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Tissue- and Gender-Specific mRNA Expression of UDP-Glucuronosyltransferases (UGTs) in Mice

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Running Title: Tissue Distribution of Mouse Ugts

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Non-Standard Abbreviations: UGT, Uridine-diphosphate glucuronosyltransferase; UDP-GA, Uridine-diphosphate glucuronic acid; UDP-GPP, UDP-glucose pyrophosphorylase; UDP-GDH, UDP-glucose dehydrogenase

Abstract

UDP-glucuronosyltransferases (UGTs) catalyze phase-II biotransformation reactions in which lipophilic substrates are conjugated with glucuronic acid to increase water solubility and enhance excretion. Currently, little information regarding tissue or gender-specific expression of mouse UGTs is available. Mice are increasingly popular models in biomedical research, and therefore, thorough characterization of murine drug metabolism is desired. The purpose of the present study was to determine both tissue- and gender-specific UGT-gene-expression profiles in mice. RNA from fourteen tissues was isolated from male and female C57BL/6 mice and UGT expression was determined by the bDNA assay. Ugts highly expressed in mouse liver include Ugt1a1, Ugt1a5, Ugt1a6, Ugt1a9, Ugt2a3, Ugt2b1, Ugt2b5/37/38, Ugt2b34, Ugt2b35, and Ugt2b36. Several isoforms were expressed in the GI tract, including Ugt1a6, Ugt1a7c, Ugt2a3, Ugt2b34, and Ugt2b35. In kidney, Ugt1a2, Ugt1a7c, Ugt2b5/37/38, Ugt2b35, and Ugt3a1/2 were expressed. Ugt expression was also observed in other tissues: lung, Ugt1a6; brain, Ugt2b35; testis and ovary, Ugt1a6 and Ugt2b35; and nasal epithelia, Ugt2a1/2. Male-predominant expression was observed for Ugt2b1 in liver, Ugt2b5/37/38 in kidney, and Ugt1a6 in lung. Female-predominant expression was observed for Ugt1a1 and Ugt1a5 in liver, Ugt1a2 in kidney, Ugt2b35 in brain, and Ugt2a1/2 in nasal epithelia. UDP-glucose pyrophosphorylase was highly expressed in liver, kidney, and GI tract, whereas UDP-glucose dehydrogenase was highly expressed in the GI tract. In conclusion, marked differences in tissue- and gender-specific expression patterns of Ugts exist in mice, potentially influencing drug metabolism and pharmacokinetics.

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UDP-glucuronosyltransferases (UGTs) catalyze phase-II biotransformation reactions, conjugating lipophilic substrates with glucuronic acid, thereby increasing hydrophilicity and enhancing excretion through bile and urine. Glucuronidation reactions are generally considered detoxification reactions, in which a potentially toxic substrate is conjugated to glucuronic acid. The addition of the glucuronic acid moiety occurs at hydroxyl, carboxylic acid, amine (primary, secondary, and tertiary), carbonyl, and thiol groups (Dutton, 1980). Endogenous UGT substrates comprise numerous steroids and metabolic by-products, including bilirubin, testosterone, thyroxine, estradiol, androsterone, and hyodeoxycholic acid (HDCA). Additionally, numerous xenobiotics serve as UGT substrates, including drugs such as acetaminophen, morphine, propofol, chloramphenicol, and non-steroidal anti-inflammatory drugs (NSAIDs), as well as environmental compounds, such as plant-derived dietary flavanoids, carcinogens, and other pollutants (Dutton, 1980; Radominska-Pandya *et al.*, 1999; Tukey and Strassburg, 2000).

The UGT superfamily of genes is divided into two families, UGT1 and UGT2, based on sequence similarity at the amino acid level. UGT1 family genes are encoded from a unique first exon (I) spliced with common exons 2-5 (II-V), creating common C-terminal domain and unique N-terminal domains; each UGT1A family member contains gene-specific promoter regions (Mackenzie et al., 1997; Mackenzie et al., 2005). In humans, thirteen unique first exons have been identified (UGT1A1-13), encoding nine functional enzymes and four pseudogenes (UGT1A2, 11, 12, 13), whereas in rats, nine first exons have been identified (UGT1A1-9), with two coding pseudogenes (UGT1A4 and UGT1A9)(Emi *et al.*, 1995; Gong *et al.*, 2001). In mice, the Ugt1a subfamily

contains fourteen first exons, coding nine enzymes (Ugt1a1, 2, 5, 6, 6a, 7c, 8, 9, and 10) and five pseudogenes (Ugt1a3, 4, 7a, 7b, and 11)(Mackenzie et al., 2005).

Unlike UGT1 genes, each UGT2 gene is encoded by six individual exons and are further divided into two families, UGT2A and UGT2B. UGT2A1 mRNA is expressed in the nasal epithelia of both humans and rats (Jedlitschky *et al.*, 1999; Heydel *et al.*, 2001). Recent reports indicate that the gene duplication product of UGT2A1 in humans, UGT2A2, is expressed in liver (Heydel et al., 2001; Jedlitschky et al., 1999; Tukey and Strassburg, 2001). Analysis of the mouse genome indicates that there are three Ugt2a gene duplication products, Ugt2a1, Ugt2a2, and Ugt2a3 (Mackenzie et al., 2005). Humans, rats, and mice each contain seven unique UGT2B genes (Mackenzie et al., 2005). The seven Ugt2b genes in mice include: Ugt2b1, 2b5, 2b34, 2b35, 2b36, 2b37, and 2b38. In addition, a novel UGT3A family has been identified in humans and rodents, giving rise to two individual gene products in mice, Ugt3a1 and Ugt3a2, located on mouse Chromosome 15 (Mackenzie et al., 2005). In general, UGT1A genes share homology between species (human UGT1A1 is homologous to rat and mouse Ugt1a1). Conversely, the UGT2B family does not share orthologs between species, except for UGT2B1 in rats and mice (Mackenzie et al., 2005).

Expression of UGT mRNA and protein, and thus glucuronidation, occurs primarily in liver, gastrointestinal tract, and kidney. Human UGT1A transcripts are highly expressed in liver, kidney, and portions of the GI tract, including gastric tissue, small intestine, and colon, which are primarily responsible for glucuronidation of dietary substances and drugs (Basu et al., 2004; Fisher et al., 2001; Mojarrabi and Mackenzie, 1998; Strassburg et al., 2000; Strassburg et al., 1998a; Strassburg et al., 1998b).

Likewise, mRNA expression of rat UGT1A isoforms is predominant in liver and intestine (Emi *et al.*, 1995; Grams *et al.*, 2000; Shelby *et al.*, 2003). However, human and rat UGT2B members are expressed not only in liver and intestine, but also in steroid-target tissues such as testis, uterus, mammary tissue, and brain, where they conjugate numerous endogenous steroids (Beaulieu et al., 1996; Beaulieu et al., 1998; Levesque et al., 1997; Levesque et al., 1999; Shelby et al., 2003; Turgeon et al., 2001). Hence, tissue-specific expression of individual UGT members contributes to the organism's ability to selectively and preferentially glucuronidate chemicals in specific tissues.

