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HUMAN UDP-GLUCURONOSYLTRANSFERASES: Metabolism, Expression, and Disease

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■ **Abstract** In vertebrates, the glucuronidation of small lipophilic agents is catalyzed by the endoplasmic reticulum UDP-glucuronosyltransferases (UGTs). This metabolic pathway leads to the formation of water-soluble metabolites originating from normal dietary processes, cellular catabolism, or exposure to drugs and xenobiotics. This classic detoxification process, which led to the discovery nearly 50 years ago of the cosubstrate UDP-glucuronic acid (19), is now known to be carried out by 15 human UGTs. Characterization of the individual gene products using cDNA expression experiments has led to the identification of over 350 individual compounds that serve as substrates for this superfamily of proteins. This data, coupled with the introduction of sophisticated RNA detection techniques designed to elucidate patterns of gene expression of the UGT superfamily in human liver and extrahepatic tissues of the gastrointestinal tract, has aided in understanding the contribution of glucuronidation toward epithelial first-pass metabolism. In addition, characterization of the *UGT1A* locus and genetic studies directed at understanding the role of bilirubin glucuronidation and the biochemical basis of the clinical symptoms found in unconjugated hyperbilirubinemia have uncovered the structural gene polymorphisms associated with Crigler-Najjar's and Gilbert's syndrome. The role of the UGTs in metabolism and different disease states in humans is the topic of this review.

INTRODUCTION

The catalytic reaction that utilizes UDP-glucuronic acid (UDPGlcUA) as a cosubstrate for the formation of lipophilic glucuronides from non–membrane-associated substrates, such as steroids, bile acids, bilirubin, hormones, dietary constituents, and thousands of xenobiotics that include drugs, environmental toxicants, and carcinogens, has evolved as a highly specialized function in higher organisms.

The UDP-glucuronosyltransferases (UGTs) (EC 2.4.1.17) utilize UDPGlcUA as a sugar acceptor and transfer glucuronic acid to available substrates, a process that forms β-glucuronidase–sensitive β-D-glucopyranosiduronic acids (glucuronides). These β-D-glucuronides can be formed through hydroxyl (alcoholic, phenolic), carboxyl, sulfuryl, carbonyl, and amino (primary, secondary, or tertiary) linkages. This type of structural diversity in substrate specificity allows for the acceptance of thousands of agents to be targeted for glucuronidation. Although there are examples of glucuronides possessing biological activity, such as the analgesic action of morphine 6-glucuronide, this pathway is primarily catabolic and is therefore generally regarded as a ''detoxification'' reaction (1). Thus, glucuronidation serves as an integral step in transforming lipophilic substrates into hydrophilic glucuronides, a process that increases their ability to partition into the aqueous intra- and extracellular compartments of the body, facilitating the transport to excretory organs and subsequent elimination through the bile and urine.

The past decade has seen significant advances in the applications of recombinant methodologies toward the investigation of the genetic multiplicity of the UGT gene families (2). For example, investigations into the specificity of bilirubin glucuronidation have identified the genes (3) involved in the hereditary metabolic errors associated with Crigler-Najjar syndrome type I (4), which is transmitted as an autosomal recessive trait in humans and characterized by an inability to glucuronidate bilirubin (reviewed in 5, 6). The accumulation of unconjugated bilirubin (hyperbilirubinemia) in Crigler-Najjar syndrome type I leads to nonhemolytic icterus within the first few days of life and is followed eventually by the accumulation of bilirubin in nerve terminals and glial cells, a clinical condition termed kerinicterus. The homozygous inheritance of nonfunctional bilirubin UGT and the onset of Crigler-Najjar syndrome type I results in early childhood death. The discovery of the genetic defect and the development of chimeraplasty, a form of gene transplantation, is being evaluated as potential therapy for the deadly disease (6a).

The realization that most vertebrates are capable of generating UGT-directed glucuronides from a virtual plethora of structurally divergent substances fostered the belief that this unique catalytic pathway involved a family of UGTs, with the catalytic potential for a wide range of substrate specificities. From early experiments in protein purification (7–11) that led to the characterization of several animal UGTs, and which were followed by the first cloning of cDNAs encoding rodent UGTs (12, 13), over 50 vertebrate UGT cDNAs (14) have been characterized and deposited in the National Institutes of Health Genbank and European Molecular Biology Laboratory. To further define the substrate specificities of proteins encoded by the cDNAs, stable and transient expression experiments in a variety of cell lines has allowed investigation of the potential substrate specificities of individual UGTs (15). Although there are potential drawbacks in attempting to compare the significance of results generated in different laboratories,

because they are defining their own biological tools for protein expression, a good understanding of the role of the UGTs in endogenous and xenobiotic metabolism is starting to occur (16).

For the most part, investigations that have elaborated on human glucuronidation have focused primarily on hepatic tissue, because of the greater availability of this tissue source and the well-understood role of the liver in drug metabolism. However, methodologies sensitive enough to detect RNA transcripts, e.g. polymerase chain reaction (PCR) technology and information garnered from unique DNA sequences obtained from cDNA or gene sequence databases make it possible to identify precisely the potential of different human tissues to carry out glucuronidation (17, 18). In combining the ease of obtaining substrate specificity information for any single UGT through in vivo expression experiments, conclusions about the importance of human tissue in specific glucuronidation patterns can be obtained. These data are important in understanding the role of glucuronidation in metabolism and human disease, particularly when defining the role of glucuronidation in different tissues.

Since the discovery and characterization in 1953 of UDPGlcUA as the donor nucleotide sugar acting as cosubstrate in the generation of o-aminophenol and (−)menthol glucuronides (19), and the localization of the catalytic activity to the microsomal fraction of liver homogenates (20), many outstanding reviews and monographs have been written addressing the regulation and function of the UGTs (e.g. 1, 5, 21–24). Although glucuronidation has been identified in various vertebrates (1), this review addresses the recent advances that have helped to elucidate the regulatory and functional aspects of glucuronidation in man. We have elected to focus primarily on the contributions that have defined the genetics and the multiplicity of the *UGT* gene families, and how this information has helped in advancing a greater understanding of the role these enzymes play in metabolism and disease.

NOMENCLATURE

In this article, we define the UDP-glucuronosyltransferases as UGTs, in contrast to the classification as UDP-glycosyltranferases, as previously recommended (14). This decision to modify the recommended nomenclature is based upon several considerations.

Although the characterization of over 50 vertebrate UGTs has been described, those localized in the endoplasmic reticulum (ER) have been grouped into a gene family that consists of (*a*) several other known vertebrate UTP-sugar acceptor proteins and (*b*) a diverse number of invertebrate proteins that have not been characterized. This grouping relied both on limited homology (as low as 10% identity between some sequences) and on a predicted UDP-glycosyltransferase signature sequence that is conserved among several UTP-sugar glycosyltransfer-

Identity	Accession Number		Identity	Accession Number
C elegans	AAC69026		UGT1A8h	AAB84259
Drosophila	AAD22028		UGT1A8r	Q64634
Felis1	BAA24692		UGT1A9h	AAB19791
Felis2	AAB96667		UGT1B1p	CAA52214
Plaice	CAB51369		UGT2A1b	No Number
UGT18A1	CAA99957		UGT2A1h	CAB41974
UGT18B1	CAA99955		UGT2A1r	P36510
UGT18C1	CAA99956		UGT2B10h	P36537
UGT18D1	CAA94871		UGT2B11h	CAA44961
UGT18D2	CAA94870		UGT2B12r	P36511
UGT18E1	CAA99954		UGT2B131	P36512
Ugt1a1	Q63886		UGT2B141	P36513
UGT1A10h	AAB81537		UGT2B15h	AAA83406
UGT1A1h	P22309		UGT2B161	AAB71494
UGT1A1r	Q64550		UGT2B17h	NP 001068
Ugt1a2	P70691		UGT2B19m	AAD24435
UGT1A2r	P20720		UGT2B1r	P09875
UGT1A3h	P35503		UGT2B2r	P08541
UGT1A3r	Q64637		UGT2B3r	P08542
UGT1A41	Q28612		UGT2B4h	CAA68415
UGT1A4h	P22310		Ugt2b5	P17717
UGT1A5h	P35504		UGT2B6r	P19488
UGT1A5r	Q64638		UGT2B7h	AAA36793
Ugt1a6	Q64435		UGT2B8r	AAA86833
UGT1A61	Q28611		UGT2B9m	AAB50249
UGT1A6b	BAA23359		UGT2C11	P36514
UGT1A6h	AAA61251		UGT31B1v	AAB03658
UGT1A6o	BAA77457		UGT74ma	A54739
UGT1A6r	P08430		UGT77A1ma	P16166
Ugt1a7	Q62452		Ugt8	Q64676
UGT1A7h	AAB81536		UGT8h	Q16880
UGT1A7r	Q64633		UGT8r	Q09426

