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Quantitative Analysis of Ugt1a and Ugt2b mRNA Expression in Rat Liver and Small Intestine: Sex and Strain Differences

Takaya Kutsukake, Yoichi Furukawa, Kyoko Ondo, Saki Gotoh, Tatsuki Fukami, and Miki Nakajima

Drug Metabolism and Toxicology, Faculty of Pharmaceutical Sciences (T.K., Y.F., K.O., S.G., T.F., M.N), WPI Nano Life Science Institute (T.F., M.N), Kanazawa University, Kakuma-machi, Kanazawa 920-1192, Japan

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Running Title: Ugt1a and Ugt2b mRNA levels in rat liver and small intestine

Corresponding author: Miki Nakajima, Ph.D., Drug Metabolism and Toxicology, Faculty of Pharmaceutical Sciences, Kanazawa University, Kakuma-machi, Kanazawa 920-1192, Japan.
E-mail: nmiki@p.kanazawa-u.ac.jp

Number of text pages:

Number of tables: 0

Number of figures: 2

Number of reference: 36

Number of words in the Abstract: 239

Number of words in the Introduction: 641

Number of words in the Discussion: 1324

Abbreviations:

F344, Fischer344; PCR, polymerase chain reaction; RT, reverse transcription; UGT, UDP-glucuronosyltransferase; UDPGA, UDP-glucuronic acid; SD, Sprague-Dawley

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Abstract

UDP-glucuronosyltransferases (UGTs) catalyze the glucuronidation of numerous endogenous and exogenous compounds to facilitate their excretion from the body. Rats are most widely used in nonclinical studies. Information regarding UGT species differences between rats and humans would be helpful for understanding human pharmacokinetics. In this study, we determined the absolute mRNA expressions of Ugt isoforms in the livers and small intestines of male and female Sprague-Dawley, Fischer344 and Wistar rats. The sum of the mRNA levels of Ugt isoforms expressed in the liver was significantly ($P < 0.005$) higher than that in the small intestine regardless of the strain and sex. Ugt2b mRNA levels represented approximately 80% of the total Ugt mRNA levels in the liver, while Ugt1a mRNA levels accounted for almost 90% in the small intestine. Ugt2b2 mRNA was specifically expressed in the livers of Wistar rats, resulting in a 2-fold higher expression of the total hepatic Ugt mRNA in Wistar rats than that in the other strains. In the small intestine, Wistar rats showed prominently higher Ugt2b3 and Ugt2b8 mRNA levels than the other strains. The difference between sexes was remarkable with regards to the hepatic Ugt1a10 in any of the strains, although slight differences between the sexes were also observed in multiple Ugt isoforms. Taken together, this study revealed sex and strain differences in the mRNA levels of rat Ugts. The data shown here would be useful for the selection of rat strains in nonclinical studies.

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Introduction

UDP-glucuronosyltransferases (UGTs) are responsible for the glucuronidation of a wide range of structurally diverse endogenous and exogenous substances (Tukey and Strassburg 2000; Mackenzie et al., 2005). UGTs conjugate glucuronic acid to substrates to increase their water solubility and enhance their excretion through bile and urine (Dutton, 1980); therefore, glucuronidation is generally considered to be a detoxification reaction. UGT activities significantly affect the pharmacokinetic and pharmacodynamic profiles as well as the toxicities of drugs. In humans, 40–70% of all clinical drugs are subjected to glucuronidation by UGTs (Wells et al., 2004). UGTs are widely distributed throughout the body and have been found in the intestines, kidney, brain, pancreas, and placenta, but most of them are found in the liver, which is the primary detoxification organ (Strassburg et al., 2000; Collier et al., 2002; Izukawa et al., 2009; Knights et al., 2013).

In humans, 19 functional UGT isoforms comprise 2 families (UGT1A and UGT2) based on genetic similarity (Guillemette et al., 2010). The *UGT1A* gene consists of multiple unique first exons and common exons 2 to 5 (Gong et al., 2001). There are nine functional UGT1A isoforms (UGT1A1, UGT1A3, UGT1A4, UGT1A5, UGT1A6, UGT1A7, UGT1A8, UGT1A9 and UGT1A10). The *UGT2* gene family includes three UGT2A isoforms (UGT2A1, UGT2A2 and UGT2A3) and seven UGT2B isoforms (UGT2B4, UGT2B7, UGT2B10, UGT2B11, UGT2B15, UGT2B17 and UGT2B28) (Riedy et al., 2000; Remmel et al., 2008). Similar to UGT1A family members, UGT2A1 and UGT2A2 are encoded by differential first exons that are spliced to common exons 2 to 6 of a single gene (Sneitz et al., 2009; Mackenzie et al., 2005). UGT2A3 and UGT2B isoforms are encoded by unigenes.