Gender differences in UGT function and expression have been documented in rats. In Wistar rats, the rate of bilirubin glucuronidation in liver microsomes is 2-fold higher in females than in males (Muraca and Fevery, 1984). Castration of male rats increased bilirubin glucuronidation, and androgen treatment reduced the rate of bilirubin-glucuronide formation. Ovarectamized female rats displayed decreased bilirubin glucuronidation rate, whereas treatment with progestins increased glucuronidation rates (Muraca and Fevery, 1984). Rat UGT2B3 mRNA was expressed higher in males than females, could be reduced by castration, and restored by treatment with testosterone (Strasser *et al.*, 1997). These examples illustrate the importance of understanding gender-specific UGT expression to determine gender-specific glucuronidation profiles.

The tissue distribution of mouse Ugt members has not been determined, nor have gender differences in mouse Ugt gene expression been explored. The prevalence of knockout mice in scientific research imparts great significance in understanding the roles of normal drug metabolism in the mouse model, including that of glucuronidation.

Therefore, the present study determines the tissue- and gender-specific mRNA expression patterns of mouse Ugts as well as the enzymes responsible for the synthesis of UDP-GA.

Materials and Methods:

Animals: Male and female C57BL/6 mice (*n* = 5 per gender) 8 weeks of age were purchased from Charles River Laboratories, Inc. (Wilmington, MA). Animals were housed according to the American Animal Association Laboratory Animal Care (AAALAC) guidelines. Mice were allowed food (Harlan-Teklab) and water ad libitum, and were acclimated to the housing facility for one week. Twelve tissues were removed, including liver, kidney, stomach, duodenum, jejunum, ileum, large intestine, lung, brain, heart, and gonads (ovaries and testis). Ovaries were pooled (25 ovaries for *n* = 1), and additional ovaries were purchased from Charles River Breeding Laboratories from ovarectamized C57BL/6 mice for other experimental purposes. Placentas were collected from timed-pregnant females at gestation-day 18. Nasal olfactory epithelia was collected and pooled into a single sample for each gender (*n* = 1/gender). Stomach and intestine were dissected and rinsed in saline. All tissues were snap frozen in liquid nitrogen and stored at –80° C.

Total RNA Isolation: Total RNA was extracted from each tissue using RNA-Bee Reagent (Tel-Test Inc., Friendswood, TX) according to the manufacturers protocol. RNA was quantified by UV spectrophotometry at 260/280nm and diluted to 1μg/μl in diethyl pyrocarbamate (DEPC)-treated water. RNA samples were analyzed by formaldehyde-agarose gel electrophoresis, and integrity was confirmed by visualization of 18S and 28S rRNA bands.

Development of Oligonucleotide Probe Sets for bDNA Analysis: Multiple oligonucleotide probe sets [capture extender (CE), label extender (LE), and blocker (BL) probes] were designed to mouse Ugt cDNA sequences obtained from GenBank. Probe sets for Ugt1a1, 1a2, 1a6, and 2b5 were previously designed (Chen *et al.*, 2003). Due to high DNA sequence similarity of Ugt2b5, 2b37, and 2b38, the Ugt2b5 oligonucleotide probe set designed for Ugt2b5 by Chen *et. al.* 2003 has a high probability of cross-reacting, and has been given the annotation Ugt2b5/37/38 to note this. Probe sets for mouse Ugt1a5, 1a7c, 1a8, 1a9, 1a10, 2a1/2, 2a3, 2b1, 2b34, 2b35, 2b36, 3a1/2 were designed by the following methods from cDNA sequences from NCBI (Table 1). Target sequences were designed by Probe Designer software version 1.0 (Bayer Corp.-Diagnostics Division, Tarrytown, NY), as described in previous studies in our laboratory (Hartley and Klaassen, 2000). All probes were designed with a T_m of approximately 63°C, and to ensure minimal cross reactivity with other mouse sequences, each sequence was submitted to BLASTn for nucleotide comparison. Probes with >80% sequence homology to other mouse sequences were eliminated from the probe design. Probe sets were synthesized by Operon Technologies (Alameda, CA) or Integrated DNA Technologies (Coralville, IA) and diluted in 1X Tris-EDTA buffer (pH 8.0) and stored at –20°C. Similar to the Ugt2b5/37/38 probe set, sequence homology required probe sets for Ugt2a1/2 and Ugt3a1/2 to cross-react with, and detect, two genes; probe sets are named accordingly. A list of the newly designed oligonucleotide probe sets is located in Supplemental Data.

Branched DNA Signal Amplification (bDNA) Assay: Individual mouse Ugt mRNA transcripts were detected using the QuantiGene® bDNA signal amplification

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assay (Genospectra Inc, Fremont, CA). The bDNA assay was performed as described and validated previously (Hartley and Klaassen, 2000). Briefly, CE, LE, and BL probes were combined and diluted into Lysis buffer. Total RNA (1μg/μl; 10μl) was added to each well of 96-well plates containing 50 μl of capture hybridization buffer and 50 μl of diluted probe set, and allowed to hybridize at 53°C overnight. Plates were cooled to 46°C and rinsed twice with wash buffer. Amplifier reagent (100 μl), diluted 1:1000 in amplifier/label probe buffer, was added to each well and incubated at 46°C for 1 hr. Plates were rinsed again with wash buffer and label reagent (100 μl), diluted 1:1000 in amplifier/label probe buffer, and incubated at 46°C for 1 hr. Plates were rinsed in wash buffer, and substrate reagent (100 μl) was added to each well. Alkaline phosphatase luminescence was activated by the addition of dioxetane substrate reagent. Plates were incubated for 1 hr at 37°C, and luminescence was measured with the Quantiplex 320 bDNA Luminometer (Bayer Corp. Diagnostics Div.). Analysis of luminescence from the 96-well plates was performed by Quantiplex Data Management Software version 5.02 (Bayer Diagnostics). Luminescence for each well is reported as Relative Light Units (RLU) per 10 μg Total RNA.

Statistical Analysis: Data are presented as a mean with bars representing standard error of the mean (S.E.M.). Gender differences were determined by a two-tailed Student's t-Test. Asterisks represent statistical differences ($p \leq 0.05$) in Ugt mRNA levels between males and females.

Results:

Tissue Distribution of Mouse Ugt1a Subfamily Genes: The tissue- and gender-specific mRNA expression of Ugt1a1, 1a2, 1a5, and 1a6 genes are illustrated in Fig 1. Ugt1a1, the bilirubin-conjugating isozyme, mRNA was highly expressed in liver in a female-predominant pattern (Fig 1). Ugt1a1 mRNA expression was also observed at low levels in duodenum, jejunum, ileum, and large intestine, whereas expression was negligible in all other tissues examined. Ugt1a2 mRNA was highly, and almost exclusively expressed in female kidney (Fig 1). Ugt1a5 mRNA transcripts were expressed in a similar pattern to Ugt1a1. Ugt1a5 mRNA was expressed highest in liver, where expression was predominant in females (Fig 1). Low levels of Ugt1a5 were observed in lung and gastrointestinal tract, where males expressed more Ugt1a5 in jejunum than females. Expression of Ugt1a5 was observed at considerably lower levels in kidney, brain, gonads, and placenta. The phenol-conjugating isozyme, Ugt1a6, mRNA was more ubiquitously expressed (Fig 1). Ugt1a6 mRNA was expressed to the highest extent in liver and large intestine, followed by lung, stomach, and gonads. Low levels of Ugt1a6 were also detected in kidney and small intestine. Additionally, a gender difference was also observed for Ugt1a6 in lung where males expressed higher levels than female mice.

