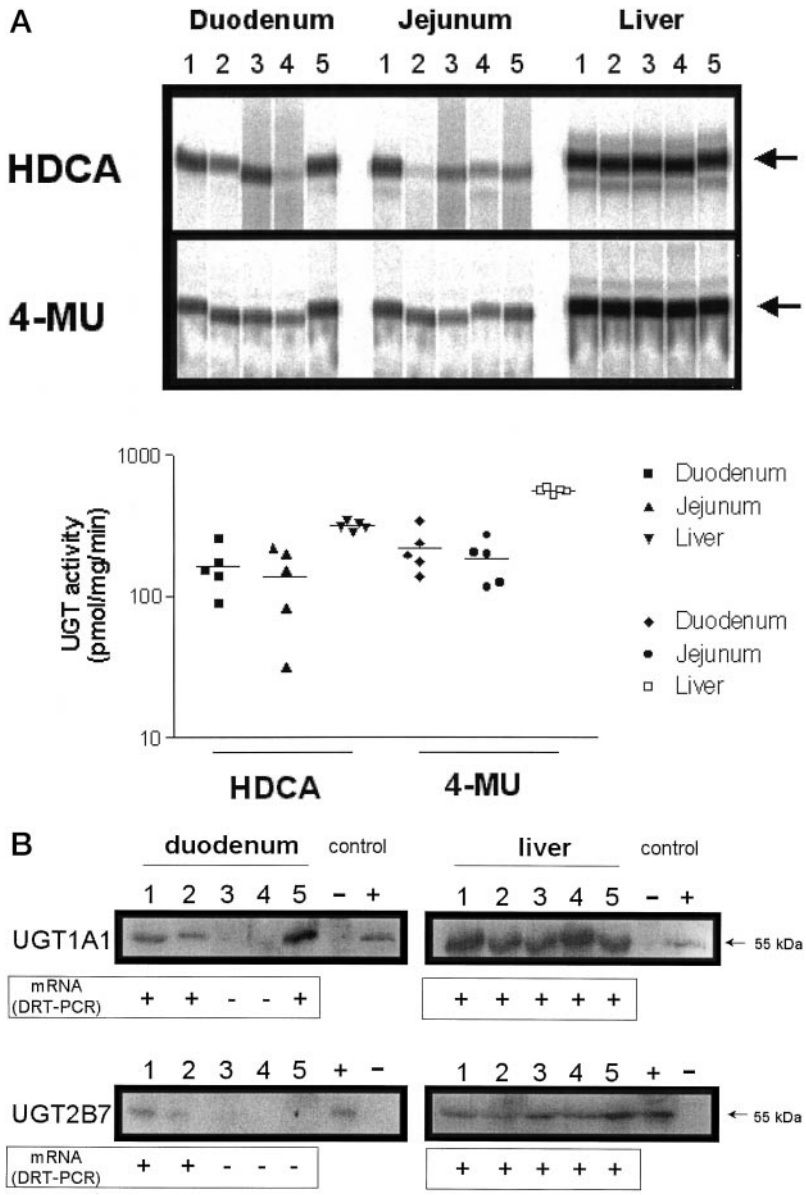

FIG. 2. **Interindividual variation of specific UGT activity in small intestine but not in liver.** *A*, five preparations of endoplasmic reticulum protein from duodenum, jejunum, and liver were analyzed for catalytic activity toward HDCA and 4-MU (*top panel*). Duodenal *sample 4* is the same as that shown in the *top panel* of Fig. 1. In the duodenal *sample 4*, HDCA glucuronidation was found to be lowest. This tissue sample lacks both UGT2B4 and UGT2B7 expression and has low levels of UGT1A3 expression (compare Fig. 1, *top panel*). In the other duodenal samples there is either expression of UGT2B4 (*sample 3*), UGT2B7 (*samples 1* and *5*), or UGT1A3 (*samples 2*, *4*, and *5*), which would lead to the observed HDCA glucuronide formation (compare Table II). The *bottom panel* demonstrates a graphic representation of interindividual variations of the measured UGT activities. *B*, Western blot analysis using 20 μg of microsomal protein from five duodenal and five liver tissue samples. The detection of UGT1A1 (*top panels*) and UGT2B7 (*bottom panels*) protein was performed using monospecific rabbit anti-human UGT1A1 and rabbit anti-human UGT2B7 antisera as described under "Experimental Procedures." The duodenal sample shown in *lane 5* (expression of UGT1A1 but not of UGT2B7) is demonstrated in the *top panel* of Fig. 1, confirming the expression pattern found at the transcript level. The − denotes a negative control using Sf9 cell extracts not expressing UGT protein; + represents Sf9 cells expressing UGT1A1 or UGT2B7, respectively. The + and − marks below the Western analysis indicate the presence or absence of UGT1A1 or UGT2B7 mRNA detectable by DRT-PCR.

in Fig. 3. The specific activity toward 1-naphthol, 4-MU, 4-nitrophenol, and HDCA was greater in liver than in the individual intestinal samples. Yet there were a greater number of compounds glucuronidated at higher rates in small intestine. This is best demonstrated by examining the glucuronidation of steroids such as 2-hydroxy estrone, β-estradiol, estrone, and also of the carcinogens PhIP, 7-hydroxy benzo(α)pyrene, and 2-hydroxyamino-1-methyl-6-phenylimidazo-(4,5-β)-pyridine, in addition to the tertiary amine antidepressant drugs imipramine and amitriptyline.