Rats are commonly used in non-clinical drug development studies to investigate the pharmacokinetic profiles or toxicity of new chemical entities. Since Ugts exert a critical influence on the pharmacokinetics of drugs, species differences in Ugt expression profiles between humans and rats should be elucidated. In rats, there are 18 functional Ugt isoforms that are classified into three subfamilies: Ugt1a, Ugt2a, and Ugt2b (Mackenzie and Rodbourn,

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1990; Haque et al., 1991). The rat *Ugt1a* gene cluster is located on chromosome 9q35 and consists of multiple unique first exons and common exons 2 to 5. There are eight Ugt1a isoforms (Ugt1a1, Ugt1a2, Ugt1a3, Ugt1a5, Ugt1a6, Ugt1a7, Ugt1a8 and Ugt1a10) with two pseudogenes (Ugt1a4 and Ugt1a9) (Emi et al., 1995). The rat *Ugt2* gene family located on chromosome 14q21 includes three members of the Ugt2a subfamily and seven members of the Ugt2b subfamily (Klaassen and Parkinson, 2001). Rat Ugt2a1 and Ugt2a2 are formed by alternative splicing of variable first exons and common exons 2 to 6, whereas the Ugt2a3 and Ugt2b subfamily (Ugt2b1, Ugt2b2, Ugt2b3, Ugt2b6, Ugt2b8, Ugt2b12 and Ugt2b34) are encoded by unigenes (Emi et al., 1995; Mackenzie et al., 2005). The tissue distribution of the mRNA expression of rat Ugt1a and Ugt2b has been previously reported (Shelby et al., 2003). However, a quantitative evaluation remains to be performed. Since the glucuronidation potential depends on the UGT isoforms expressed in the tissues and their abundances, information about the absolute expression levels of individual Ugts in rat tissues would be useful to estimate the contribution of glucuronidation to pharmacokinetics or toxicity.

Glucuronidation occurs primarily in the liver, but it has become clear that glucuronidation in extrahepatic tissues has a significant impact on the pharmacokinetics and bioavailability of UGT substrates (Fisher et al., 2001). Actually, it has been reported that rat intestinal UGT contributes to the first-pass effect (Xu et al., 2009, Furukawa et al., 2012). The purpose of this study is to determine the absolute expression levels of rat *Ugts* mRNA in the liver and small intestine as well as clarify sex and strain differences in their expression levels using male and female Sprague-Dawley (SD), Fischer344 (F344) and Wistar rats that are all commonly used in nonclinical studies.

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Materials and Methods

Materials. RNAiso Plus, random hexamer, and SYBR Premix Ex *Taq* were purchased from Takara Bio (Shiga, Japan). ROX and ReverTra Ace were from Stratagene (La Jolla, CA) and Toyobo (Tokyo, Japan), respectively. Primers were commercially synthesized at Rikaken (Tokyo, Japan). All other reagents were of the highest grade commercially available.

Animals. Male and female (7-week old) Sasco Sprague-Dawley (SD) rats (140-230 g), Fischer344 (F344) rats (110-140 g), and Wistar rats (150-230 g) were purchased from Japan SLC (Hamamatsu, Japan). The animals were housed in the institutional animal facility in a controlled environment (temperature 25°C and 12-h light/dark cycle) with access to food and water *ad libitum*. Rats were acclimatized for three days before use and maintained in accordance with the National Institutes of Health Guide for Animal Welfare of Japan, as approved by the Institutional Animal Care and Use Committee of Kanazawa University.

Total RNA from rat liver and small intestine. The total RNA was isolated from 50-100 mg rat liver and small intestinal mucosa using RNAiso plus according to the manufacturer's instructions (https://takara.co.kr/file/manual/pdf/9108_9109_e.v1301Da.pdf#search=%27RNAiso%27). Duodenum ~ upper jejunum was used as small intestine, because UGT activities in these parts are relatively higher than those in lower jejunum and ileum (Shiratani et al, 2008). The integrity of the extracted RNA was confirmed by the ratio of the band intensities of the 28S and 18S ribosomal RNAs.