The tissue- and gender-specific mRNA expression profiles of mouse Ugt1a7c, 1a8, 1a9, and 1a10 are illustrated in Fig 2. Ugt1a7c mRNA was primarily expressed in intestine and kidney (Fig 2). Ugt1a7c transcripts were highest in large intestine, followed by duodenum, kidney, jejunum, ileum, and stomach. No gender differences were observed for mouse Ugt1a7c. Ugt1a8 was expressed at very low levels in all mouse

tissues examined, exhibiting high background readings, raising concerns whether Ugt1a8 mRNA is truly expressed in tissues examined (Fig 2). Ugt1a9 mRNA was mainly expressed in liver, with very low levels detected in the other tissues examined (Fig 2). Hepatic Ugt1a9 expression tended to be higher in female than male mice, but the difference was not statistically significant. Ugt1a10 was detected at low levels in several tissues (Fig 2); most notable were kidney, liver, and stomach, where expression in kidney was female-predominant.

Tissue Distribution of Mouse Ugt2a Subfamily: Figure 3 illustrates the mRNA expression of the mouse Ugt2a subfamily. Ugt2a1/2 mRNA was highly and exclusively expressed in nasal epithelia in a female-predominant manner (Fig 3). Ugt2a3 mRNA, like human UGT2A2, was detected at high levels in liver, with lower levels expressed in duodenum and jejunum (Fig 3).

Tissue Distribution of Mouse Ugt2b Subfamily Genes: The tissue- and gender-specific expression of Ugt2b mRNAs are shown in Figure 4. Mouse Ugt2b1, named for its orthology to rat UGT2B1, was highly, and almost exclusively expressed in liver in a male-predominant pattern (Fig 4). Ugt2b5/37/38 mRNA was detected at high levels in both liver and kidney (Fig 4), whereas all other tissues examined had minimal expression. Ugt2b5/37/38 mRNA expression in kidney was higher in male than female mice. In contrast to Ugt2b5/37/38, Ugt2b34 transcripts were expressed at high levels in liver and the entire gastro-intestinal tract (Fig 4). Low levels of Ugt2b34 mRNA were also detected in kidney. Ugt2b35 was expressed, to some extent, in all tissues examined (Fig 4). Specifically, Ugt2b35 mRNA was highly expressed in liver, stomach, and duodenum; moderately expressed in kidney, stomach, jejunum, ileum, and large intestine; and

showed low expression in lung, heart, brain, gonads, and placenta. A gender difference was observed for Ugt2b35 in brain, where expression was higher in females than in male mice. Ugt2b36 mRNA, like Ugt2b1, was highly expressed in liver, with low expression levels in several tissues (Fig 4).

Tissue Distribution of Mouse Ugt3a Subfamily: Ugt3a1/2 mRNA was highly expressed in kidney, at low levels in liver, and not detected in other tissues examined (Fig 5).

Tissue Distribution of Mouse UDP-glucose pyrophosphorylase and UDP-glucose dehydrogenase: Figure 6 illustrates the tissue- and gender-specific mRNA expression patterns of the two key enzymes responsible for UDP-glucuronic acid synthesis. UDP-glucose pyrophosphorylase (UDP-gpp) mRNA was highly expressed in liver, kidney, stomach, large intestine, and heart. Moderate expression was observed in brain and ovary. Lower levels of mRNA expression were detected in lung, duodenum, jejunum, ileum, and placenta. Females expressed higher levels of UDP-gpp in gonads than males. UDP-glucose dehydrogenase (UDP-gdh) mRNA was highly expressed in the mouse gastrointestinal tract, including stomach, duodenum, jejunum, ileum, and large intestine. Lower levels of UDP-gdh mRNA were observed in liver, kidney, lung, heart, brain, gonad, and placenta. The observed level of UDP-gdh in liver was higher in male than female liver. Conversely, UDP-gdh mRNA was expressed higher in females in both brain and gonads.

Discussion:

UDP-glucuronosyltransferases conjugate and generally detoxify various lipophilic endo- and xenobiotics to more hydrophilic compounds that are readily excreted via bile and urine (Dutton, 1980). UGTs are expressed in a wide variety of tissues and are responsible for xenobiotic biotransformation and metabolism of endogenous compounds (Radominska-Pandya *et al.*, 1999; Tukey and Strassburg, 2000; Turgeon *et al.*, 2001). Therefore, glucuronidation undoubtedly influences the pharmacokinetic profiles of drugs in tissues with high glucuronidation capacity. Previous studies indicate that UGT mRNA expression, protein expression, and enzyme function correlate. In vitro, PPARα ligands increase human UGT2B4 mRNA and protein expression as well as increase glucuronidation of bile acids (Barbier et al., 2003). In UGT humanized transgenic mice, human UGT1A1, 1A4, and 1A6 mRNA and protein levels correlate in liver (Chen et al., 2005). Likewise, induction of mouse Ugt1a1 and 1a9 mRNA by PCN led to increase glucuronidation of bilirubin, etc. by liver microsomes (Chen et al., 2003). With this in mind, the bDNA assay was used in this study to determine the tissue- and gender-specific mRNA expression profiles of mouse Ugts.

Several UGT1A genes are expressed in liver of humans, rats, and mice. In adult human liver, UGT1A1, 1A3, 1A4, 1A6, and 1A9 have been detected by RT-PCR and Northern-blot analysis and are responsible for bilirubin and phenol glucuronidaton, amongst other substrates (Basu et al., 2004; Mojarrabi and Mackenzie, 1998; Radominska-Pandya et al., 1999; Strassburg et al., 1997). Previous studies indicate that rat UGT1A1 and 1A6 are highly expressed in adult liver; UGT1A3, 1A5, 1A7, and 1A8 transcripts are also detectable in liver (Emi *et al.*, 1995; Grams *et al.*, 2000; Shelby *et al.*,

2003). Our findings indicate four highly expressed Ugt1a isoforms in adult mouse liver, namely Ugt1a1, 1a5, 1a6, and 1a9. Expression of Ugt1a1, 1a5, and 1a9 was higher in liver than any other tissue examined; the abundance of Ugt1a6 in liver and large intestine were comparable. The roles of Ugt1a8 and Ugt1a10 in mouse liver, if any, are not currently known, but further information on their substrate specificity may uncover their contribution to murine hepatic metabolism. To compare rodent species, both rats and mice have highest expression of Ugt1a1 and 1a6 in liver (Shelby et al., 2003), whereas mice also highly express Ugt1a5 and 1a9 in liver. For rodent-to-human comparisons, both humans and mice express UGT1A1, 1A6, and 1A9 mRNA in liver. Table 2 summarizes data regarding the tissue distribution of human, rat, and mouse UGT isozymes.