This experiment also demonstrates that the catalytic activities were found to be greater in the jejunum than in the proximally located duodenum or the distally located ileum. In addition, the finding that the catalytic activities in the jejunum are universally greater than those found in liver would suggest that this tissue plays an important role in the metabolism of dietary and xenobiotic material.

Detection and Localization of UGT Protein in Human Intestine—To confirm the mucosal distribution of UGT protein, an indirect immunofluorescence analysis was performed using a rabbit anti-human UGT1A antibody directed against all UGT1A protein species (Fig. 4). Staining was exclusively localized to the epithelial cell layer of the intestinal mucosa (Fig. 4, *A* and *B*). No staining was observed in the submucosa or muscularis as well as with a normal rabbit serum (not shown). UGT1A protein expression was found only in the epithelilar cell layer of the crypt (Fig. 4 C) as well as the vili (Fig. 4*B*).

DISCUSSION

Human UDP-glucuronosyltransferases are expressed in a tissue-specific fashion that defines tissue-specific glucuronidation activities in metabolically active organs including the liver and the extrahepatic gastrointestinal tract (2–4, 11, 27). Microsomal glucuronidation and UGT mRNA expression have been analyzed in human esophagus, stomach, and colon, establishing the role of these external surface tissues in extrahepatic glucuronidation. Although intestinal glucuronidation has been documented (26, 33–35), UGT1A and UGT2B gene regulation and biological function have not been correlated.

Using DRT-PCR, the regulation of the *UGT1A* locus as well as the *UGT2B4*, *UGT2B7*, *UGT2B10*, and *UGT2B15* genes was analyzed in 18 tissue samples from duodenum, jejunum, and ileum. A pattern of tissue-specific gene expression was observed in small intestine, which exhibited considerable differences from that found in liver and colon (Table II and Fig. 1). Liver (UGT1A1, UGT1A3, UGT1A4, UGT1A6, and UGT1A9)