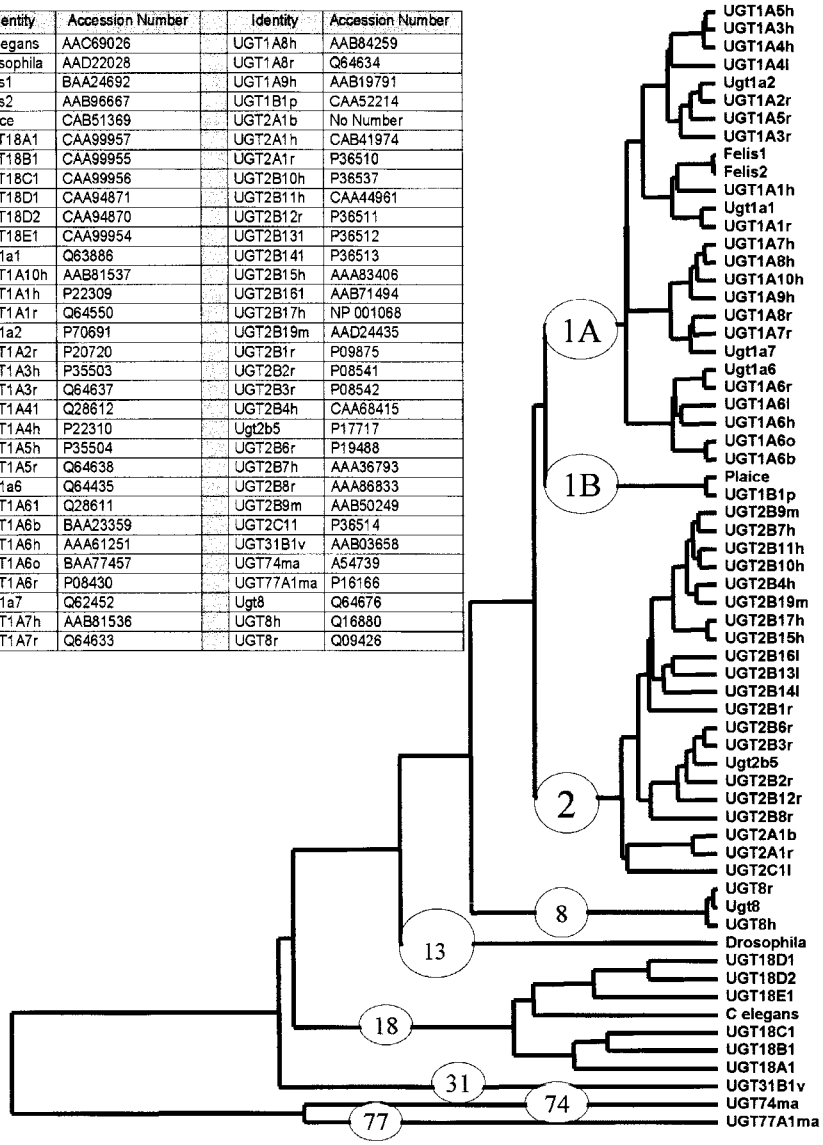

Figure 1 See next page for legend.

ases. Using the predicted prosite sequence (http://www.expasy.ch/cgi-bin/get-prosite-entry?PS00375) and computer algorithms to search protein databases, the mammalian UDP-galactose-ceramide galactosytransferases (EC 2.4.1.45), the plant flavonol O-(3)-glucosyltransferase (EC 2.4.1.91), the baculovirus ecdysteroid UDP-glucosyltransferase (EC 2.4.1.-), the prokaryotic zeaxanthin glucosyl transferase (EC 2.4.1.-), and the streptomyces macrolide glycosyltransferases (EC 2.4.1.-) have been identified. In addition, a host of other putative gene families, most without any known function and identified strictly through limited sequence similarities, have also been included. In combination with the known *UGT1* and *UGT2* gene families, an additional 31 gene families are included. A representative phylogenetic tree generated using UGT1 and UGT2 sequences and representatives of the other UDP-glycosyltransferases is shown in Figure 1. Based upon the homologies and speculated branch distances, there is most likely an evolutionary link between the vertebrate UGTs and the other vertebrate glycosyltransferase gene family (UGT8), because there exists approximately 30% identity between these gene families. However, there exists little structural or functional similarity between the ER-specific UGTs and UGT8. In addition, there is very limited identity with any of the invertebrate sequences (e.g. *Caenorhabditis elegans*, baculovirus, and plant sequences). It should also be noted that the signature sequence is not found in glycosyltransferases with the function of catalyzing the addition of the glycosyl group from UTP-sugar toward the synthesis of proteoglycans. Most important, as we think about the evolution of these genes and the specificity of the proteins to utilize UDPGlcUA as cosubstrate, there is also no similarity in amino acid or prosite sequence with the Golgi-specific UGT I, a type two glycosyltransferase that transfers GlcUA from UDPGlcUA to glycosaminoglycans (25–27).

As outlined by Mackenzie et al (14), it is being recommended that UGT be

← **Figure 1** Phylogenic tree showing divergence patterns of the 50 vertebrate UDP-glucuronosyltransferases (UGTs) (families 1 and 2) with other presumed glycosyltransferases. The table in the middle outlines the accession numbers used to obtain the protein sequences from GenBank and European Molecular Biology Laboratory. The Phylip program that we utilized was downloaded from http://evolution.genetics.washington.edu/phylip/getme.html, and the tree view program was obtained from http://taxonomy.zoology.gla.ac.uk/rod/treeview.html. The sequences were aligned using ClustalX (ftp://ftp.ebi.ac.uk/pub/software/dos/clustalw/clustalx.) and the dendrogram was generated with the average linkage clustering method (UP6MA), which assumes an evolutionary clock. The species represented can be identified as follows: Ugt, mouse; h, human: 1, lagomorph; r, rat; o, sheep; b, bovine; felis, cat; p, flounder; m, monkey; d, drosophila; v, veres; ma, maize; UGT18, *Caenorhabditis elegans.* The sequences for Felis1 -2, Plaice, *Drosophila*, and *C. elegans* have not been assigned to a UGT family but were identified in the databases. With the exception of UGT1 and UGT2, not all families are represented as previously outlined (14).

used as the synonym to reference UDP-glycosyltransferases. This poses potential problems in accurately describing the well-characterized UGTs. (*a*) The utilization of amino acid similarities to predict evolutionary relatedness to all UDP-glycosyltransferases will result in the exclusion of other important UTP-sugar–accepting proteins (and gene families), as indicated above. This has been noted by Mackenzie et al (14). Thus, the use of a defined term such as UGT to link all UDP-glycosyltransferases is not representative of all the gene families of proteins that utilize UTP-sugars as cosubstrates. (*b*) There is no experimental evidence that any of the nonvertebrate-related UDP-glycosyltransferases or those proteins that are distantly related in evolution to the UGTs utilize UDPGlcUA as cosubstrate. The ability to incorporate UDPGlcUA as cosubstrate in a biochemical reaction that catalyzes the formation of small-molecule glucuronides has evolved as a highly selective process in higher organisms, as is evident from the identification and function of these enzymes only in vertebrates.

Although the remnants of some conserved sequences are found in different phyla and species (i.e. families UGT18, UGT31, and UGT77 in Figure 1), evolution has resulted in the formation of the UGT gene family, whose function is unique to other distantly related glycosyltransferases. For these reasons, we propose that the synonym UGT represent UDP-glucuronosyltransferase. The criteria for classification as we would recommend might rely on the following. (*a*) All protein sequences grouped into the UGT supergene family would share at a minimum 45% similarity in sequence identity, a parameter similar to those of the cytochrome P450s (28). In the absence of function, preliminary assignment of nomenclature to a family could be established based upon relative similarity of amino acid sequence to the other UGTs. (*b*) All of the protein sequences within a UGT family would maintain approximately 55% similarity. Based upon this designation, the Plaice (Flounder) sequences (UGT1B), which share less than 50% identity to the other UGT1A sequences, would be assigned to a new UGT family, such as UGT3. (*c*) The sequences within a family could be arbitrarily grouped into subfamilies based upon predictions of phylogenetic divergence. As it now stands, the sequences within the UGT1A and UGT2 subfamilies share 60% similarity in amino acid identity. (*d*) The designated UGTs must utilize UDPGlcUA as cosubstrate. This is appropriate because evolution has naturally selected a large family of proteins to utilize UDPGlcUA for the conjugation of small lipophilic substances. This distinguishing property would separate the family of UGTs from other UTP-sugar–accepting glycosyltransferases. Under these guidelines and what is available in the present protein and DNA sequence databases, only the vertebrate sequences would constitute the present UGT supergene family. Based upon the lack of functional relatedness and the significant divergence in sequence homology to the ER-based UGTs, it may be more appropriate to assign the functional glycosyltransferases (i.e. UDP-galactose-ceramide galactosyltransferase) and the predicted glycosyltransferases (i.e. *C. elegans*) into different families.

HUMAN UDP-GLUCURONOSYLTRANSFERASES

Currently, 15 human UGT cDNAs have been identified (Figure 2), eight UGT1A proteins encoded by the *UGT1A* locus and seven proteins encoded by *UGT2* genes. Structural information from the *UGT2B17* (29) and *UGT2B4* (30) genes demonstrates that these genes consist of six exons spanning approximately 30 kb. Conservation in exon/intron organization is maintained in rodents, as several *UGT2* genes have been characterized and shown to contain the same exon/intron branch points (31, 32). Transcripts encoding UGT2A1 (33), UGT2B4 (34–36), UGT2B7 (36–39), UGT2B10 (35), UGT2B11 (40), UGT2B15 (41, 42), and UGT2B17 (29, 43) have been characterized, and these genes have been mapped to human chromosome 4-q13 (44, 45) and 4q28 (41). Three of the *UGT2* genes are tightly clustered within a 195-kb region and maintain a provisional ordering as *UGT2B7* (previously *UGT2B9*)-*UGT2B4*-*UG2B15* (44). The clustering of gene families on the same chromosome is evidence for gene duplication events. Note in Figure 1 that the UGT2B sequences from primates do not cluster with the rodent or lagomorph forms, which suggests that following speciation, the *UGT2B* genes have evolved from a common ancestrial lineage in each species by independent gene duplication and selective pressure events. This would account for the divergence in function of these proteins in the different species. There is little evidence for truly orthologous UGT2B proteins between the different species. In humans, there is considerable tissue specific regulation with the *UGT2* genes, as evident from the identification of *UGT2A* gene products in olfactory tissue (33), and the differential regulatory patterns of the *UGT2B* genes throughout the gastrointestinal tract (discussed below). Little is known about the mechanisms that contribute to *UGT2* tissue-specific regulation.