Similar to UGT1A genes, several UGT2B genes are expressed in liver. In humans, UGT2B4, 2B7, 2B10, 2B11, 2B15, and 2B17 are expressed in liver and conjugate endogenous androgens, estrogens, and bile acids, in addition to numerous xenobiotics (Beaulieu et al., 1996; Beaulieu et al., 1998; Levesque et al., 1997; Levesque et al., 1999; Radominska-Pandya et al., 1999; Shelby et al., 2003; Turgeon et al., 2001). Shelby *et al.* (2003) concluded that UGT2B1, 2B2, 2B3, 2B6, and 2B12 are highly expressed in rat liver. Our results indicate that each of the five probe sets detecting Ugt2b genes exhibit high expression in liver, including the rat ortholog Ugt2b1 as well as 2b5/37/38, 2b34, 2b35, and 2b36. Like UGT2B1 in rat, mouse Ugt2b1 is almost exclusively expressed in liver, but is expressed in a female-predominant manner in rats (Shelby et al., 2003) and male-predominant manner in mice. Like UGT1A genes, quantitative comparisons between rodent and human mRNA cannot be accurately made

due to detection techniques. However, as noted above, several UGT2B genes are expressed in livers of both humans and mice. The substrate specificity of the mouse Ugt2b family has yet to be determined, therefore more data is needed to determine functional significance. Based on gene expression data, it is obvious that the mouse Ugt2b subfamily's role in biotransformation largely occurs in liver.

Several Ugts were expressed in the mouse gastrointestinal tract. Numerous studies have shown the importance of human UGTs in the gastrointestinal tract, implicating the role of intestinal UGTs in the conjugation and detoxification of numerous food-derived compounds such as flavonoids, alcohols, aldehydes, and ketones, particularly UGT1A7, 1A8, and 1A10 (Basu et al., 2004; Cheng et al., 1998; Mojarrabi and Mackenzie, 1998; Strassburg et al., 1998a). In rats, UGT1A1, 1A3, 1A6, and 1A7 are highly expressed in portions of the small and large intestine (Emi *et al.*, 1995; Grams *et al.*, 2000; Shelby *et al.*, 2003). Only one mouse gene, Ugt1a7c, was most highly expressed in the GI tract, decreasing distally through the small intestine, but reaching highest levels in large intestine. Ugt1a6 was expressed highly in the large intestine where levels were similar to that in liver. Additionally, only two Ugt2b genes, 2b34 and 2b35, were noticeably expressed in stomach as well as small and large intestine. Mouse Ugt1a7c and rat UGT1A7 share considerable expression similiarities; both are predominantly expressed in small and large intestine (Shelby *et al.*, 2003), whereas human UGT1A7 is expressed in esophagus and stomach but not in intestine (Strassburg et al., 1998b). Both humans and mice express, to some extent, UGT1A1, 1A6, 1A7, and 1A10 along the gastrointestinal tract (Strassburg et al., 1998b). As for the UGT2B subfamily, UGT2B7, 2B10, and 2B15 are expressed in human esophagus (Tukey and

Strassburg, 2000), whereas murine Ugt2b34 and 2b35 are present in stomach and large intestine.

Mouse Ugt1a8 expression was expected in intestine. However, Ugt1a8 was not detected above background signals in all tissues, raising concerns that mouse Ugt1a8 mRNA may not be expressed. Mouse Ugt1a8 probe set was designed to a portion of the mouse BAC RP23-396I23 (Accession number: AC087801; Bases: 91725-92580) that is highly homologous to rat UGT1A8. No expressed cDNAs or expressed sequence tags (ESTs) were found by BLASTn on the NCBI website. Therefore, mouse Ugt1a8 may represent a psuedogene.

Mouse Ugts are widely expressed in a variety of other tissues (Table 2), some of which are involved in pharmacokinetics and others involved in endogenous steroid metabolism. Kidney is well known for urinary excretion. Several studies in humans and rats have identified the presence of UGT isoforms in kidney, specifically human UGT1A3, 1A9, 2B4, 2B7, 2B10, 2B11, 2B15, and 2B17 and rat UGT1A1, 1A6, 1A7, and 2B8 (Beaulieu et al., 1996; Beaulieu et al., 1998; Emi et al., 1995; Grams et al., 2000; Levesque et al., 1997; Levesque et al., 1999; Shelby et al., 2003; Turgeon et al., 2001). In the present study, six members of mouse Ugt1a and Ugt2b families were highly expressed in kidney. Both rats and mice express Ugt1a7 in kidney, suggesting species similarities amongst rodents.

In lung, six human UGT2B (4, 7, 10, 11, 15, and 17) isozymes have been detected (Turgeon et al., 2001), whereas three, UGT1A6, 1A7 (Emi et al., 1995) and 2B12 (Emi et al., 1995; Shelby et al., 2003), were detected in rat lung. In contrast, only one mouse

isoform, Ugt1a6, was moderately expressed in lung. The presence of Ugt1a6 in lung may be attributed to the demand for glucuronidation of phenolic compounds from inspired air.

Mouse Ugt expression was detected in steroid-sensitive tissues. Human UGT enzymes, particularly the UGT2B family, are expressed in steroid-sensitive tissues such as breast (mammary tissue), placenta, uterus, adrenal, prostate, and testis, and have been found to conjugate endogenous hormones (testosterone, DHT, androsterone, estriol, and some phenolic xenobiotics)(Turgeon *et al.*, 2001). As reviewed by Turgeon et al. (2001), four human UGT2B isoforms (4, 10, 15, and 17) are expressed in testis, whereas UGT1A1, 1A6, and 1A7 have been detected in rat testis (Emi *et al.*, 1995). Rat UGT1A6 is expressed in ovary, similar to what was observed here for mouse Ugt1a6 (Auyeung *et al.*, 2001). The same laboratory also determined that the tissue-specific expression of rat UGT1A6 is dependent on basal activation of two separate 5′-proximal-promoter regions, either P1 in spleen, lung, and ovary, or the P2 promoter in liver, kidney, and intestine (Auyeung *et al.*, 2001). In mice, Ugt1a6, the phenol conjugating isoform, was moderately expressed in testis and ovary, and only one Ugt2b gene, Ugt2b35, was expressed at low levels in mouse testis and ovary. Expression in testis and ovary may implicate mouse Ugt1a6 involvement in endogenous steroid conjugation. Mice may have a similar mechanism of controlling Ugt1a6 expression as noted above for rats based on its ubiquitous expression profile and the predicted alternative start site sequences (www.ensembl.org). In human placenta, six UGT2B members were expressed, however in this study, only Ugt2b35 was barely detectable in mouse placenta, implicating differences in placental biotransformation between species (Collier *et al.*, 2002). RNA

from other steroid-sensitive tissues was not analyzed in this study, leaving in question the expression of Ugts in such tissues.

Mouse Ugts were detected in whole brain homogenates. Glucuronidation of endogenous neurotransmitters, and possibly toxicants, occurs in the brain at the blood-brain barrier (King *et al.*, 1999). In humans, UGT1A6 and 2B7 are expressed in adult cerebellum (King *et al.*, 1999). Rats have minimal UGT expression in cerebellum and cerebral cortex (Shelby *et al.*, 2003). In this study, low expression of Ugt2b35 was detected in homegenates of whole mouse brain.

Human and rat UGT2A1 genes were cloned from and are exclusively expressed in nasal epithelia (Heydel et al., 2001; Jedlitschky et al., 1999; Shelby et al., 2003). Tukey and Strassburg (2001) illustrated that human UGT2A1 underwent gene duplication to form UGT2A2, whose tissue distribution differs from UGT2A1; UGT2A2 is expressed in small intestine and liver. Mouse Ugt2a1 and Ugt2a2 share high sequence homology and were detected together by a single bDNA probe set exclusively in nasal epithelia. However, Ugt2a3 is expressed in liver and small intestine, similar to human UGT2A2. Likewise, a novel subfamily of Ugt3a gene transcripts, named Ugt3a1 and 3a2 (Mackenzie et al., 2005), share significant sequence homology and were detected by a single probe set in liver and kidney in this study.