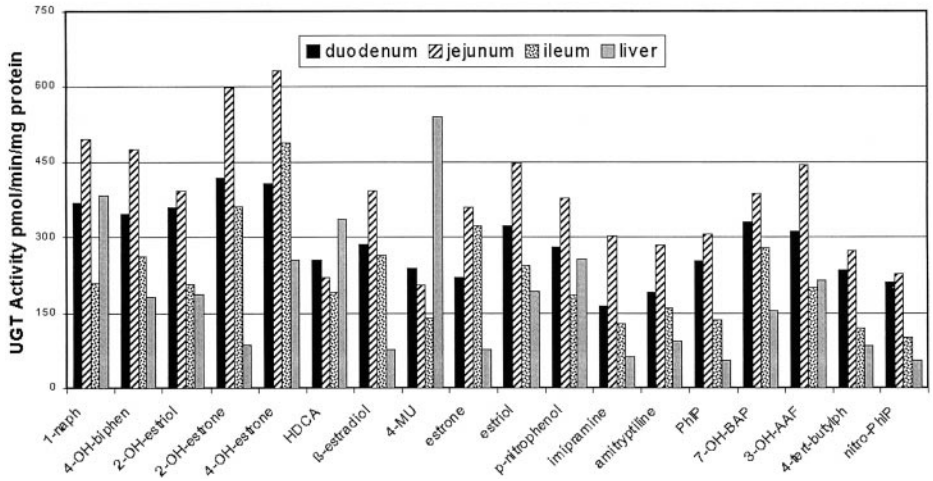

FIG. 3. **Catalytic UGT activities in small intestine and liver.** Graphic representation of the average ($n = 2$) specific UGT activities in duodenum, jejunum, ileum, and liver using 18 substrates as described under "Experimental Procedures." *1-naphth*, 1-naphthol; *4-OH biphen*, 4-hydroxybiphenyl; *2-OH-estriol*, 2-hydroxyestriol; *4-OH-estrone*, 4-hydroxy estrone; *p-nitrophenol*, 4-nitrophenol; *7-OH-BAP*, 7-hydroxy benzo(α)pyrene; *3-OH-AAF*, 3-hydroxy acetylaminofluorene; *4-tert-butylph*, 4-*tert*-butylphenol; *Nitro-PhIP*, 2-hydroxyamino-1-methyl-6-phenylimidazo-(4,5-β)-pyridine.