Real-time RT-PCR. cDNA was synthesized from 4 μg of total RNA extracted from the rat liver and small intestine using ReverTra Ace with random hexamer. cDNA samples from each individual rat were pooled within groups (3 rats per each strain and sex). A 1 μL portion of the pooled cDNA was added to a PCR mixture containing 10 pmol of each primer

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(Supplementary Table 1), 12.5 μL of SYBR Premix Ex Taq solution and 75 nM Rox in a final volume of 25 μL. For Ugt1a mRNAs, sense primers were set at the specific region in the exon of each isoform, and the antisense primer set at exon 2 was common for all Ugt1a isoforms, except for Ugt1a5 (Supplementary Table 1). For Ugt1a5, another antisense primer was designed because the amplification efficiency with the former antisense primer was low. For Ugt2b mRNAs, sense and antisense primers were designed at the specific regions within exon 1 and exon 2, respectively.

The PCR conditions were as follows: after an initial denaturation at 94°C for 25 sec, the amplification was performed by denaturation at 94°C for 7 sec, while the annealing temperature and extension time are listed in Supplementary Table 1 for 40 cycles. The real-time RT-PCR was performed using a Mx3000P instrument (Stratagene, La Jolla, CA) with the MxPro QPCR software. Amplified PCR products were monitored by measuring the increase of the fluorescence intensity of the SYBR Green I. A calibration curve was constructed using known amounts of PCR product. The copy number was calculated for each sample based on the calibration curve. Specific amplification was confirmed by digestion of PCR amplicon with appropriate restriction enzymes as needed. Percentage of the expression level of each Ugt isoform was calculated using the sum of the copy numbers of all Ugt isoforms.

Statistical analyses. A three-factor ANOVA model with factors for sex, strain, tissue, and their interactions was used to compare group means. Further analyses involving either one-way ANOVA (followed by Tukey's HSD for pairwise comparisons) or two sample t-tests were performed when significant interactions were observed among the factors in the three-way model. $P < 0.05$ was considered to be statistically significant.

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Results

Absolute expression levels of Ugt1a mRNAs in the rat liver and small intestine.

Absolute Ugt1a mRNA levels in the rat liver and small intestine are shown in Supplementary Table 2, and visualized in Fig. 1A. Ugt1a1 mRNA levels in the liver were 3- to 29-fold higher than those in the small intestine in any strain. Regardless of the sex, F344 rats and Wistar rats have the highest Ugt1a1 level in the liver and small intestine, respectively. In SD and F344 rat livers as well as SD and Wistar rat small intestines, the Ugt1a1 mRNA was significantly higher in the females than in the males. Whereas, in F344 rat intestines, the Ugt1a1 mRNA was significantly higher in the males than in the females, and no sex difference was observed in Wistar rat livers. Ugt1a2 mRNA levels in the small intestines were 46- to 211-fold higher than that in the livers of any strain. The Ugt1a2 mRNA level was the highest in F344 male rat small intestines. In SD and Wistar rat small intestines, the Ugt1a2 mRNA levels were significantly higher in females than in males. Higher expression in females was also observed for hepatic Ugt1a2 levels. Whereas, an opposite difference between sexes was observed in F344 rat small intestines. Ugt1a3 mRNA levels were higher in the small intestine than in the livers in any strain. The hepatic Ugt1a3 mRNA levels were significantly higher in females than in males. Such sex differences were also observed in SD and Wistar rat intestines, whereas the opposite sex difference was observed in F344 rat small intestines. Ugt1a5 mRNA levels were 16-fold higher in the livers than in the small intestines in any strain. A difference between sexes was observed in the Ugt1a5 levels in SD rat livers. In females, SD rats showed significantly higher hepatic Ugt1a5 levels than F344 and Wistar rats.