The *UGT1A* locus in humans (2) is located on chromosome 2-q37 (46–48) and encodes UGT1A1 (2, 49), UGT1A3 (50), UGT1A4 (2), UGT1A5 (to date, no active transcript), UGT1A6 (51), UGT1A7 (18), UGT1A8 (52–53), UGT1A9 (55), and UGT1A10 (18, 54, 56). Although only eight active gene transcripts have been identified, the *UGT1A* locus evolved with the potential to encode 12 separate UGT RNA transcripts. A representation of the organization of the *UGT1A* locus is shown in Figure 3 (see color insert). The *UGT1A* locus has been estimated to span 160 kb, with four 3′ exons flanked 5′-ward by 12 cassette exons, each encoding one of the UGT1A first exon RNAs (5). The 5′ flanking region of each first-exon cassette contains appropriate promoter elements that would attract PolII and the transcriptional initiation factors. At the 3′ end of each first-exon cassette can be found consensus 5′ splice sequences, recognized by the RNA spliceosome. Transcription of each individual first exon leads to a strategy of exon sharing, combining the first exon sequences with common exons 2–5. Thus, all of the UGT1A proteins are identical in the carboxyl 245 amino acids, which is encoded by exons 2–5. This transcriptional mechanism is often mistakenly referred to as alternative splicing. The process of alternative RNA splicing can

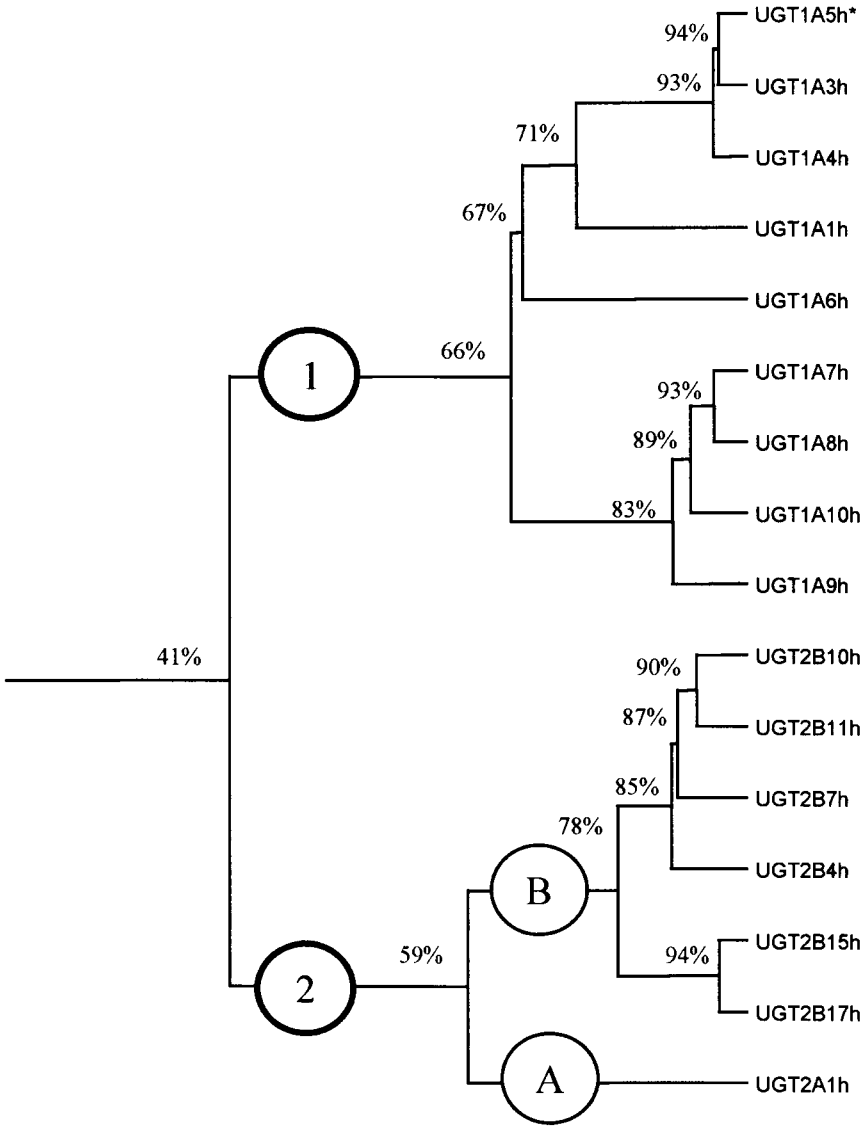

Figure 2 Phylogenetic tree showing the relationships between the human UDP-glucuronosyltransferases (UGTs). The median percentage identity of amino acid sequences between UGTs split by a mode is shown. (*Asterisk*) Indicates the gene product has not been identified.

only occur if mature or cryptic 5′ and 3′ consensus splice sites are transcribed as RNA and recognized in a regulated fashion to serve as substrates for splicing reactions catalyzed in spliceosomes (57). This most likely does not occur with the UGT1A RNA transcripts. Based upon the structure of the *UGT1A* locus, each of the UGT1 transcripts are produced independently following transcriptional initiation. Note, 3′ consensus splice sites do not exist until the presence of exon 2. Thus, during transcriptional elongation, the spliceosome must wait until the sequence encoding exon 2 is transcribed, at which time the process of RNA splicing begins. For example, if transcription commences at the *UGT1A7* gene, the sequences encoding *UGT1A6-UGT1A1* is simply considered intronic. The RNA encoding UGT1A6–1A1 is processed by normal RNA splicing mechanisms. Thus, the regulatory sequences flanking each of the exon 1 regions dictate the unique aspects of expression of the *UGT1A* genes.

The human UGTs range from 529 amino acids to 534 amino acids in length, with several highly conserved domains that are important for membrane targeting and activity (reviewed in 58). Although the UGT1 proteins are encoded by five exons and UGT2 proteins are encoded by six exons, the UGTs share a high degree of similarity in the carboxyl end, which spans the last 250 amino amino acids. The amino terminal 280 amino acids are divergent. With the exception of UGT1A10, all of the UGTs contain an N-terminal signal peptide (18), which is removed following insertion of the proteins into the ER (59). Although anchorage of the UGTs to the ER is predicted to be facilitated by a membrane retention signal at the carboxyl end of the proteins (60), Meech & Mackenzie recently demonstrated that other regions of the UGTs participate in securing the protein to the membrane (61). Studies carried out using chimeric constructs of different UGT cDNAs followed by expression studies indicate that the amino terminal may be important in substrate specificity of the different UGTs (62), whereas other studies have clearly shown that the carboxyl region of the UGTs are also critical for activity (36, 63, 64). As a result of the high degree of similarity in the carboxyl portion of the UGTs, it has been proposed that this region controls the conformational properties that underlie the binding of the cosubstrate UDPGlcUA, but this remains to be conclusively demonstrated. Under this type of model, it is presumed that a substrate or aglycone binding pocket and a separate UDPGlcUA domain interact to coordinate transfer of glucuronic acid to the facilitating substrate. Such a model would indicate that the UDPGlcUA binding domain and possibly the secondary structure responsible for forming this region is closely related in all of the UGTs.

Functional Diversity of the Human UDP-Glucuronosyltransferases

In the absence of modern molecular biology, it had been known that there existed a variety of chemically divergent agents that were conjugated in humans. These

range from the many hundreds of endogenous substrates and xenobiotics to many clinically relevant drugs (23, 24, 65). Glucuronides contain glucopyranuronosyl linked to -O.R, -S.R, -N.R′R″, or -C.R groups (for review see 1). A significant advantage of the utilization of recombinant DNA methodologies is the ability to express the different UGT cDNAs in cell lines that express minimal levels of the respective proteins. All the human UGT cDNAs shown in Figure 2 have been expressed to evaluate their potential to metabolize endogenous and xenobiotic substances. Examination of classes of compounds known to be glucuronidated by expressed UGTs are shown in Table 1, with several examples of their structure presented in Figure 4. Methods of choice include standard transient transfections of recombinant DNA where the cells are harvested approximately 48 h after transfection, or following the stable introduction of the DNA into the genome following selection of the cells with an appropriate antibiotic (15, 58). Other laboratories have utilized the expression of UGTs in insect *Spodoptera frugiperda* cells following infection with UGT-recombinant AcMNPV viruses (53, 66–68).

As discussed by Remmel & Burchell (15), estimations of the catalytic activity attributed to any single enzyme is dependent on a number of critical factors, such as the method of choice used for transfection (i.e. transient or stable), the type of cell line, or the choice of membrane preparation (i.e. microsomes or whole cell extracts). Even when different laboratories use the same cell line for stable expression experiments, the concentration of expressed protein can vary considerably.

TABLE 1 Xenobiotic substrates glucuronidated by expressed human UDP-glucuronosyltransferases (UGTs)

Human glucuronides	Substrates[a]
Linkage through -O-	
Aryl hydroxy (ether)	Simple and complex phenols, anthraquinones and flavones, opioids and steroids, hydroxylated coumarins
Aryl or alkyl enolic	Coumarins, steroid-dione structures
Alkyl hydroxy	Primary, secondary, tertiary alcohols
Acyl hydroxy (carboxylic esters)	Bilirubin, carboxylic acids
Linkage through -S-	
Aryl and Alkyl thiols	No examples reported
Linkage through -C-	No examples reported
Linkage through -O-	
Sulfonamides	No examples reported
Nonquaternary	Primary and secondary amines, arylamine N-OH, tetrazoles
Quarternary	Cyclic tertiary, alicyclic tertiary, imidazoles, pyridines, triazoles

[a]Examples of chemical classes used by different laboratories to demonstrate specificity toward expressed human UGTs.