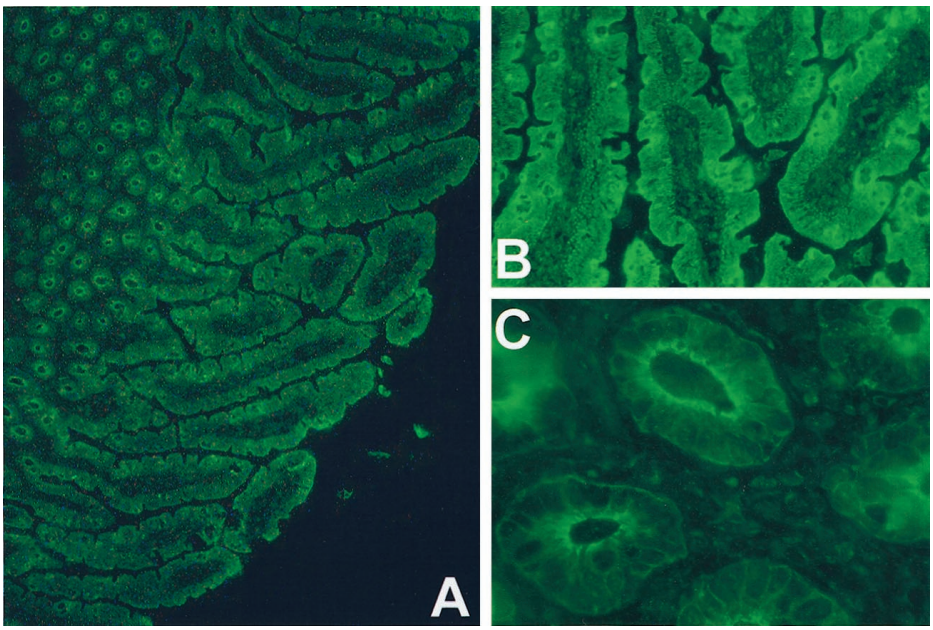

FIG. 4. **Immunofluorescence detection of UGT1A protein in the intestinal mucosa.** Indirect immunofluorescence using a rabbit anti-human UGT1A antibody is shown with cryostat sections of ileum tissue. *A*, UGT1A protein is localized to the epithelila cell layer and the crypts (magnification, 40×). UGT staining of the vili is homogeneous, and the crypts exhibit a ring-like pattern. *B*, high power magnification of a vilus section confirming staining of the epithelial cells but not of the submucosa (magnification, 400×). *C*, high power magnification of the cross-section of a mucosal crypt. UGT1A protein is concentrated in the apical portions of the crypt enterocytes toward the luminal surface (magnification, 400×). UGT protein is detected at the surfaces of direct contact between intestine and xenobiotic matter.

and colon (UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A8, UGT1A9, and UGT1A10) tissue have been characterized to express specific UGT1A transcript patterns without variation (4, 27, 36). In intestine, gene expression included UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A10, UGT2B4, UGT2B7, and UGT2B15 transcripts, and the absence of UGT1A5, UGT1A7, UGT1A8, and UGT1A9, and most UGT2B10 transcripts. However, the regulation of all UGT1A and UGT2B transcripts with the exception of UGT1A10 was polymorphic with variations between individuals and between the proximal and distal sections of the small intestine. As an example, the *UGT1A6* gene was expressed in fewer jejunum samples, UGT2B4 mRNA was expressed more often in the ileum, and UGT2B7 transcripts were expressed more often in the jejunum.

To confirm the data observed at the gene transcript level, immunodetection of the polymorphically regulated UGT1A1 and UGT2B7 gene products was analyzed with monospecific antisera. This analysis confirmed the presence of UGT1A1 protein in three of the five samples and of UGT2B7 protein in two of the five samples (Fig. 2*B*) as detected by DRT-PCR at the mRNA level (Table II). To assess the biological effect of the identified polymorphic regulation in small intestine catalytic UGT activity assays using HDCA and 4-MU and endoplasmic reticulum protein prepared from the mucosa of small intestine and from liver tissue were performed. HDCA glucuronidation has been identified for UGT2B4, UGT2B7 (30, 31), and UGT1A3 (32), whereas 4-MU glucuronidation can be catalyzed by most UGT1A proteins (5). As predicted from the polymorphic expression of UGT2B4 and UGT2B7 transcripts in the duodenum and the jejunum, HDCA glucuronidation varied 7-fold between individuals, whereas 4-MU glucuronidation in the same samples varied little but clearly more than the absence of interindividual variation seen in liver tissue. This finding is explained by a greater redundancy of UGT proteins active with the substrate 4-MU than with the substrate HDCA. Importantly, both 4-MU and HDCA glucuronidation showed no significant variation between individual hepatic samples, a finding that reflects the absence of polymorphic UGT transcript regulation in human liver. The biological effect is best demonstrated by the analysis of the sample shown in Fig. 2*A* (*Duodenum*, *lane 4*). In this individual, neither UGT2B7 nor UGT2B4 are expressed, whereas UGT1A3 transcripts are merely present at low levels (Fig. 1, *top panel*). As a result of the absence or reduced levels of the UGTs with specificity for

HDCA, this duodenum sample was found to have dramatically reduced HDCA glucuronidation activity. In combination, the mRNA, Western blot and catalytic activity data provide evidence for the finding that the polymorphic regulation of *UGT* genes in the small intestine represents a molecular biological basis of interinidividual variations of mucosal glucuronidation activity.