It is generally known that phase-II conjugation reactions require high-energy co-substrates. For glucuronidation, UDP-glucose pyrophosphosphorylase (UDP-gpp) and UDP-glucose dehydrogenase (UDP-gdh) metabolize glucose-1-phosphate to the high-energy co-substrate donor UDP-glucuronic acid (UDP-GA). UDP-gpp was expressed in a wide range of mouse tissues, including liver, kidney, stomach, small and large intestine,

heart, brain, and ovaries. This is not surprising due to the importance of UDP-glucose in general cellular intermediary metabolism, especially in liver. However, UDP-gdh was highly expressed in mouse GI tract, suggesting the importance for high-capacity glucuronidation of orally ingested xenobiotics in intestine, perhaps because of a decreased capacity to store UDP-GA in intestinal tissue versus liver.

The present study suggests potential gender differences in C57BL/6 mice. Female-predominant expression of Ugt1a1 in mouse liver correlates with gender differences in *in vivo* bilirubin glucuronidation in Wistar rats (Muraca and Fevery, 1984), suggesting potential functional gender differences in mouse bilirubin metabolism. Further evidence indicates rat UGT1A1 is repressed by testosterone in male rats and induced by progestins in female rats, highlighting the roles of sex hormones in regulating UGT basal transcription (Muraca and Fevery, 1984). Additionally, rat UGT2B3 is predominant in males, in which testosterone exhibited an inductive effect, and estrogen slightly reduced basal expression (Strasser *et al.*, 1997). In mouse liver, Ugt2b1 is male-predominant, whereas Ugt1a1 and 1a5 are female-predominant. In mouse kidney, Ugt2b5/37/38 is male-predominant, and conversely, Ugt1a2 and 1a10 are female-predominant. Whether these genes are repressed or induced by sex hormones, or alternative pathways such as growth-hormone-pattern secretion, has yet to be determined. Taken together, the results indicate that mouse Ugts are regulated by sex hormones.

Tissue-specific transcription factors control basal expression of UGTs. MacKenzie et al. (2003) reviewed tissue-specific transcription factors regulating human and rat UGTs. Those include Hepatocyte Nuclear Factor 1 alpha (HNF1α), CAAT-enhancer Binding Protein (C/EBP), Octamer transcription factor 1 (Oct1), and putative

pre-B cell homeobox-2 (Pbx 2), which can confer specific expression of distinct UGT isoforms in liver and intestine. Additionally, mice lacking C/EBP have deficiencies in hepatic expression of Ugt1a1 and Ugt2b (Lee *et al.*, 1997). These factors undoubtedly contribute to tissue-specific Ugt expression patterns observed in mice.

In the present study, eleven mouse Ugts were expressed in liver, seven in GI tract, and six in kidney. Gender-specific gene expression was observed in liver and kidney. Similarities in expression patterns between species (humans, rats, and mice) were observed in tissues and between orthologous isozymes. Taken together, these data can potentially be utilized to determine tissue-specific/dependent glucuronidation based upon substrate specificity and tissue localization of individual Ugt isoforms. Additional insights into gender-dependent differences in drug metabolism in vivo are shown here as well.

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Figure Legends:

Figure 1. Messenger RNA expression of Ugt1a1, 1a2, 1a5, and 1a6 in various tissues of male and female mice (n = 5/gender) by the bDNA assay. Data is expressed as Relative Light Units/ 10 μg of total RNA. Values are expressed as mean ± S.E.M.. Asterisks (*) indicate statistically significant differences between genders ($p \leq 0.05$).

Figure 2. Messenger RNA expression of Ugt1a7c, 1a8, 1a9, and 1a10 in various tissues of male and female mice (n = 5/gender) by the bDNA assay. Data is expressed as Relative Light Units / 10 μg of total RNA. Values are expressed as mean ± S.E.M.. Asterisks (*) indicate statistically significant differences between genders ($p \leq 0.05$).

Figure 3. Messenger RNA expression of Ugt2b1, 2b5/37/38, 2b34, 2b35 and 2b36 in various tissues of male and female mice (n = 5/gender) by the bDNA assay. Data is expressed as Relative Light Units / 10 μg of total RNA. Values are expressed as mean ± S.E.M.. Asterisks (*) indicate statistically significant differences between genders ($p \leq 0.05$).

Figure 4. Messenger RNA expression of Ugt2a1/2 and Ugt2a3 in various tissues of male and female mice (n = 5/gender) by the bDNA assay. Nasal epithelium is five replicates of a pooled sample for each male and female. Data is expressed as Relative Light Units / 10 μg of total RNA. Values are expressed as mean ± S.E.M.. Asterisks (*) indicate statistically significant differences between genders ($p \leq 0.05$).

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Figure 5. Messenger RNA expression of Ugt3a1/2 in various tissues of male and female mice (n = 5/gender) by the bDNA assay. Data is expressed as Relative Light Units / 10 μg of total RNA. Values are expressed as mean ± S.E.M.. Asterisks (*) indicate statistically significant differences between genders ($p \leq 0.05$).

Figure 6. Messenger RNA expression of mouse UDP-glucose pyrophosphorylase and UDP-glucose dehydrogenase in various tissues of male and female mice (n = 5/gender) by the bDNA assay. Data is expressed as Relative Light Units / 10 μg of total RNA. Values are expressed as mean ± S.E.M.. Asterisks (*) indicate statistically significant differences between genders ($p \leq 0.05$).

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Table 1. Nomenclature and Accession Numbers for Mouse Ugts.

Gene	Accession #
Ugt1a1	L02333
Ugt1a2	D87866
Ugt1a5	AY227196
Ugt1a6	U16818
Ugt1a7c	AY227199
Ugt1a8	AC087801[a]
Ugt1a9	L27122
Ugt1a10	AY227201
Ugt2a1	NM_053184
Ugt2a2	BC058786
Ugt2a3	NM_028094
Ugt2b1	NM_152811
Ugt2b5	X06358
Ugt2b34	NM_153598
Ugt2b35	NM_172881
Ugt2b36	NM_132178
Ugt2b37	NM_053215
Ugt2b38	NM_133894
Ugt3a1	NM_207216
Ugt3a2	BC034837
UDP-gpp	NM_139297
UDP-gdh	NM_009466

[a]**Bases 91725-92580**

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Table 2. Summary and comparison of the tissue distribution of Human, Rat, and Mouse UGTs in liver, stomach, small intestine, colon, and kidney

Note: Genes listed for human are based upon presence in tissue. Genes listed for rodents are based on high expression from bDNA data.