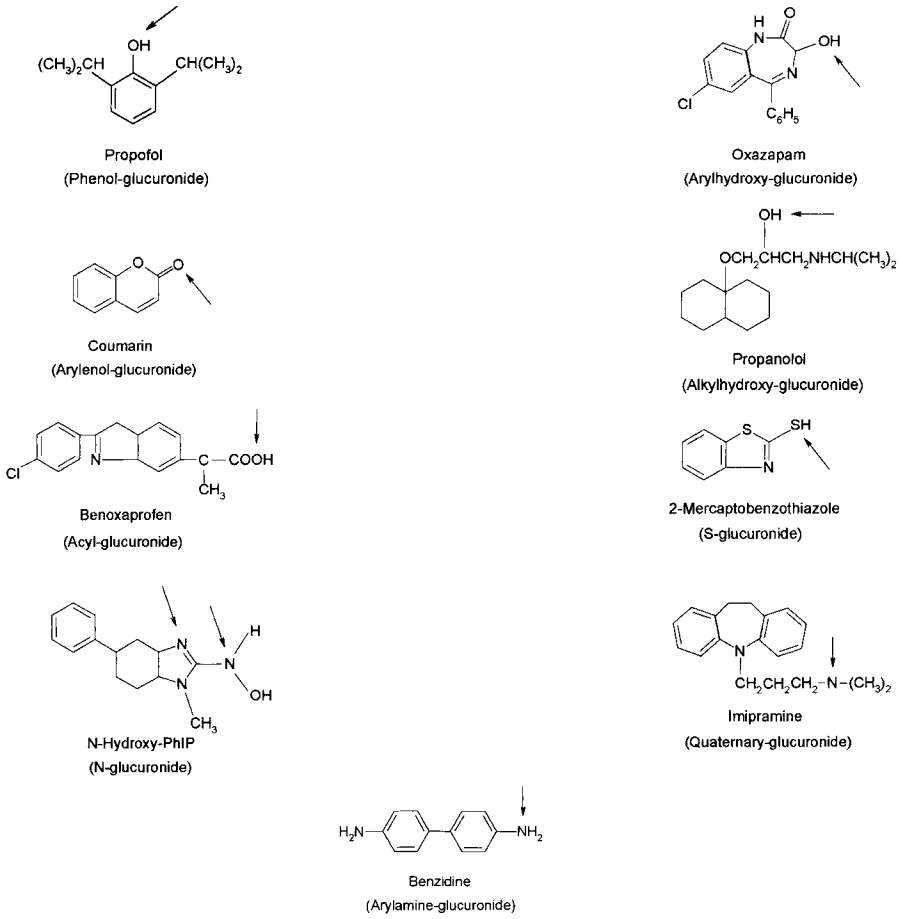

Figure 4 Chemical structures of compounds that form glucuronides. (*Arrows*) The position on each molecule where glucuronidation occurs.

Coupled with the fact that expression experiments are performed with variations in assay conditions, concentrations of substrates, and concentrations of the cosubstrate UDPGlcUA, estimations of substrate UGT activity can vary significantly. However, a picture is emerging that defines the ability of the different enzymes to glucuronidate the many different classes of substrates. To evaluate the specificity and diversity of the UGTs, we have assembled a database of human UGT activities compiled from expression experiments. Our database, assembled in Microsoft Access, covers over 350 substrates and is available on the Web (www.AnnualReviews.org). For evaluation purposes, we have taken the highest

reported activities within the different classes of chemical substrates and condensed this information into Table 2. Based upon the predicted substrate specificities, several unique patterns of expression can be observed.

O-Linked Glucuronidation As outlined in the historic monograph on glucurondiation by Dutton (1), the different classes of O-glucuronides predominate those compounds that serve as substrates for the UGTs. The predominant substrates in this list are those that form the aryl-O-(phenolic)-glucuronides, whereas substrates susceptible to acyl-O-glucuronidation (carboxylic acids) as well as aryl- and alkyl-O-(enolic)-glucuronidation (coumarins) have been extensively investigated. Glucuronidation through O-linked moieties (acyl, phenolic, hydroxy) predominates the diversity in substrate recognition, and all of the UGTs are capable of forming O-linked glucuronides, albeit with different efficiencies and turnover rates. The small molecules that form such phenolic-hydroxy glucuronides as 4-nitrophenol and 1-napthol and the coumarin derivative 4-methylumbelliferone serve as substrates for most of the UGT proteins but are catalyzed most efficiently by the UGT1 proteins, with the exception of UGT1A4. The simple and complex phenols are also efficiently glucuronidated by the nasal mucosa-specific UGT2A1 (33), but overall they are glucuronidated at reduced rates by UGT2B4 (35), UGT2B7 (39, 69), and UGT2B15 (70). Small phenolic substrates such as p-nitrophenol and 4-methylumbelliferone are often used for analysis of total UGT activity in tissue microsomes, but when compared with the catalytic activities of the UGT1 proteins, these substrates are glucuronidated at rates that are 10- to 20-fold lower by UGT2B4 (35), UGT2B7 (39, 41), and UGT2B15 (70). There is considerable substrate-specific redundancy in phenolic glucuronidation between UGT1A1, UGT1A3, UGT1A7, UGT1A8, UGT1A9, and UGT1A10, as evident from the glucuronidation activities observed with small and bulky phenols as well as the hydroxy-glucuronide forming polynuclear hydrocarbons, anthraquinones, flavones, and coumarins. The slightly larger bulky phenols (i.e. 4-tert-butyl phenol) and those that include the naturally occurring anthraquinones and flavanoids, although excellent substrates for most of the UGT1 proteins, are not readily glucuronidated by UGT1A6 (71). Because the *UGT1A* locus is highly conserved in other mammals and the individual UGT1 proteins are differentially expressed throughout the gastrointestinal tract, we can predict that these proteins have evolved to facilitate the metabolism of digested matter, much of which exists as simple and complex phenols.

Phenanthrene Glucuronidation The glucuronidation of morphine and other structurally similar opioids are metabolized at relatively low rates by UGT1A1 (72), UGT1A8 (73), and UGT2A1 (33). However, Coffman et al (38, 69) indicate that UGT2B7 is the major isoform responsible for the glucuronidation of opioids of the morphinan and oripavine class. Morphine, a phenanthrene alkaloid, is glucuronidated at the phenolic 3-hydroxyl as well as the alcoholic 6-hydroxyl group,

TABLE 2 UDP-glucuronosyltransferases (UGT) glucuronidation activity with selected substrate classes[a]

Chemical class	1A1	1A3	1A4	1A6	1A7	1A8	1A9	1A10	2A1	2B4	2B7	2B15	2B17
Simple phenols	1900	239	30	2400	175	1346	5300	88	735	0.4	5	167	38
Complex phenols	420	299	11	13300	480	2217	1200	85	2440	0.2	3	176	7
Aliphatic alcohols	ND	0	75	ND	ND	0	270	ND	1290	0	388	41	ND
Anthraquinones/flavones	1720	1072	0	0	57	1534	2500	35	320	ND	ND	103	ND
Courmarins	800	1970	0	1100	220	4970	1500	11	898	0	4	170	0
Bilirubin	400	0	2	0	0	ND	0	ND	ND	0	0	0	0
Bile acids	0	10[b]	0	0	ND	ND	0	0	ND	1.8	20	0	0
Carboxylic acids	0	121	0	ND	0	0	170	0	68	0	1.8	0	ND
Primary amines	0.3	84	540	10600	0	42	1800	0	22	ND	2.5	0	ND
Secondary amines	0	12	240	ND	ND	15	ND	20	ND	ND	ND	0	ND
Tertiary amines	0	87	165	1	0	0	0	0	ND	0	0	0	0
Heterocyclic amines	0	49	ND	50	3	71	91	156	ND	ND	ND	ND	ND
Opioids	0	130	0	0	ND	126	0	ND	73	0	3462	0	ND
C_{18} steroids	350	313	25	0	6	711	450	48	40	0.3	980	14	0
C_{19} steroids	0	0	110	0	0	43	0	4	207	0	2	73	15
C_{21} steroids	0	ND	130	ND	ND	0	ND	ND	53	0	0	ND	8
Sapogenins	0	0	330	ND	ND	0	ND	ND	ND	ND	ND	ND	ND

[a]Represented are maximal specific activities (in picomoles per minute per milligram of protein) using substrates that can be defined for each of the different chemical classes. ND, Not determined; 0, enzyme preparations that have been tested with no detectable activity. Table generated from the following reports for expressed UGT: UGT1A1 (49, 67, 82, 84a, 86, 109, 139, 156, 157); UGT1A3 (67, 79, 84, 86, 88, 139); 1A4 (67, 81, 83, 84a, 86, 139); UGT1A6 (51, 67, 74, 84a–87, 138, 139, 158–160); UGT1A7 (53, 68, 86); UGT1A8 (52, 73, 139); UGT1A9 (55, 55, 67, 71, 84a, 86, 99, 109, 139, 156, 161); UGT1A10 (52, 53, 67, 139, 139); UGT2A1 (33); UGT2B4 (34, 35, 41, 67, 78, 80, 86, 162); UGT2B7 (36–39, 67, 69, 80, 84a, 86, 139); UGT2B11 (40); UGT2B15 (41, 67, 70, 139); UGT2B17 (29, 43).