Ugt1a6 mRNA levels in the small intestine were slightly higher than those in the liver in any strain. The largest difference was observed in female SD rats (2.7-fold). In both the livers and small intestines, Ugt1a6 mRNA levels were highest in male Wistar rats, with a significant difference between the sexes. Similar differences between the sexes were observed in F344 rat livers and small intestines, but an opposite difference between sexes was observed

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in the Ugt1a6 levels in SD rat small intestines. The Ugt1a7 mRNA levels in small intestines were 13- to 34-fold higher than those in the livers of any strain. The level was the highest in male Wistar rats, with a significant difference between the sexes. Similar sex differences were observed in F344 rat small intestines and Wistar rat livers, but an opposite difference between the sexes was observed in SD rat small intestines. Ugt1a8 mRNA was only detected in male Wistar rat livers at a very low level. Ugt1a10 mRNA in the liver was significantly higher in females than in males in F344 and Wistar rats. The highest level was observed in female SD rats, whereas it was not detected in male SD rats. Ugt1a10 mRNA was not expressed in the small intestine in any strain.

Absolute expression levels of Ugt2b mRNAs in the liver and small intestine of rats.

Ugt2b mRNA levels in the rat liver and small intestine are shown in Supplementary Table 2, and visualized in Fig. 1B. Ugt2b1 mRNA was expressed in the livers but not in the small intestines. The highest level was observed in the livers of male Wistar rats. In SD and F344 rats, the Ugt2b1 mRNA level was significantly higher in females than in males, whereas an opposite difference between the sexes was observed in Wistar rat livers. Ugt2b2 mRNA was highly expressed in Wistar rat livers. The level was 1.4-fold higher in males than in females. Trace levels of Ugt2b2 were detected in SD and F344 rat livers with no difference between the sexes. Ugt2b2 was not expressed in the rat small intestine. Ugt2b3 mRNA levels in the liver were higher than in the small intestine in any strain. In SD and Wistar rat livers, the levels were significantly higher in males than in females. In the small intestine, the Ugt2b3 mRNA levels in Wistar rats were 15-fold higher than those in SD and F344 rats regardless of the sex. The Ugt2b6 mRNA level was the highest in the livers of male SD rats. The hepatic Ugt2b6 mRNA level in female SD rats was 4-fold lower than that in the male SD rats. The levels in F344 and Wistar rats were comparable with that in female SD rats, with no difference between the sexes. In the small intestine, a trace level of Ugt2b6 expression was detected in Wistar rats with a difference between sexes (1.5-fold higher in males than females) but not in SD and F344 rats.

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Ugt2b8 was expressed in the small intestine but not in the liver. The highest level was detected in Wistar rats. The levels in SD and F344 rats were 3- to 7-fold lower than those in Wistar rats. In their levels, no difference between the sexes was observed. Ugt2b12 mRNA levels in the liver were 14- to 116-fold higher than those in the small intestine. In Wistar rats, Ugt2b12 levels in the liver and small intestine were significantly higher in males than those in females. Meanwhile, no difference between the sexes was observed in the other strains. Ugt2b34 mRNA levels were higher in the livers than those in the small intestines in any strain. Hepatic Ugt2b34 levels in males were equal in the three strains, while in females, the levels in SD rats were significantly higher than those in the other strains. In SD and F344 rats, the levels were significantly higher in females than in males. In the small intestine, the levels in females were equal in the three strains, while in males, the levels in F344 rats were higher than those in the other strains. In F344 rats, the levels were significantly higher in males than in females.

Comparison of Ugt expression profiles in the livers and small intestines of the three rat strains.

Fig. 2A shows the sum of the mRNA levels of individual Ugts in the liver and small intestine. The sums of all Ugts in the liver were 2.1- to 6.3-fold higher than those in the small intestine. Wistar male rats show the highest levels in both the liver and small intestine. In the liver, the sums of all Ugts in Wistar rats were 2-fold higher than those in the other strains, which is due to abundant Ugt2b2 expression. In SD and F344 rat livers, the sums of all Ugts were slightly higher in females than in males. Meanwhile, in Wistar rats, the sums of all Ugts were higher in males than in females. In the small intestine, the sums of all Ugts were higher in Wistar rats than the other strains. In SD rat small intestine, the sums of all Ugts were slightly higher in females than in males. Meanwhile, the opposite difference between sexes was observed in F344 and Wistar rats.

The percentages of the mRNA levels of individual Ugts relative to the total Ugt levels were calculated (Supplementary Table 3), and visualized in Fig. 2B. In the livers, Ugt2b

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accounted for ~80% of the total Ugt levels. The most abundant isoform was Ugt2b12 (~50% of total Ugts) in SD and F344 rat livers. In Wistar rat livers, Ugt2b2 (40% of the total Ugts) was most abundant, followed by Ugt2b12 (~35% of total Ugts). Ugt2b1, Ugt2b3 and Ugt1a1 accounted for ~10% of the total Ugts. In the small intestines, Ugt1a accounted for ~90% of the total Ugt level. The most abundant isoform was Ugt1a7 (50% of the total Ugts) regardless of the strain and sex, followed by Ugt1a2 and Ugt1a6. Moderate expression of Ugt2b3 (14% of the total Ugts) was characteristic in Wistar rat small intestine.