Tissue	UGT Family	Human[a]	Rat[a]	Mouse
Liver	*1A*	1,3,4,6,9	1,5,8	1,5,6,9
	2A/B	B4,7,10,11,15,17	B1,2,3,6,12	a3, b1,5,34,35,36,37,38
	3A	—	—	1,2
Stomach	*1A*	3,6,7,10	1	—
	2B	10	8	34,35
Small	*1A*	1,3,4,5,6,10	1,2,3,6,7	7
	2A/B	A2, B4,7,10,15,17	8	*b34* ,35
Large Intestine	*1A*	1,3,4,6,8,9,10	1,3,6,7	6,7
	2B	7,10,15	—	34,35
Kidney	*1A*	9	1,6,8	2,7,10
	2B	4,7,10,11,15,17	12	5,35
	3A	—	—	1,2

[a]References for Human (Beaulieu *et al*., 1996, 1998; Levesque *et al*., 1997,1999; Strassburg *et al*., 2000; Tukey and Strassburg, 2001; Turgeon *et al*., 2001; Basu *et al*., 2004) and Rat (Shelby *et al*., 2003)

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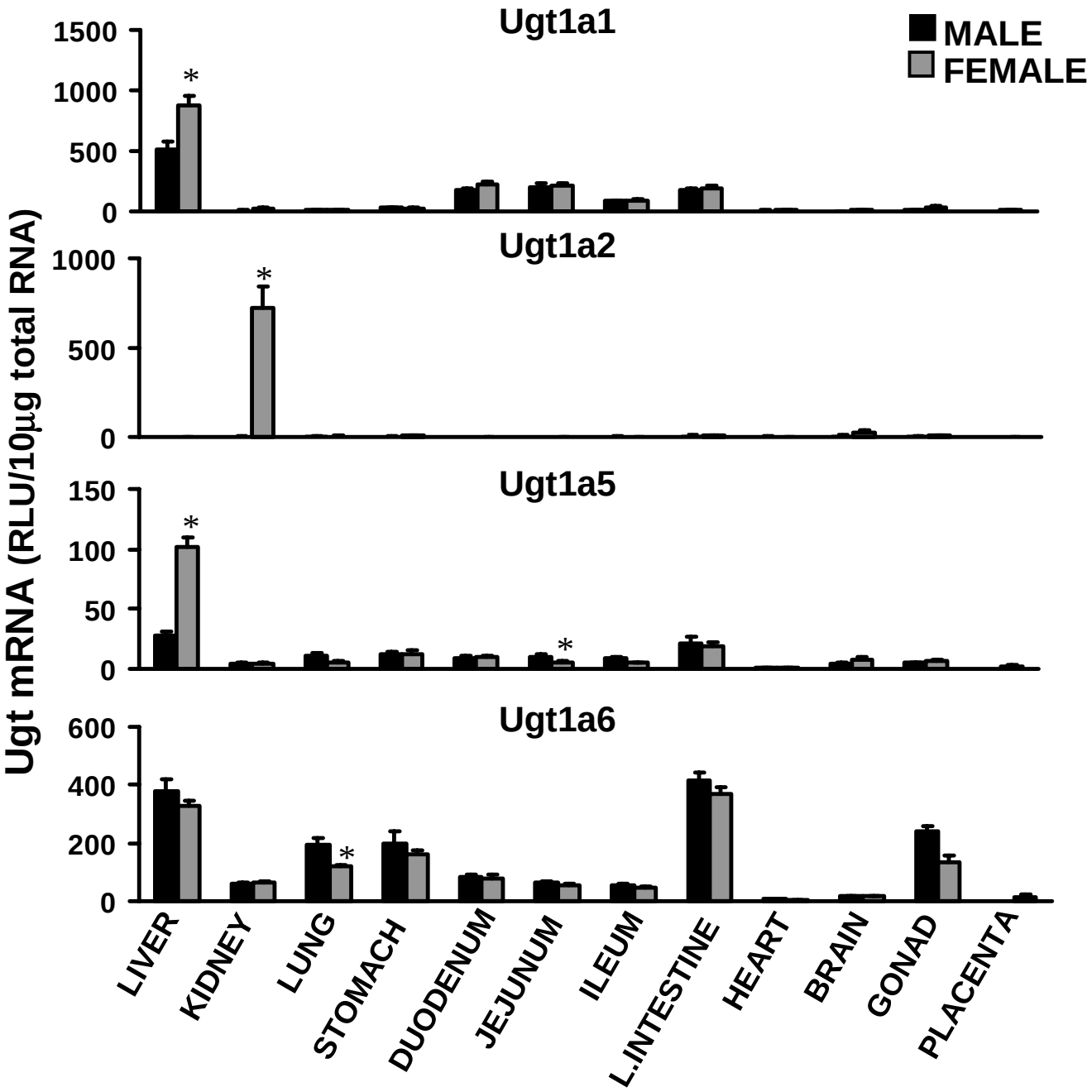

Fig 1

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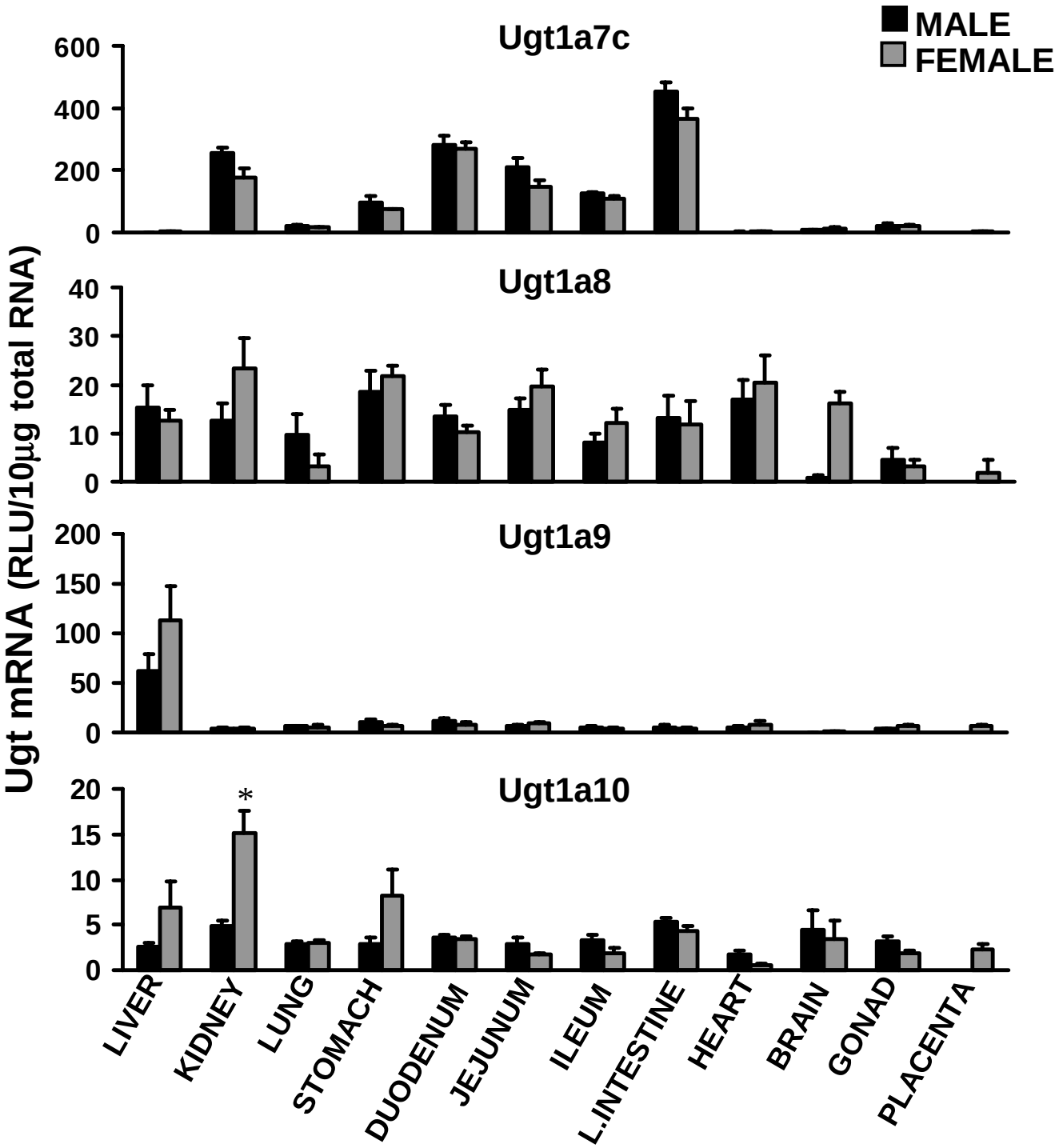