[b]Value for hyodeoxycholic acid conducted in the authors laboratory.

at a ratio of approximately 7:1 (38). It is interesting to note that the 6-O-glucuronide is a more potent analgesic than the parent compound, whereas the 3-O-glucuronide contains no biological activity. It is significant that UGT2B7 has been found recently also in the brain, where metabolism to the 6-O-glucuronide may facilitate analgesic activity (74).

Steroid Glucuronidation Steroid glucuronidation is an important pathway for removing biologically active endogenous ligands, such as the androgens (C_{19} steroids), estrogens (C_{18} steroids), progestins (C_{21} steroids), and bile acids (C_{24} steroids), and both the UGT1 and UGT2 family of proteins participate in these reactions (75–77). Hyodeoxycholic acid, one of the bile acids that is actively glucuronidated, was first demonstrated to serve as a substrate for UGT2B4 (34, 78) and was later found to be more efficiently conjugated by UGT2B7 (36). These are the only UGTs known to conjugate hyodeoxycholic acid. Human UGT2B7 (37, 39, 79) and to a lesser degree UGT2B4 and UGT2B15 (35, 36, 70) are also capable of glucuronidating through the hydroxylated A-ring of the steroid a number of estrogenic derivatives, including catechol-estrogens. A comprehensive survey of the regional and stereo-selectivity of UGT2B4 and UGT2B7 to glucuronidate C_{19} and C_{21} steroids has recently been conducted (80), and it is evident that UGT2B7 is 10- to 20-fold more active in conjugating hydroxyandrogens and pregnanes. UGT2B15 and UGT2B17 are also active toward a number of androgens and are the only two UGTs to glucuronidate testosterone (43, 70). Glucuronidation of C_{19} and C_{21} steroids has also been observed with expressed UGT1A4 (81), and all of the UGT1 (49, 53, 73, 79) proteins with the exception of UGT1A6 are capable of glucuronidating some of the C_{18} estrogenic steroids. On the other hand, UGT1A3 (50) and UGT1A10 (53) are the only two proteins known to glucuronidate estrone. Although the UGT2B proteins are often perceived to play the central role in steroid-like glucuronidation, it is clear that steroid metabolism by the UGTs is an extremely diversified process, and substrate specificities of the many different steroid metabolites cannot be considered exclusive to any one or group of proteins.

Carboxylic Acid Glucuronidation One of the first characterized aglycone-GlcUA linkages was the carboxylic acid (ester) linkage of anthranilic acid. Many therapeutic agents, such as aglycones that are aryl, primary, secondary, tertiary aliphatic, or heterocyclic (1), are metabolized to acyl-O-glucuronides (carboxylic esters). As shown in Table 2, the majority of these substrates are glucuronidated principally by UGT1A3 and UGT1A9, although valproic acid is subject to glucuronidation by the nasal-specific UGT2A1 (33). Bilirubin, which is also metabolized to an acyl-O-glucuronide, is selectively glucuronidated by UGT1A1. However, bilirubin glucuronidation by UGT1A1 is a rare example of the ability of this enzyme to form carboxylic ester glucuronides, because other carboxylic acids are not glucuronidated by this protein (82). Although UGT1A7 and

UGT1A10 have not been examined extensively for their ability to glucuronidate carboxylic acids, UGT1A8 is not active toward these substrates (73). UGT1A4, which is active in the N-glucuronidation of tertiary amines, also possesses no activity toward carboxylic acids. It is interesting to note that cynomologous monkey UGT2B9, which is 89% identical in amino acid sequence to human UGT2B7, catalyzes the glucuronidation of a number of nonsteroidal anti-inflammatory agents at the carboxylic acid moiety. An allelic variant of UGT2B7 (37) has been shown to possess a minimal amount of catalytic activity toward several carboxylic acid–containing nonsteroidal anti-inflammatory agents.

N-Glucuronidation The UGT1 family of proteins are primarily responsible for N-glucuronide formation. The formation of N-glucuronides can be classified into two groups: those compounds that form nonquaternary N-conjugates (heterocyclic aromatic amines, primary and secondary amines), and those that form quaternary glucuronides (cyclic tertiary amines, alicyclic tertiary amines, aromatic heterocyclic amines) (65). Many clinically useful drugs, such as antihistiminics (i.e. tripelenamine), antipsychotic (chlorpromazine), and tricyclic antidepressants (i.e. amitryptyline), contain aliphatic tertiary amine moieties that are substrates for UGT quaternary N-glucuronidation, whereas secondary amines such as desipramine and nortriptyline form nonquaternary glucuronides. A host of primary aromatic amines that have been shown to be carcinogenic, such as benzidine and 1/2-naphthylamine, are also glucuronidated through the primary amine. In examining the UGT1 proteins to form N-glucuronides, there have been no reported activities for UGT1A7 and UGT1A10 (53). Primary and/or secondary amine glucuronidation is seen with UGT1A1 (84a), UGT1A3 and UGT1A4 (83, 84, 84a), UGT1A6 (84a, 85), UGT1A8 (73), and UGT1A9 (71, 84a). Glucuronidation of a new anticonvulsant, retigabine, at the primary amino group has been demonstrated with UGT1A1, UGT1A3, UGT1A4, and UGT1A9 (86). UGT1A6 is also capable of forming direct N-glucuronides from N-containing heterocylic compounds, such as methylbiphenyl-tetrazole (87). Benzidine is also metabolized by UGT2B7 (84a). However, the formation of quaternary ammonium glucuronides is selective and has only been observed when tertiary amines serve as substrates for UGT1A3 (84) and UGT1A4 (83). Several excellent discussions on the role of amine glucuronidation have recently been published (65, 88).

EXTRAHEPATIC UDP-GLUCURONOSYLTRANSFERASES GENE EXPRESSION

Until recently, glucuronidation has been considered to represent a metabolic pathway performed mainly by the liver (1). However, multiple studies have indicated that UGT activity toward bile acids, phenols, and bilirubin was resident in human intestinal (89–93), kidney (91, 92, 94–96), and colon tissue (89, 97). Although

the liver is the organ with the most diverse metabolic capabilities and a central locus of catabolic metabolism, contact with xenobiotic material is first established in surface epithelia of the gastrointestinal tract and respiratory system prior to resorption. Thus, glucuronidation is likely to represent an important mechanism of moving lipophilic nutrients out of the digestive tract membranes. In addition, it is well known that enterohepatic circulation requires gastrointestinal means of glucuronidation to counteract the activities of bacterial β-glucuronidases. An appreciation of the multiplicity and genetics of extrahepatic UGTs has made it possible to define the role of glucuronidation in extrahepatic tissues.

UGT1A Locus

The development of specific reverse transcriptase (RT)-PCR–based methodology made it possible to distinguish single base pair differences between highly homologous sequences, as evident between the *UGT1A* gene products (18). This has made it possible to identify the precise expression patterns in liver as well as in other extrahepatic tissues (Figure 5, see color insert). The *UGT1A* locus in human liver is defined by UGT1A1, UGT1A3, UGT1A4, UGT1A6, and UGT1A9 mRNA expression (54). Of significance, UGT1A7 and UGT1A10 (18) were discovered and cloned from gastric tissue and UGT1A10 in biliary tissue (18, 53, 54), indicating these RNAs are exclusively extrahepatic *UGT1A* gene products (54). The detection of UGT1A10 also in human colon (53, 56) confirmed that UGT1A10 is expressed in multiple extrahepatic organs of the gastrointestinal tract. We now know that UGT1A10 appears to be expressed in all tissues of the gastrointestinal tract except liver. This is significant because UGT1A10 has one of the widest range of substrate specificities of any of the UGTs, encompassing the small phenolics to steroids, an indication that it may play a vital role in most extrahepatic tissues for the glucuronidation of endogenous and xenobiotic substrates.

The analysis of human esophagus demonstrated a pattern of expression that focused exclusively on the UGT1A7–10 cluster of gene products (67). It is interesting to note that UGT1A7 is expressed only in the proximal tissues of the gastrointestinal tract, such as the esophagus and stomach (18, 53, 98). Analysis of extrahepatic tissues outside the gastrointestinal tract have documented the expression of UGT1A9 mRNA in kidney (99), and following the identification of UGT1A6 in rat brain (100), UGT1A6 mRNA was also detected in human brain (74). Expression of the *UGT1A* locus in colon represents one of the more diverse patterns of expression, with RNA detected for UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A8, UGT1A9, and UGT1A10 (53). In addition, UGT1A8 (53) was expressed in human jejunum and ileum (52, 73). These recent findings demonstrate tissue-specific patterns of expression of *UGT1A* gene products and support the hypothesis that glucuronidation requirements of different metabolically active tissues are tightly regulated (17).