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Discussion

Glucuronidation is a major conjugation pathway of a large variety of endogenous and xenobiotic compounds. UGTs play a significant role in the control of the pharmacokinetics, bioavailability, and toxicity of drugs. The expression profiles of human UGTs at mRNA and protein levels in several tissues have been well-characterized (Strassburg et al., 1997; Tukey and Strassburg, 2001; Nakamura et al., 2008). As for rat Ugts, a previous paper reported the tissue distribution (liver, kidney, lung, stomach, duodenum, jejunum, ileum, large intestine, cerebellum, and cerebral cortex) of Ugts at the mRNA level in SD rats by branched DNA signal amplification analysis (Shelby et al., 2003). The quantitative evaluation of rat Ugt expression remains to be performed. In this study, we sought to determine the absolute expression of Ugt1 and Ugt2b mRNA levels in the rat liver and small intestine by qRT-PCR to clarify the sex and strain differences.

The sum of the expressions of all Ugts in the liver was significantly ($P < 0.005$) higher than that in the small intestine. This phenomenon was consistent with the finding in human Ugts (Ohno and Nakajin, 2008; Court et al., 2012). In the rat livers, Ugt2b accounted for ~80% of the total Ugt mRNA level, which was consistent with the abundant expression of Ugt2b in human livers (70-90% of the total Ugts mRNA) (Ohno and Nakajin., 2008, Izukawa et al., 2009; Court et al., 2012). In the rat small intestines, Ugt1a accounted for ~90% of the total Ugt mRNA level. This observation was consistent with a previous report for human Ugt by Court et al (2012) (~90% of the total Ugts), although Ohno and Nakajin (2008) had reported that Ugt1a accounts for 30% of the total Ugts in the human small intestine. Based on a recent study, the expression profiles of UGT1A and UGT2B in the liver and small intestine appear to be similar between humans and rats.

Particularly interesting findings of this study are the strain differences in Ugt expression profiles. Ugt2b2 is abundantly expressed in only Wistar rat livers, leading to 2-fold higher expression of the total Ugts in the liver compared with the other strains. As for the strain differences, it has been reported that the glucuronidations of androsterone and lithocholic acid, which are the substrates of Ugt2b2, were detected in liver microsomes from SD rats but were

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hardly detected in those from F344 rats (Radominska et al., 1994). Richardson and Klaassen (2010) have reported that the glucuronidation of triiodothyronine, which is a substrate of Ugt2b2, was detected in liver microsomes from Wistar rats, but it was hardly detected in those from F344 rats. According to these results, it has been recognized that Ugt2b2 is deficient in F344 rats (Haque et al., 1991). Our study showed that the Ugt2b2 mRNA level in F344 rat livers was extremely low, supporting the results of previous studies. It has been reported that Wistar rats were divided into two groups on the basis of hepatic microsomal glucuronidation of androsterone: those with high activity and those with low activity (Matsui and Hakozaki, 1979). The discontinuous variation was due to the genetic heterogeneity of Ugt (Beetstra et al., 1991). It would be plausible that the Wistar rats used in the present study belong to former group. When we measured the hepatic Ugt2b2 level in an additional 8 individual Wistar rats (4 males and 4 females) purchased from Charles River (Hino, Japan), the levels were comparable to the level shown in Fig. 1B (data not shown). Thus, we could not find individual- and colony-dependent differences in the Ugt2b2 expression in the total 14 Wistar rats. Previous studies (Matsui and Hakozaki, 1979, Green et al., 1985) have reported that discontinuous variation in androsterone glucuronidation was not observed in the SD strain. Shelby et al (2003) have reported substantial expression of Ugt2b2 mRNA in SD rat livers. Meanwhile, our study demonstrated that the Ugt2b2 mRNA level was extremely low in SD rat livers. As for the discrepancy, two reasons are considered. First, the probes to detect Ugt2b2 used in the study by Shelby et al (2003) might react with other Ugt isoforms such as Ugt2b3 and Ugt2b6 because of their high homologies with Ugt2b2 (~90%). Supporting this suspicion, the tissue distributions of Ugt2b2, Ugt2b3 and Ugt2b6 were similar (Shelby et al, 2003). In this study, we carefully designed primers that can specifically detect only Ugt2b2 mRNA, and we confirmed that the amplicon was surely Ugt2b2 by cleavage with some restriction enzymes. Second, the glucuronidation of androsterone and lithocholic acid observed in SD rat microsomes might be due to other Ugt isoforms because evaluation using recombinant rat Ugts was not sufficient enough in the past.