Although UGT activity located in the mucosa of the small intestine is characterized by interinidividual variation caused by polymorphic gene regulation, control of hepatic glucuronidation remains constant. The biological significance of this finding may be reflected in the rate of metabolism in these tissues. As a consequence the therapeutic efficacy or toxicity of pharmaceutical compounds could be influenced directly by extrahepatic glucuronidation during or prior to resorption from the substantial surface of the small intestine in humans. Analysis of gene expression provides evidence for the strictly individual regulation of *UGT1A* genes, which share common exons 2–5 in the *UGT1A* gene complex, and of the *UGT2B* genes in humans. Polymorphic regulation of human UGT2B transcripts represents the second example of polymorphic expression of the human *UGTs* identified to date. In human gastric epithelium, the polymorphic regulation of UGT1A3, UGT1A4, and UGT1A6 transcripts in contrast to a constitutive expression of UGT1A7 and UGT1A10 mRNA was recently reported (14). The polymorphic expression of *UGT* genes in the gastrointestinal tract indicates that these enzymes may be regulated by a general biochemical mechanism contributing to interindividual differences in drug and xenobiotic metabolism (14). Importantly, this finding differs from the principle of bimodally distributed genetic polymorphisms reported for other drug metabolizing enzymes (37, 38).

In a recent study, the expression of UGT2B7 but not of UGT1A6 and UGT2B4 were reported in intestinal tissue by RT-PCR (26). In our analysis, UGT2B4 transcripts were detectable in 8 out of the 18 small intestinal tissue samples. When the analysis is subdivided into the different segments of the small intestine, the UGT2B4 gene was expressed in one out of the five duodenal samples. Similarly UGT1A6 transcripts were identified in 11 out of the 18 tissue samples, but in jejunum UGT1A6 mRNA was only present in one out of the five samples. Both genes were expressed more frequently at other levels of the small intestine. The data presented in this manuscript provide evidence for the expression of the *UGT1A6* and *UGT2B4* genes in human small intestine. In light of the identified polymorphic regulation of both genes, sample number as well as the biopsy position in the small intestine are likely to influence the detection of individual UGT transcripts and represent a likely explanation for the contrasting findings.

The detection of UGT1A8 mRNA in jejunum, ileum, and colon was recently reported (11). In experiments presented here, UGT1A8 transcripts were not detectable in any of the 17 intestinal specimens. In samples removed near the ileo-cecal valve, we were able to detect UGT1A8 transcripts in the cecal portions of the mucosa but not in the terminal ileum (data not shown). Our data suggest that the UGT1A8 transcripts are expressed in esophagus (3) and colon (2) but are not expressed in small intestine. However, genetic or evolutionary differences of patient cohorts of different geographic origin may account for differences in UGT1A8 gene expression. Specimen sampling in the area of the ileocecal valve may additionally influence the detection of UGT1A8 mRNA.

Although the human UGT proteins exhibit a considerable overlap of substrate specificity, the regulation of individual UGT genes in a tissue allows for a prediction of overall catalytic UGT activity toward different substrates. In additional experiments, 18 specific UGT activities in small intestine were determined to demonstrate that extrahepatic glucuronidation in small intestine can function to complement hepatic glucuronidation, which would represent an important consequence in light of the discovered polymorphic regulation of *UGT* genes. Specific activities were predicted based on the gene expression, because UGT1A3 and UGT1A10 display catalytic activity toward steroid hormones (2, 8); UGT1A4 catalyzes the glucuronidation of tertiary amine substrates such as antidepressants (39), and UGT1A10 exhibits UGT activity with putative tobacco carcinogens (3). The hepatic UGT activity profile favored steroids such as 4-hydroxy-estrone and phenolics such as 1-naphthol, 4-methylumbelliferone, and 4-nitrophenol. Interestingly, specific activities in the small intestine toward commonly used drugs such as imipramine and amitriptyline, as well as steroids such as estrone and putative tobacco carcinogen metabolites such as 7-hydroxybenz(α)pyrene and *N*-hydroxy-PhIP exceeded the UGT activities measured in liver. Specific UGT activities followed a gradient which peaked in the jejunum (Fig. 3) and demonstrate that the specific activity of steroid hormone and putative tobacco carcinogen metabolite glucuronidation is highest in the jejunum, where it exceeds the specific UGT activities of the liver. In comparison with other extrahepatic tissues such as the esophagus, the stomach, and the colon, jejunal UGT activity is identified to represent some of the highest specific glucuronidation activities in the gastrointestinal tract (3, 14, 27). Polymorphic gene regulation in this tissue may therefore, more than in other epithelia, have a significant impact on human xenobiotic metabolism. Intestinal glucuronidation is capable of determining the extrahepatic metabolism of pharmacologically active drugs and may also serve as metabolic barrier for mutagen-associated genotoxicity and cytotoxicity, which is implicated by the presented characterization of specific UGT activites toward tobacco carcinogen metabolites in small intestine. The cellular and subcellular localization of UGT protein exclusively in intestinal villi and crypts forming this putative barrier was demonstrated by indirect immunofluorescence analysis and is in aggrement with the data obtained at the transcript and functional levels (Fig. 4).