UGT2 Gene Products

The expression of *UGT2* genes also follows a tissue-specific pattern (Table 3, Figure 5). The human olfactory UGT2A1, which has been shown to be one of the more versatile of the UGTs by recognizing all of the major classes of substrates, is restricted in expression to olfactory tissue, although RT-PCR experiments detected minimal levels also in brain and fetal lung (33). One could predict that UGT2A1 has evolved for the need to serve as a first line of metabolic defense for many substances that enter the body through the nasal mucosa, and possibly to terminate olfactory signals efficient to ensure a rapid adaptation to changing olfactory stimuli and signals. In human liver, cDNAs have been identified for UGT2B4, UGT2B7, UGT2B10, UGT2B11, and UGT2B15 (35, 40, 67, 70, 101, 102). A significant observation is that UGT2B transcripts are abundantly expressed in steroid-sensitive target tissues such as prostate and mammary gland. For example, UGT2B10, UGT2B11, UGT2B15, and UGT2B17 gene transcripts have been identified in human prostate, and UGT2B11 is also expressed in mammary gland tissue. A wide expression pattern including liver, kidney, breast, prostate, skin, adipose tissue, adrenal tissue, and lung has been shown for UGT2B11

TABLE 3 Expression of human UDP-glucuronosyltransferases (UGTs) mRNA in the human body

UGT	Tissues	References
UGT1A1	Liver, bile ducts, stomach, colon	53, 98, 109
UGT1A3	Liver, bile ducts, stomach, colon	2, 18, 50, 53, 98
UGT1A4	Liver, bile ducts, colon	18, 53, 109
UGT1A5	Not detected	53, 67
UGT1A6	Liver, bile ducts, stomach, colon, brain	18, 51, 53, 74
UGT1A7	Esophagus, stomach	67, 98
UGT1A8	Esophagus, ileum, jejunum, colon	53, 67, 73
UGT1A9	Liver, colon, kidney	53, 55, 99
UGT1A10	Esophagus, stomach, bile ducts, intestine, colon	53, 54, 56
UGT2A1	Olfactory epithelium, brain, fetal lung	33
UGT2B4	Liver	67, 101
UGT2B7	Esophagus, liver, intestine, colon, brain, kidney, pancreas	39, 67, 74, 103, 104, 163
UGT2B10	Esophagus, liver, mammary gland, prostate	35, 67, 103
UGT2B11	Liver, kidney, mammary gland, prostate, adrenal, skin, adipose tissue, lung	40
UGT2B15	Esophagus, liver, prostate	40, 67, 69, 102
UGT2B17	Prostate	29, 45

(40). The presence of UGT2B17 may have a significant impact on cancer of the prostate gland by glucuronidating androgens and thus protecting this tissue from the carcinogenic actions of these steroids. Similarly, UGT2B7 transcripts are found in liver, intestine, esophagus, brain, kidney, and pancreas (74, 103, 104). In the gastrointestinal tract, a differential expression pattern is also evident with UGT2B4 and UGT2B7. Although UGT2B7 is expressed in intestine, esophagus, and pancreas (67, 74, 104), no UGT2B4 transcripts have been identified in intestine or esophagus (67, 104).

Glucuronidation in the Gastrointestinal Tract

It is apparent that the human digestive tract is capable of significant glucuronidation activity, as evident from detection of gene products (Figure 5) and the analysis of catalytic activities, which appear to function complementary to hepatic glucuronidation. What is the role of glucuronidation in the physiological functions of the gastrointestinal tract? Glucuronidation most likely represents a metabolic barrier function of the gastrointestinal mucosa. Lipophilic compounds enter the body as components of our diet and are likely to diffuse into the membranes of resorptive tissues. UGTs can fulfill a dual role at this critical localization of the resorption process. First, digestive material can be transformed into water-soluble glucuronides and remain in the lumen of the digestive tract, or second, it can be resorbed and transported to the kidney for excretion or ultimately targeted to the biliary tract for elimination. There is no conclusive experimental evidence measuring endoluminal nonbile glucuronide formation to determine which of the illustrated pathways is more significant in humans. However, the presence of bacterial β-glucuronidases in the lumen of the colon suggests that epithelial UGT proteins may serve to protect against deconjugation of compounds designated to exit the body. These considerations would assign a critical role to the human colon. Immunofluorescence analysis has confirmed that UGT protein is expressed selectively in the epithelial cell layer of the human colon (17), where the resorbed compounds would be available as substrates for the large pool of UGTs. In addition, UGT1A protein detected by Western blot has been found to be expressed at levels comparable to the human liver. The colon may therefore exert a ''scavenger function'' at the distal end of the digestive system. For compounds that are reabsorbed in the colon, the battery of UGTs would assure the formation of glucuronides as a final detoxification step, with the water-soluble metabolites targeted for biliary or renal excretion. A yet-unexplained observation is the significantly lower glucuronidation rates in colon compared with liver (17, 89, 93, 97).

The discovered regulation of the *UGT* genes provides first insight in the potential role of first pass kinetics of orally administered drugs. Given the diversity of glucuronidation enzymes in the gastrointestinal tract, prehepatic metabolism may be significantly underestimated and may be the biochemical basis of differences

in resorption rates between individuals frequently observed in standardized clinical drug treatment regiments. For example, in human stomach, the polymorphic expression of the UGT1A isoforms UGT1A1, UGT1A3, and UGT1A6 has been demonstrated (98). These variations correlate with a fourfold interindividual variation of glucuronidation activities for a number of phenolic compounds between individuals. It could be predicted that a UGT1A6 polymorphism may be related to the interidividual variations seen in acetaminophen toxicity (105). Acetaminophen toxicity at doses as low as 6000 mg is observed to lead to acute liver failure. In some individuals, extremely high doses are tolerated without liver failure. In our experience, the highest dose survived was 20,000 mg, which suggests that polymorphisms in drug metabolism may have a significant impact on acetaminophen tolerance and metabolism. Also observed in gastric mucosa was a qualitative variation in the hyodeoxycholic acid glucuronidation (98), which corresponds to polymorphic alleles identified for UGT2B7 (69). The biochemical and physiological basis of these phenomena are emerging as the glucuronidation capabilities of the human digestive system and other tissues are elucidated.

UDP-GLUCURONOSYLTRANSFERASES IN HUMAN DISEASES

Inherited Unconjugated Hyperbilirubinemia

Jaundice is one of the most striking and stigmatizing symptoms of human disease. Hyperbilirubinemia becomes clinically evident when serum bilirubin levels exceed 35 μM/liter. Given a daily production rate of about 500 μM in a 70-kg adult, the necessity of effective means of bilirubin transport and quantitative elimination from the body are obvious (106). Bilirubin is a breakdown product of heme containing proteins, 80% of which originates from the catalysis of circulating hemoglobin, and 20% of which originates from hepatic heme-containing proteins such as cytochrome P450s, tryptophan pyrrolase, and catalase as well as from the body's pool of free heme. Bilirubin is highly hydrophobic and mainly exists bound reversibly to albumin. Its hydrophobicity is the reason why elimination from the body requires additional metabolic steps (107). The failure of either transport or conjugation leads to the saturation of albumin and consequently to tissue accumulation of bilirubin. When serum levels exceed 300 μm/liter, bilirubin can pass the blood brain barrier and lead to a fatal necrosis of neurons and glial tissue (kernicterus). Only 5% of the bilirubin pool exists as water-soluble bilirubin diglucuronide. This metabolite is key to the elimination of bilirubin from the body, and therefore, the glucuronidation of bilirubin by UGT1A1 is an essential metabolic pathway of human metabolism.

The symptoms of hyperbilirubinemia can have multiple etiologies, including viral, toxic, or autoimmune liver disease (108) as well as biliary obstruction and

hemolysis. However, the observation of physiological hyperbilirubinemia in neonates and the hereditary unconjugated hyperbilirubinemias in children and adults have led to the investigation of bilirubin metabolism and have ultimately proved to be the driving force of the discovery of the human *UGT1A* gene locus (2, 109, 110). Although the human *UGT1A* locus potentially encompasses nine functional transferase genes, only one isoform, UGT1A1, is involved in inherited diseases of bilirubin metabolism (2, 109, 111). With the exception of minor bilirubin UGT activity detected in vitro with expressed UGT1A4 (109), only UGT1A1 is capable of forming bilirubin glucuronides. Because a number of patients suffering from a complete loss of bilirubin glucuronidation exhibit homozygous mutations of the *UGT1A1* first exon only, it is not likely that additional bilirubin UGTs exist in humans (112). Mutations of the *UGT1A1* first exon (113–119) lead to a selective effect on the *UGT1A1* gene product. Because bilirubin glucuronidation can be completely abrogated by such a mutational event (117), there appears to be no substitute isoform capable of bilirubin glucuronidation and the reported bilirubin activity of UGT1A4 by in vitro experiments does not appear to have any biological significance.

In humans, three forms of inheritable unconjugated hyperbilirubinemic diseases exist: Crigler-Najjar syndrome type I, Crigler-Najjar syndrome type II, and Gilbert's syndrome (Table 4). Crigler-Najjar syndrome type I is diagnosed as a complete lack of bilirubin glucuronidation, and therefore, no conjugated bilirubin is clinically detectable in duodenal biliary secretions. Crigler-Najjar syndrome type II is diagnostically differentiated by the presence of low amounts of bilirubin diglucuronides and monoglucuronides in duodenal biliary secretions and a response to induction therapy with phenobarbital (106). The biochemical basis of this disease variant is UGT1A1 deficiency with at least 10% of normal activity remaining. The therapeutic approach for Crigler-Najjar syndrome type I and type II is different (Table 4). Crigler-Najjar syndrome type I patients invariably require immediate orthotopic liver transplantation as a surgical means of gene therapy capable of replacing the defective UGT1A1 alleles, whereas Crigler-Najjar syndrome type II patients can frequently be treated by induction therapy or phototherapy for prolonged periods of time. The third condition with jaundice and UGT involvement was described by Gilbert and Lereboullet in 1901 (120). This benign condition of young adults does not require therapy and is characterized by fluctuating unconjugated hyperbilirubinemia in response to psychological stress, infection, fasting, or physical activity. The levels of unconjugated serum bilirubin are lower than in Crigler-Najjar's syndrome, and the hepatic bilirubin UGT activity is reduced to 60–70% of an unaffected individual.