In addition to Ugt2b2, a distinct expression profile was observed in the intestinal

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Ugt2b3. The Ugt2b3 mRNA levels in the small intestine of Wistar rats were prominently higher than those in the other strains, resulting in higher expression of the total Ugt level in the small intestine of Wistar rats. Rat Ugt2b3 has been reported to catalyze the glucuronidation of aromatic primary amines (Irshaid and Tephly, 1987). Therefore, the strain differences in the intestinal Ugt2b3 expression level may contribute to strain differences in the pharmacokinetics of such drugs. Taken together, we demonstrated that Ugt expression profiles in Wistar rats were different from those in other strains. This information may provide a hint for the selection of rat strains.

In humans, UGT1A7, 1A8 and 1A10 are expressed in the gastrointestinal tracts but not in the liver (Tukey and Strassburg, 2001; Nakamura et al., 2008). The present study revealed that in rats, Ugt1a7 was highly expressed in the intestine and slightly expressed (~5% of the total Ugts) in the liver. Meanwhile, Ugt1a8 was hardly expressed both in the liver and intestine. In the study by Shelby et al (2003), UGT1A8 mRNA was detected in the liver but at levels barely above the detection limit. Their probes for Ugt1a8 in branched DNA assays might react with other Ugt isoforms showing high sequence similarity with Ugt1a8. Rat Ugt1a10 was not expressed in the intestine but was in the liver at a trace level (0.1-1% of the total Ugts). These results demonstrated that the tissue distributions of UGT1A7, UGT1A8 and UGT1A10 were different between humans and rats. It has reported that the tissue-specific distribution of human UGT1A7, UGT1A8, and UGT1A10 is regulated by transcription factors such as CDX2 and HNF4α (Gregory et al., 2004; Mubarokah et al., 2018). Species differences in the tissue distribution might be due to the differences in the trans or cis factors between human and rats. The mechanisms of the tissue specific expression of rat Ugts is an issue to be solved in the future.

As for differences between the sexes, hepatic Ugt1a10 levels were significantly higher in females than in males in all strains, although the copy number was much lower than other isoforms. In Wistar rats, the sum of the expression levels of hepatic Ugt2b isoforms was higher in males than in females. In F344 rats, the sum of the expression levels of intestinal Ugt1a isoforms was higher in males than in females. Similar to the previous report by Shelby

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et al (2003), hepatic Ugt1a5 and Ugt2b1 mRNA levels in SD rats were 2-fold higher in females than in males. Meanwhile, Emi et al (1995) reported no remarkable differences between the sexes in hepatic Ugt1a mRNA levels. It has reported that sex hormones such as 17β-estradiol are the modulators of some Ugt expression (Jeong et al, 2008; Chen et al, 2009). Therefore, the menstrual cycle may affect Ugt expression in females.

In conclusion, this is the first study to comprehensively quantify hepatic and intestinal mRNA levels of Ugt isoforms in SD, F344 and Wistar rats, which are commonly used for nonclinical studies. We provided basic information on species differences in hepatic and intestinal UGT expression between rats and humans as well as strain differences. Our results would be helpful for pharmaceutical scientists in choosing rat strains and the sex.

Authorship Contributions:

Participated in research design: Nakajima, Fukami, and Furukawa

Conducted experiments: Furukawa

Contributed new reagents or analytic tools: Furukawa

Performed data analysis: Furukawa, Kutsukake, and Ondo

Wrote or contributed to the writing of the manuscript: Kutsukake, Gotoh, and Nakajima

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Footnotes

Takaya Kutsukake and Yoichi Furukawa contributed equally to this work.