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REFERENCES

1. Dutton, G. J. (1980) *Glucuronidation of Drugs and Other Compounds*, CRC Press, Boca Raton, FL
2. Strassburg, C. P., Manns, M. P., and Tukey, R. H. (1998) *J. Biol. Chem.* **273,** 8719–8726
3. Strassburg, C. P., Strassburg, A., Nguyen, N., Li, Q., Manns, M. P., and Tukey, R. H. (1999) *Biochem. J.* **338,** 489–498
4. Strassburg, C. P., Oldhafer, K., Manns, M. P., and Tukey, R. H. (1997) *Mol. Pharmacol.* **52,** 212–220
5. Tukey, R. H., and Strassburg, C. P. (2000) *Annu. Rev. Pharmacol. Toxicol.* **40,** 581–616
6. Burchell, B., Brierley, C. H., Monaghan, G., and Clarke, D. J. (1998) *Adv. Pharmacol.* **42,** 335–338
7. Ritter, J. K., Crawford, J. M., and Owens, I. S. (1991) *J. Biol. Chem.* **266,** 1043–1047
8. Mojarrabi, B., Butler, R., and Mackenzie, P. I. (1996) *Biochem. Biophys. Res. Commun.* **225,** 785–790
9. Harding, D., Fournel-Gigleux, S., Jackson, M. R., and Burchell, B. (1988) *Proc. Natl. Acad. Sci. U. S. A.* **85,** 8381–8385
10. Wooster, R., Sutherland, L., Ebner, T., Clarke, D., Da Cruz e Silva, O., and Burchell, B. (1991) *Biochem. J.* **278,** 465–469
11. Cheng, Z., Radominska-Pandya, A., and Tephly, T. R. (1998) *Arch. Biochem. Biophys.* **356,** 301–305
12. Mojarrabi, B., and Mackenzie, P. I. (1998) *Biochem. Biophys. Res. Commun.* **247,** 704–709
13. Mojarrabi, B., and Mackenzie, P. I. (1997) *Biochem. Biophys. Res. Commun.* **238,** 775–778
14. Strassburg, C. P., Nguyen, N., Manns, M. P., and Tukey, R. H. (1998) *Mol. Pharmacol.* **54,** 647–654
15. Monaghan, G., Clarke, D. J., Povey, S., See, C. G., Boxer, M., and Burchell, B. (1994) *Genomics* **23,** 496–499