The inheritable unconjugated hyperbilirubinemias are all the result of either mutant *UGT1A1* alleles (112–115, 117, 119) or *UGT1A1* promoter polymorphisms (121, 122). To date, 33 mutant *UGT1A1* alleles have been identified (14, 117, 123) (Table 5, Figure 3). Nine of these mutations have been located within the unique first exon of the UGT1A1 gene (113, 119). All the other polymorphic

TABLE 4 The clinical classification of unconjugated hyperbilirubinemia[a]

Determinants	Crigler-Najjar type 1	Crigler-Najjar type 2	Gilbert (meulengracht) disease
Incidence	Rare	Very rare	7% of population
Bilirubin	Unconjugated	Unconjugated (conjugated)	Unconjugated (conjugated)
Serum liver function tests	Normal	Normal	Normal
Liver histology	Normal	Normal	Normal
Inheritance	Autosomal recessive	Autosomal recessive	Autosomal recessive
Hemolysis	Absent	Absent	Absent
Affected gene	*UGTIA* coding region and/or promoter	*UGTIA* coding region and/or promoter	*UGTIA* coding region and/or promoter
Effect of mutation	Absence of activity	10% of activity	60% of activity
Clinical response to induction	No induction	Induction with phenobarbital	Induction with phenobarbital, self-limiting, stress induced
Therapy	Blood exchange, transfusion, liver transplantation	Phototherapy, sometime liver transplantation	Not necessary
Prognosis	Untreated: death	Variable	Excellent

[a]Listed are the pertinent clinical and genetic findings that discriminate between the three known syndromes that lead to unconjugated hyperbilirubinemia.

TABLE 5 Allelic polymorphism of the human UGT1A1 gene and association with unconjugated hyperbilirubinemia[a]

Allele	Nucleotide changes	Protein changes	Type	Exon	Disease	Reference
UGT1A1*1	Wild type	—	—	—	—	109
UGT1A1*2	879 del 13	Truncation	Deletion	2	CN1	3
UGT1A1*3	1124 C→T	S375F	Missense	4	CN1	124
UGT1A1*4	1069 C→T	Q357X	Nonsense	3	CN1	126
UGT1A1*5	991 C→T	Q331 del 44	132 nt deletion	2	CN1	124
UGT1A1*6	221G→A	G71R	Missense	1	Gilbert	113
UGT1A1*7	145 T→G	Y486D	Missense	5	CN2	130
UGT1A1*8	625 C→T	R209W	Missense	1	CN2	114
UGT1A1*9	992 A→G	Q331R	Missense	2	CN2	128
UGT1A1*10	1021 C→T	R341X	Nonsense	3	CN1	129
UGT1A1*11	923 G→A	G308E	Missense	2	CN1	125, 127
UGT1A1*12	524 T→A	L175Q	Missense	1	CN2	115
UGT1A1*13	508 del 3	F170del	Deletion	1	CN1	116
UGT1A1*14	826 G→C	G276R	Missense	1	CN1	115
UGT1A1*15	529 T→C	C177R	Missense	1	CN2	115
UGT1A1*16	1070 A→G	O357R	Missense	3	CN1	127
UGT1A1*17	1143 C→G	S381R	Missense	4	CN1	127
UGT1A1*18	1201 G→C	A401P	Missense	4	CN1	127
UGT1A1*19	1005 G→A	W335X	Missense	3	CN1	127
UGT1A1*20	1102 G→A	A368T	Missense	4	CN1	127
UGT1A1*21	1223 ins G	Frameshift	Frameshift	4	CN2	127
UGT1A1*22	875 C→T	A292V	Missense	2	CN1	127
UGT1A1*23	1282 A→G	K426E	Missense	4	CN1	127
UGT1A1*24	1309 A→T	K437X	Missense	5	CN1	127
UGT1A1*25	840 C→A	C280X	Missense	1	CN1	112
UGT1A1*26	973 del G	Frameshift	Frameshift	2	CN2	115
UGT1A1*27	686 C→A	P229Q	Missense	1	Gilbert	119
UGT1A1*28	TAATA7	Transcription	Insertion	Promotor	Gilbert	121
UGT1A1*29	1099 C→G	R367G	Missense	4	Gilbert	119
UGT1A1*30	44 T→G	L15R	Missense	1	CN2	118
UGT1A1*31	11609 CC→GT	P387R	2 nt missense	4	CN1	105
UGT1A1*32	1006 C→T	R336W	Missense	3	CN1	123
UGT1A1*33	881 T→C	I294T	Missense	2	CN2	123

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alleles have differences located in exons 2–5 (3, 115, 124–131). It is debatable whether the distinction of Crigler-Najjar syndrome type I and type II and of Gilbert's disease, which is based on serum bilirubin levels and the clinical course of the metabolic error, should be regarded as a single disease entity with a combination of functionally relevant or silent allelic polymorphisms of the *UGT1A1* gene. Studies have shown that homozygous or compound heterozygous mutations can lead to Crigler-Najjar syndrome type I (112) and type II (130). Promoter polymorphisms plus mutant coding region alleles can lead to Crigler-Najjar syndrome type I and type II (123) and to Gilbert's disease (121) as well as to no detectable disease at all (121, 123, 132). Given the theoretically unpredictable impact of an individual mutant allele or of the combination of itself with a different mutant allele or a promoter polymorphism, a database of all identified allelic variants is likely to serve as a decision tool to predict the course and management of patients with unconjugated hyperbilirubinemia, in addition to gaining insight into the functional properties of the UGT1A1 protein. However, for a clinically apparent hyperbilirubinemic state, homozygous or compound heterozygous mutant alleles are required. The presence of a single mutant allele without other abnormalities does not result in clinically detectable disease.

UDP-Glucuronosyltransferases in Carcinogenesis

Chemical carcinogenesis is considered to be one of the most prevalent mechanisms of neoplastic transformation and is linked to cancer of the esophagus, stomach, bladder, liver, colon, lung, and pleura (133–135). In addition, steroid metabolism is implicated as a factor of neoplastic transformation for prostate and breast cancer development (29, 136, 137). A potential model for neoplastic transformation predicts that enzymes involved in normal detoxification of potential mutagens and gene modulators such as sulphation, acetylation, and glucuronidation may be modified in diseased tissues. This has been demonstrated in Gunn rats, where a mutation in the *UGT1A* allele renders the entire locus inactive. The production of benzo(a)pyrene glucuronides is dramatically reduced, leading to elevated levels of DNA adducts. The ability to alter the mutagenic actions of polycyclic aromatic hydrocarbons through glucuronidation is good evidence that the UGTs may represent a metabolic defense against environmental carcinogens. One approach to examine the actions of the UGTs in diseased tissues is to identify the cancer and quantitatively identify expression patterns in normal and cancer tissue.

[a]List of all identified polymorphic alleles of the human *UGT1A1* gene and their association with different forms of unconjugated hyperbilirubinemias. Note that although an individual allele may have been identified in a specific disease, e.g. Crigler-Najjar disease type 1, different combinations of alleles as compound heterozygous traits may result in a differing clinical picture. CN1, Crigler-Najjar type 1 disease; CN2, Crigler-Najjar type 2 disease; Gilbert, Gilbert's disease; del, deletion; ins, insertion; nu, nucleotide.

The human gastrointestinal tract represents one of the largest external surface organs involved in immediate contact and the metabolism of xenobiotic material. The five most frequent sites of carcinogen-associated cancer development are esophagus, stomach, liver, biliary tree, and distal colon. Comparisons of surrounding healthy tissue with the *UGT1A* locus has led to the identification of a pattern of down-regulation of UGT1A mRNA and microsomal UGT catalytic activity in gastrointestinal tumors of the esophagus (67), stomach (98), liver (54), and bile ducts (54). The *UGT1A* genes are regulated individually. Although some are up-regulated (98) and some are not regulated at all (54), in malignant tissues the majority of gene expression is reflected in dramatic down-regulation of specific gene transcripts (54). For example, differential down-regulation of UGT1A mRNA is observed in the early stages of cancer, as represented by expression in pre-malignant adenomatous hyperplasia of the liver, as well as in the more advanced malignant hepatocellular carcinoma (54). In contrast, no such regulation was seen in benign tumorgenesis, represented by focal nodular hyperplasia of the liver. In addition, down-regulation of UGT transcripts in malignant tissue coincides with a reduction in UGT1A-specific protein, as identified by Western blot analysis, as well as differences in microsomal catalytic activity when assayed with polycyclic aromatic hydrocarbons. It should be noted, however, that although there is clearly a pattern of expression, which supports the hypothesis that the UGTs may be involved in genoprotection, it remains to be determined whether these events are an early marker in cancer development or a result of neoplastic events that lead to tissue transformation.

The availability of catalytically active preparations of the UGTs makes it possible to examine the ability of these proteins to participate in the glucuronidation of potential carcinogens. For example, analysis of recombinant UGTs has confirmed that primary amines, which are known environmental mutagens, are glucuronidated by UGT1A3 (84), UGT1A4 (81, 83), UGT1A6 (85), UGT1A8 (73), UGT1A9 (71, 85), and UGT2A1 (33) (see Table 2), whereas several of the benzo(a)pyrenes have been identified as substrates for UGT1A6 (138), UGT1A7 (53), UGT1A8 (52), UGT1A9 (138), UGT1A10 (52, 53), and UGT2B7 (138). We demonstrated that 2-hydroxyamino-1-methyl-6-phenylimidazo-(4,5-β) pyridine (N-hydroxy PhIP), a heterocyclic amine and mutagen found in food as well as in tobacco smoke, was glucuronidated in human esophagus by UGT1A7, UGT1A9, and UGT1A10 (67), but that additional forms of UGTs were most likely involved in other human tissues. This is supported by recent findings demonstrating that N-hydroxy PhIP is also subject to glucuronidation by UGT1A3 and UGT1A8 (139). Clearly, these findings underline the hypothesis that multiple UGT isoforms participate in the metabolism and elimination of potential direct or indirect human carcinogens.