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Figure legends

Fig. 1. Absolute quantification of Ugt mRNAs in the liver and small intestine from male and female SD, F344 and Wistar rats. (A) Ugt1a mRNA levels. (B) Ugt2b mRNA levels. The mRNA copy numbers were calculated from the standard curve of each Ugt. Each column represents the mean ± SD of triplicate determinations. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$, compared with males. $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$ and $^{\dagger\dagger\dagger}P < 0.001$, compared with SD rats. $^{\S}P < 0.05$, $^{\S\S}P < 0.01$ and $^{\S\S\S}P < 0.001$, compared with F344 rats. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$, compared with liver. ND: Not detectable. NS: Not significant.

Fig. 2. Comparative evaluation of the expression levels of Ugt mRNA in the liver and small intestine from male and female SD, F344 and Wistar rats. (A) Sum of the absolute mRNA expression levels of Ugt1a and Ugt2b in rats. (B) Percentage of each Ugt isoform mRNA level in the sum of all of the Ugt levels (%). Males and females are shown as m and f, respectively.

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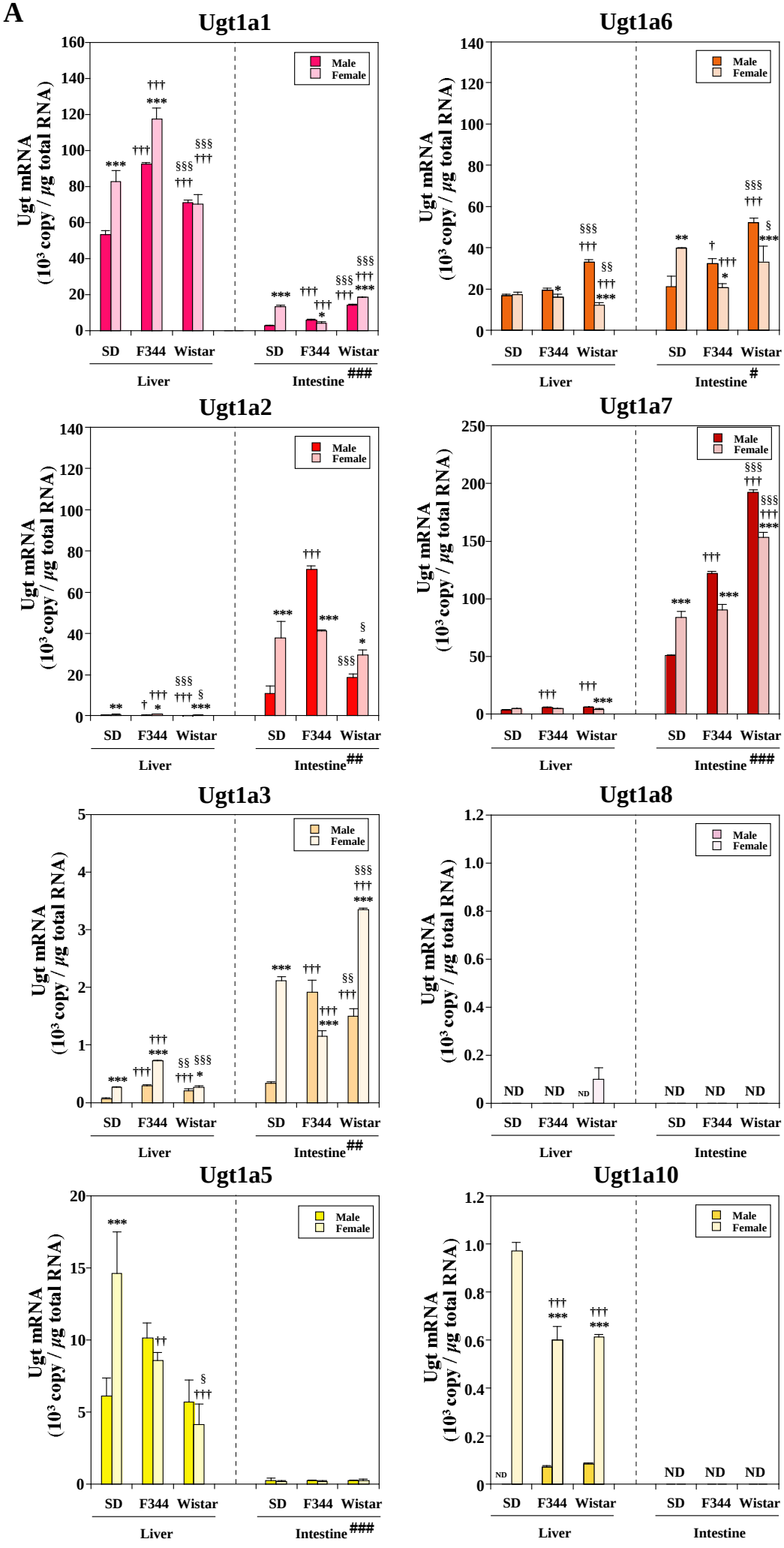
Fig. 1