Fig 2

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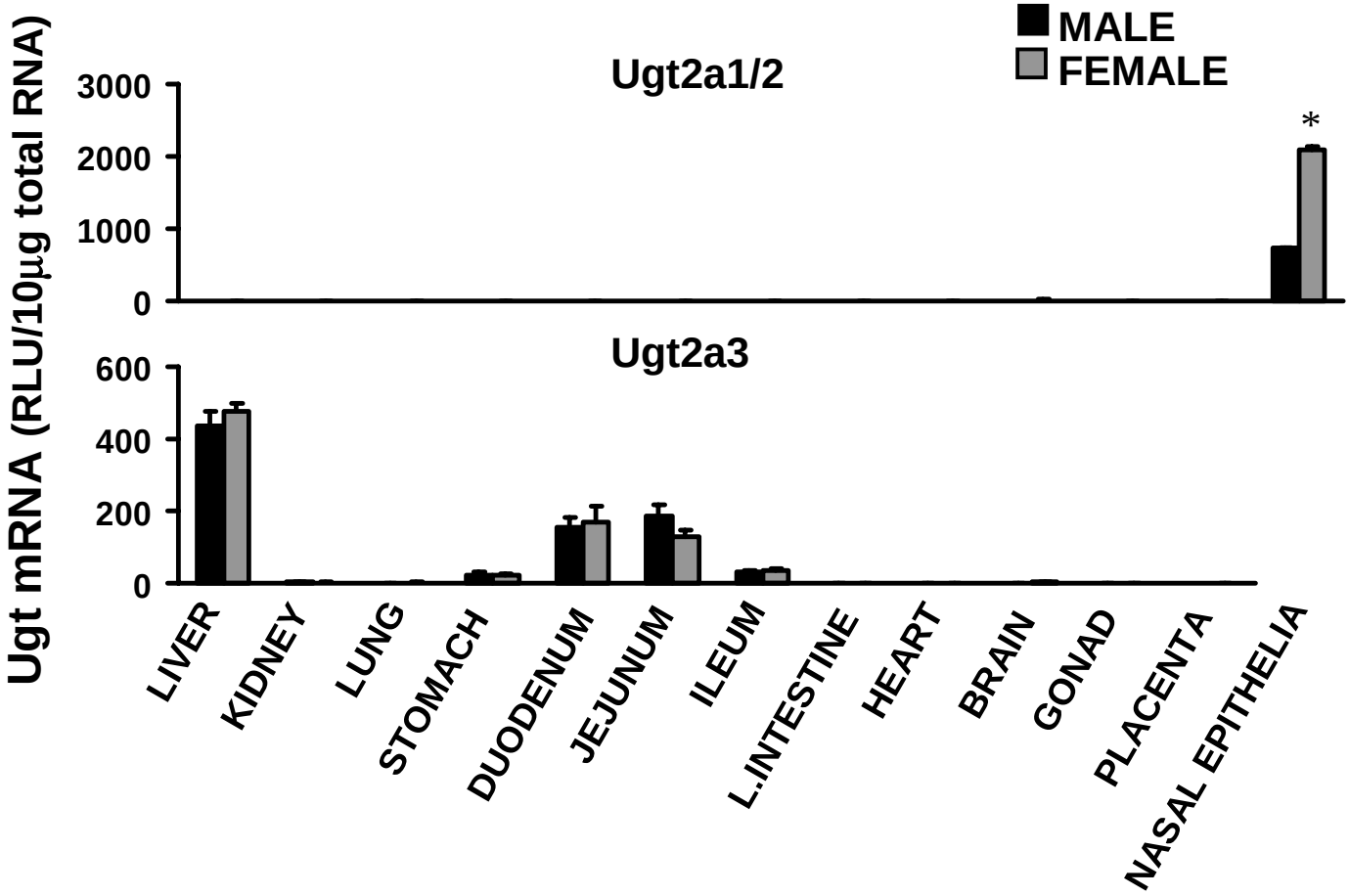

Fig 3

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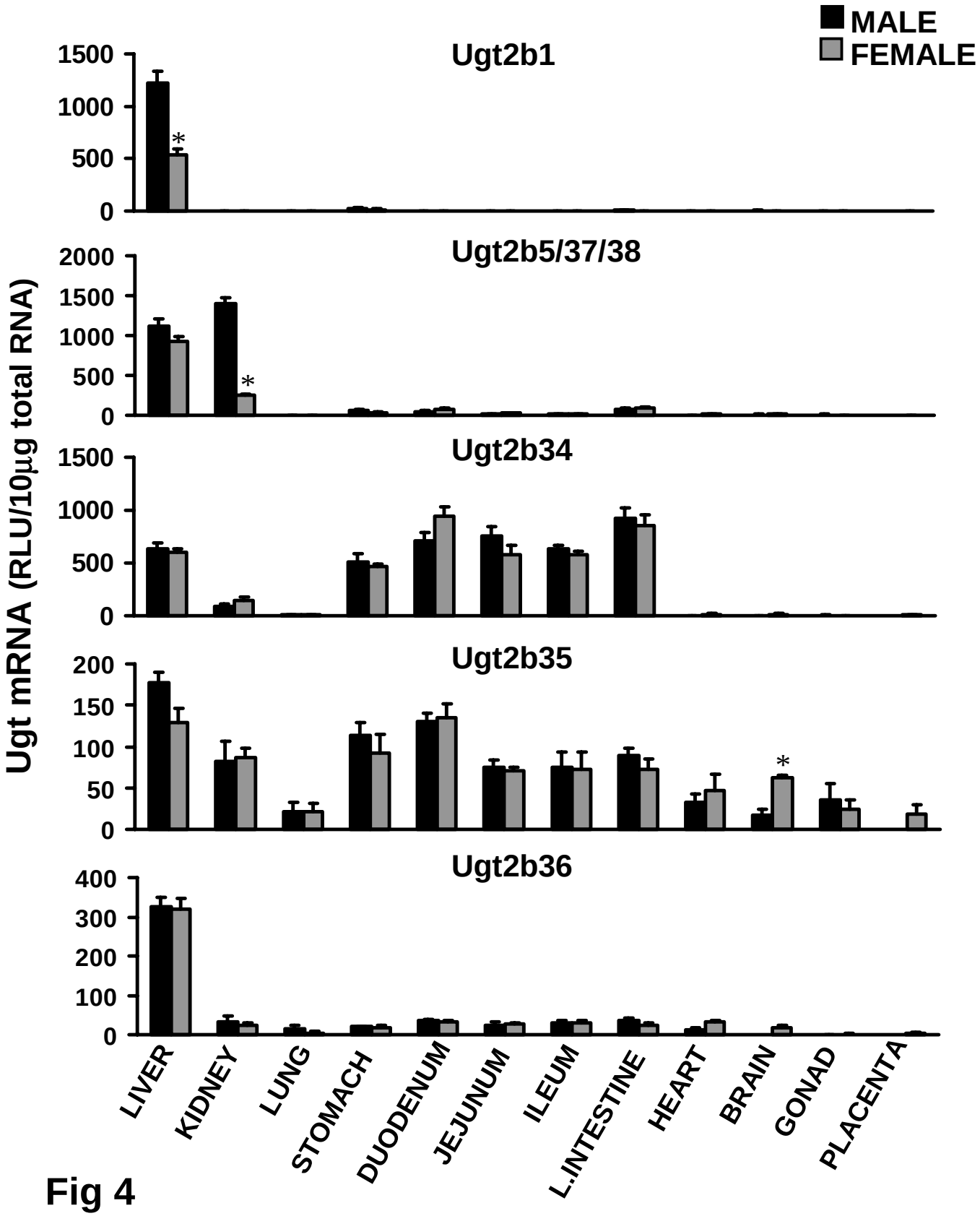