Other clues that suggest the UGTs may play a significant role in genoprotection come from results of gene expression and catalytic activity recorded in colonic mucosa. Colon cancer accounts for 9.7% of all newly diagnosed cancers and is

the second most common cancer site in humans (140). The majority of malignant digestive system tumors develop in the distal portions of the colon, whereas the intestine is virtually free of cancer development. It is interesting to note that analysis of gastrointestinal UGT activities in humans has demonstrated a sharp decrease of catalytic UGT activity from intestinal tissue to colon tissue (93), a pattern that is inversely proportional to tumor formation. The low UGT activity levels in colon may therefore represent an example of diminished genoprotection in this tissue. It is interesting to note that colon tissue displays an abundance of *UGT1A* gene expression represented by UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A8, UGT1A9, and UGT1A10 gene transcripts that correspond to similar levels of UGT protein, as detected by Western blot analysis (17). However, the high levels of *UGT1A* gene expression are not concordant with the dramatic reduction in many of the substrate-specific UGT activities in human colon, which suggests that this tissue is subject to a novel mechanism that abruptly diminishes the glucuronidation potential and conversely may predispose this tissue to the proneoplasitic effects of potential mutagens.

Although environmental carcinogens that enter the body through the lungs or gastrointestinal tract mainly act as direct carcinogens with genotoxic capablilities, other tumors are stimulated by nongenotoxic carcinogens, which directly affect growth control. Among these are the steroid hormone–sensitive neoplasms, including prostate and breast cancer. Standard treatment in prostate carcinoma involves the removal of androgen production by orchiectomy, or the administration of lutenizing hormone releasing hormone agonists with androgen blockers such as flutamide (29, 136). This strategy limits the proneoplastic effects of androgens on prostate carcinoma tissue. Steroid hormone UGT activity was detected in typically steroid-sensitive tissues, including prostate, mammary gland, and ovary. The analysis of human prostatic cell lines and prostate tissue has led to the cloning and characterization of UGT2B15 and UGT2B17 (43, 103). Humans have measurable levels of circulating C19 androgen glucuronides. It has been proposed that these circulating conjugates reflect the peripheral conversion of adrenal and gonadal C19 steroids to potent androgens, in particular to dihydrotestosterone. Catalytic UGT activity with C19 steroid hormones has been demonstrated for UGT2B15 and UGT2B17 (Table 2) (29). These findings suggest that steroid UGT activity functions to terminate activation of steroid-sensitive tissues by generating water-soluble hormone glucuronides. For the etiology and progression of prostate cancer, this may be an important mechanism, because the reduced elimination of androgens from prostatic tissue would enhance the growth signal and promote neoplastic transformation or lead to progression of cancer. Recent analyses have demonstrated that in the steroid-sensitive prostate carcinoma cell line LNCaP, the administration of interleukin 1 was capable of downregulating UGT2B17 expression and protein levels, as well as dihydrotestosterone glucuronide formation (141). The regulation of UGT proteins, therefore, appears to play a critical role in steroid-sensitive tissues and their neoplasms.

UDP-Glucuronosyltransferases in Autoimmunity

Autoimmune hepatitis was first described in 1950 (142) and represents a chronic inflammatory disease of the liver characterized by a loss of self tolerance toward the liver. The etiology of this life-threatening condition is not known, and the diagnosis is reached by exclusion of viral, toxic, metabolic, or inherited errors of metabolism, as well as by the detection of circulating autoantibodies and additional markers of immune-mediated pathophysiology (108). In autoimmune hepatitis as well as viral hepatitis C and D, drug metabolizing enzymes have been identified as human hepatocellular autoantigens. Among these are autoantibodies directed against proteins of the ER, which are identified by immunofluorescence detection of antigens expressed in liver and the distal renal tubules and are, therefore, termed liver/kidney microsomal antibodies (LKM) (143). LKM autoantibodies have been characterized to target cytochromes CYP2D6 (LKM1) (143–147), CYP2C9 (LKM2) (148), and CYP1A2 (LM, for staining of the liver tissue only) (148–151). A third group of LKM autoantibodies (LKM3) was identified as targeting UGT1A proteins (68, 152, 153). These autoantibodies serve as diagnostic markers for autoimmune hepatitis type II (143–145), the autoimmune polyendrocrine syndrome type I (APS1) (150, 151), and drug induced hepatitis (148, 149), and they also serve as markers to discriminate between viral hepatitis C and D and idiopathic autoimmune hepatitis (154). In humans, LKM3 autoantibodies have been identified as targeting UGT1A1, UGT1A6, UGT1A4, and rabbit UGT1A6, as well as targeting a minor reactivity with UGT2B isoforms (68, 153). It has been suggested that in drug-induced hepatitis, the formation of drug adducts with CYP proteins and their subsequent recognition by the immune system, generation of a B-cell response, and production of autoantibodies is a pathophysiological mechanism (155). However, in genuine autoimmune hepatitis, the reasons why drug metabolizing enzymes serve as hepatocellular autoantigens remain unclear.

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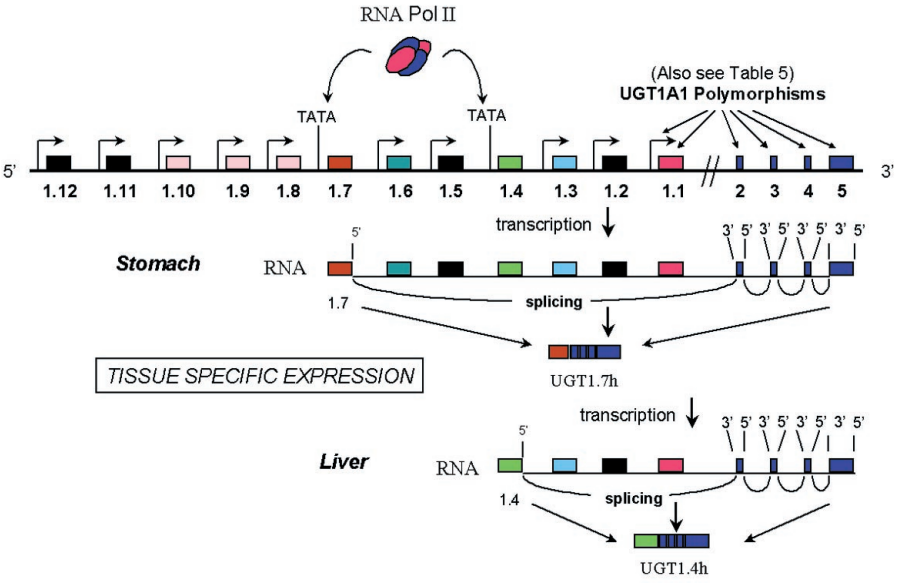

Figure 3 Representation of the organization of the *UGT1A* locus and an example of how different UGT1A RNAs are processed. Four common exons, 2-5, are shown in all the the UGT1A RNAs. The 5′ portion of the locus contains sequences encoding the divergent portion of each UGT1A protein, represented by exons 1.1 through 1.12. Following the initiation of transcription at promotors that flank each of the exon-1 sequences, the 5′ and 3′ consensus splice sites are recognized by the spliceosome and the intervening sequences are removed. In the example show, the tissue specific expression of *UGT1A7* is regulated in gastric epithelium, and UGT1A4 is expressed in hepatic tissue. Also shown by arrows are the different exon sequences that contain mutations and that have been linked clinically to unconjugated hyperbilirubinemia (see also Table 5).

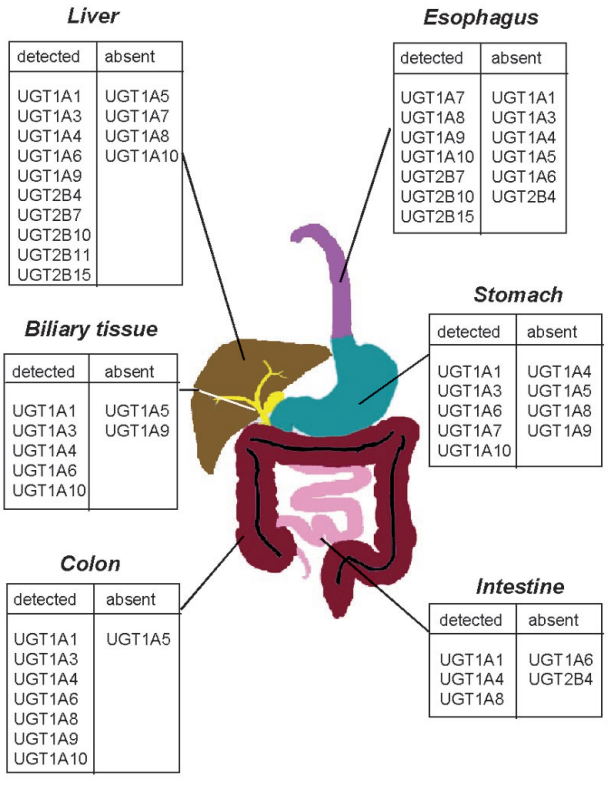

Figure 5 Representation of the tissues of the gastrointestinal tract that express the different UGTs. The majority of the data obtained for this figure was acquired by cloning the respective cDNAs from the different tissues, or gene transcripts quantitated by RT-PCR methodologies.