A



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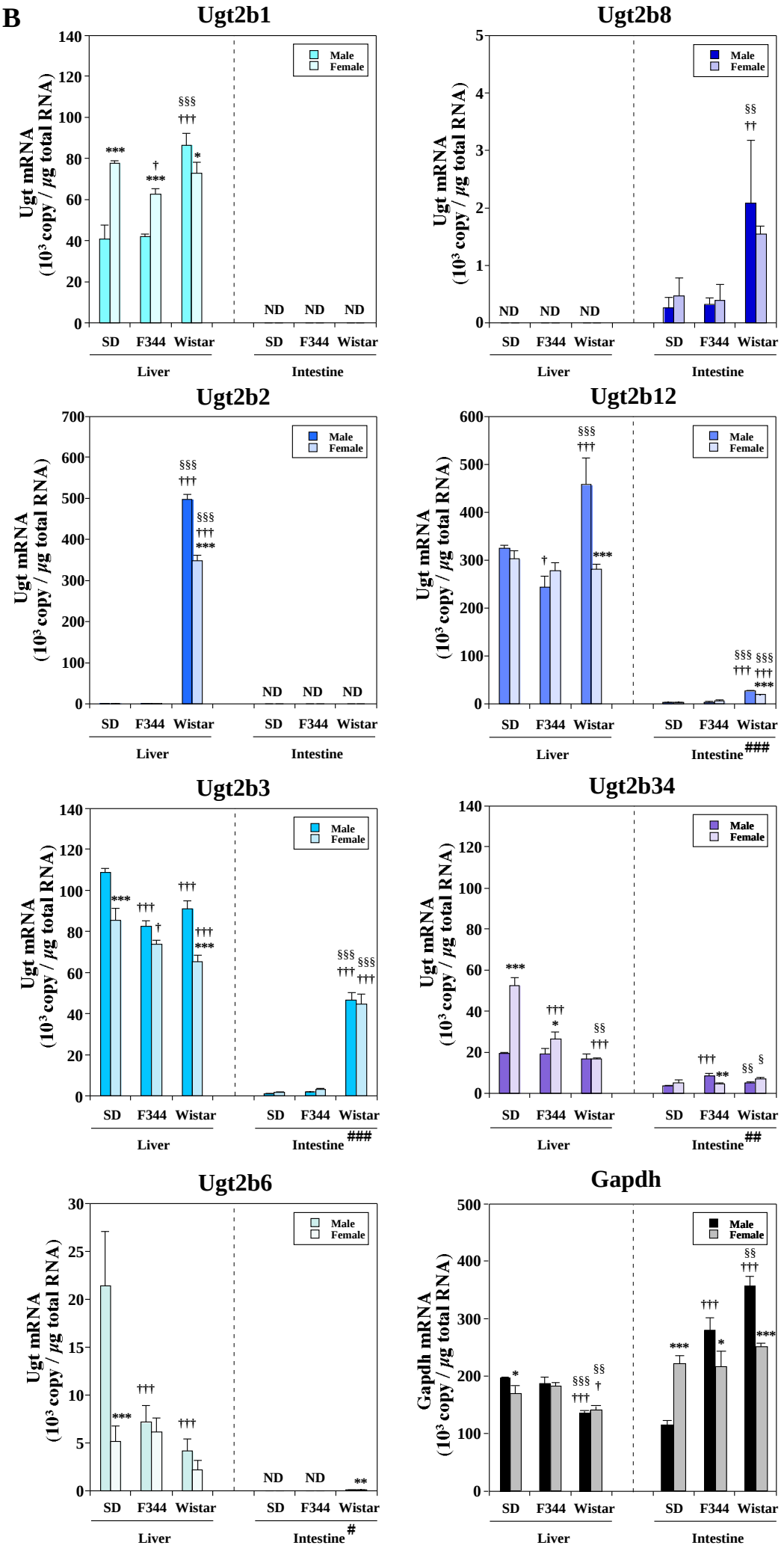
B



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