16. Beaulieu, M., Levesque, E., Tchernof, A., Beatty, B. G., Belanger, A., and Hum, D. W. (1997) *DNA Cell Biol.* **16,** 1143–1154
17. Jedlitschky, G., Cassidy, A. J., Sales, M., Pratt, N., and Burchell, B. (1999) *Biochem. J.* **340,** 837–843
18. Jackson, M. R., McCarthy, L. R., Harding, D., Wilson, S., Coughtrie, M. W., and Burchell, B. (1987) *Biochem. J.* **242,** 581–588
19. Ritter, J. K., Sheen, Y. Y., and Owens, I. S. (1990) *J. Biol. Chem.* **265,** 7900–7906
20. Jin, C. J., Miners, J. O., Lillywhite, K. J., and Mackenzie, P. I. (1993) *Biochem. Biophys. Res. Commun.* **194,** 496–503
21. Beaulieu, M., Levesque, E., Hum, D. W., and Belanger, A. (1998) *Biochem. Biophys. Res. Commun.* **248,** 44–50
22. Chen, F., Ritter, J. K., Wang, M. G., McBride, O. W., Lubet, R. A., and Owens, I. S. (1993) *Biochemistry* **32,** 10648–10657
23. Beaulieu, M., Levesque, E., Hum, D. W., and Belanger, A. (1996) *J. Biol. Chem.* **271,** 22855–22862
24. Belanger, A., Hum, D. W., Beaulieu, M., Levesque, E., Guillemette, C., Tchernof, A., Belanger, G., Turgeon, D., and Dubois, S. (1998) *J. Steroid Biochem. Mol. Biol.* **65,** 301–310
25. King, C. D., Rios, G. R., Assouline, J. A., and Tephly, T. R. (1999) *Arch. Biochem. Biophys.* **365,** 156–162
26. Radominska-Pandya, A., Little, J. M., Pandya, J. T., Tephly, T. R., King, C. D., Barone, G. W., and Raufman, J. P. (1998) *Biochim. Biophys. Acta* **1394,** 199–208
27. Strassburg, C. P., Nguyen, N., Manns, M. P., and Tukey, R. H. (1999) *Gastroenterology* **116,** 149–160
28. Strassburg, C. P., Kasai, Y., Seng, B. A., Miniou, P., Zaloudik, J., Herlyn, D., Koprowski, H., and Linnenbach, A. J. (1992) *Cancer Res.* **52,** 815–821
29. Strassburg, C. P., Alex, B., Zindy, F., Gerken, G., Luttig, B., Meyer zum Buschenfelde, K. H., Brechot, C., and Manns, M. P. (1996) *J. Hepatol.* **25,** 859–866
30. Ritter, J. K., Chen, F., Sheen, Y. Y., Lubet, R. A., and Owens, I. S. (1992) *Biochemistry* **31,** 3409–3414
31. Fournel-Gigleux, S., Jackson, M. R., Wooster, R., and Burchell, B. (1989) *FEBS Lett.* **243,** 119–122
32. Gall, W. E., Zawada, G., Mojarrabi, B., Tephly, T. R., Green, M. D., Coffman, B. L., Mackenzie, P. I., and Radominska-Pandya, A. (1999) *J. Steroid Biochem. Mol. Biol.* **70,** 101–108
33. Hartmann, F., and Bissell, D. M. (1982) *J. Clin. Invest.* **70,** 23–29
34. Matern, S., Matern, H., Farthmann, E. H., and Gerok, W. (1984) *J. Clin. Invest.* **74,** 402–410
35. Parquet, M., Pessah, M., Sacquet, E., Salvat, C., Raizman, A., and Infante, R. (1985) *FEBS Lett.* **189,** 183–187
36. Strassburg, C. P., Manns, M. P., and Tukey, R. H. (1997) *Cancer Res.* **57,** 2979–2985
37. Lampe, J. W., Bigler, J., Horner, N. K., and Potter, J. D. (1999) *Pharmacogenetics* **9,** 341–349
38. Meyer, U. A., and Zanger, U. M. (1997) *Annu. Rev. Pharmacol. Toxicol.* **37,** 269–296
39. Green, M. D., and Tephly, T. R. (1998) *Drug Metab. Dispos.* **26,** 860–867