Fig 4

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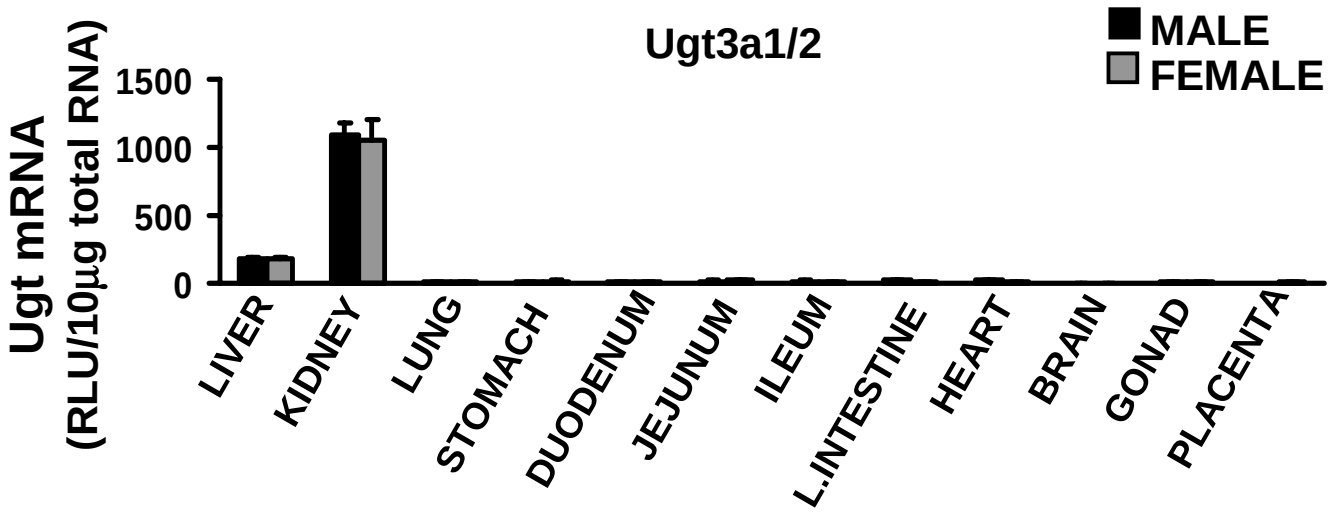

Fig 5

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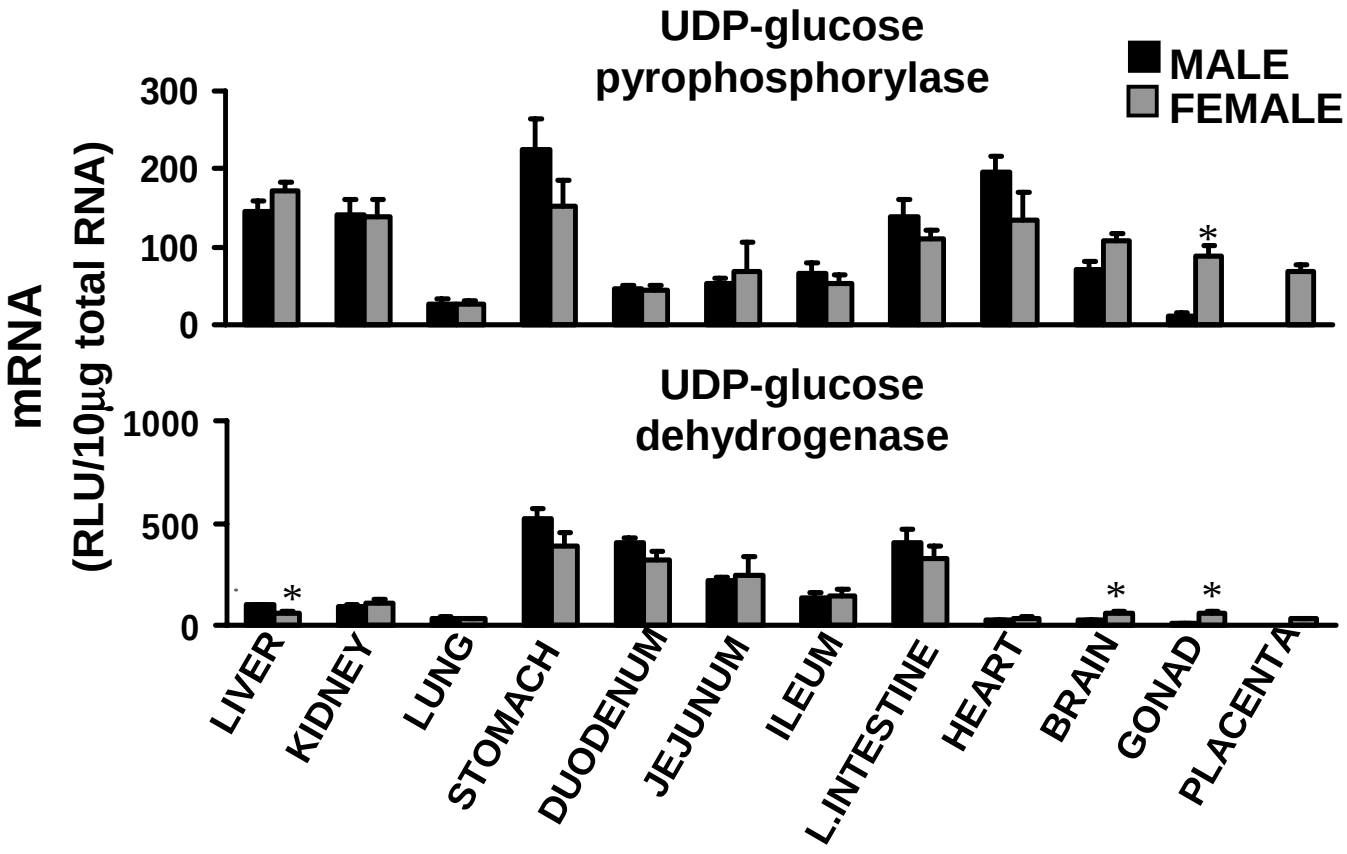

Fig 6