1521-009X/43/7/1071–1076$25.00
DRUG METABOLISM AND DISPOSITION

http://dx.doi.org/10.1124/dmd.115.063719
Drug Metab Dispos 43:1071–1076, July 2015

Expression of *UDP-Glucuronosyltransferase 1* (*UGT1*) and Glucuronidation Activity toward Endogenous Substances in Humanized *UGT1* Mouse Brain[S]

Yuki Kutsuno, Rika Hirashima, Masaya Sakamoto, Hiroko Ushikubo, Hirofumi Michimae, Tomoo Itoh, Robert H. Tukey, and Ryoichi Fujiwara

Department of Pharmaceutics (Y.K., R.H., M.S., T.I., R.F.), Department of Molecular Pharmacology (H.U.), and Division of Biostatistics (H.M.), School of Pharmacy, Kitasato University, Minato-ku, Tokyo, Japan; and Laboratory of Environmental Toxicology, Department of Pharmacology, University of California San Diego, La Jolla, California (R.H.T.)

Received February 5, 2015; accepted May 7, 2015

ABSTRACT

Although UDP-glucuronosyltransferases (UGTs) are important phase II drug-metabolizing enzymes, they are also involved in the metabolism of endogenous compounds. Certain substrates of UGTs, such as serotonin and estradiol, play important roles in the brain. However, the expression of UGTs in the human brain has not been fully clarified. Recently, humanized *UGT1* mice (*hUGT1* mice) in which the original *Ugt1* locus was disrupted and replaced with the human *UGT1* locus have been developed. In the present study, the expression pattern of UGT1As in brains from humans and *hUGT1* mice was examined. We found that UGT1A1, 1A3, 1A6, and 1A10 were expressed in human brains. The expression pattern of UGT1As in *hUGT1* mouse brains was similar to that in human brains. In addition, we examined the expression of UGT1A1 and 1A6 in the cerebellum, olfactory bulbs, midbrain, hippocampus, and cerebral cortex of *hUGT1* mice. UGT1A1 in all brain regions and UGT1A6 in the cerebellum and cerebral cortex of 6-month-old *hUGT1* mice were expressed at a significantly higher rate than those of 2-week-old *hUGT1* mice. A difference in expression levels between brain regions was also observed. Brain microsomes exhibited glucuronidation activities toward estradiol and serotonin, with mean values of 0.13 and 5.17 pmol/min/mg, respectively. In conclusion, UGT1A1 and UGT1A6 might play an important role in function regulation of endogenous compounds in a region- and age-dependent manner. Humanized *UGT1* mice might be useful to study the importance of brain UGTs in vivo.

Introduction

UDP-glucuronosyltransferases (UGTs) catalyze glucuronidation of compounds by transferring glucuronic acid from a cosubstrate, UDP-glucuronic acid (UDPGA), to substrates (Dutton, 1980). UGT isoforms have been identified to date belonging to 1A, 2A, 2B, 3A, and 8A subfamilies (Mackenzie et al., 2005). The human *UGT1* gene is located on chromosome 2q37 and encodes multiple unique exon 1s and common exons 2–5, producing nine functional UGT1A isoforms: UGT1A1, UGT1A3, UGT1A4, UGT1A5, UGT1A6, UGT1A7, UGT1A8, UGT1A9, and UGT1A10 (Ritter et al., 1992). Each UGT enzyme exhibits substrate specificity and is expressed in a tissue-specific manner (Tukey and Strassburg, 2000). Although UGTs are important phase II drug-metabolizing enzymes (Dutton, 1980), they are also involved in the metabolism of endogenous substances. UGT1As are involved in the metabolism of many endogenous substances such as bilirubin, estradiol, bile acids, thyroid hormone, and serotonin (de Leon, 2003).

This work was supported by a Grant-in-Aid for Encouragement of Young Scientists B to R.F. [Grant 26870562]. This work was also supported in part by the National Institutes of Health National Institute of Environmental Health Sciences [Grant P42-ES010337] and National Institute of General Medical Sciences [Grant R01-GM100481].
dx.doi.org/10.1124/dmd.115.063719.
[S]This article has supplemental material available at dmd.aspetjournals.org.

Liver is the major organ of glucuronidations, as UGTs are mainly expressed in the liver (Court et al., 2012). However, several UGT isoforms are also expressed in extrahepatic organs such as the intestine, kidney, lung, and skin. Therefore, extrahepatic tissues can also play an important role in glucuronidation of exogenous and endogenous substances. For example, the kidney could substantially contribute to the elimination of propofol, as glucuronidation clearance of propofol in the kidney is relatively comparable to that in the liver (Al-Jahdari et al., 2006). Furthermore, it was recently reported that UGT1A1 expressed in the skin could be involved in glucuronidation of bilirubin and play a protective role against neonatal hyperbilirubinemia (Sumida et al., 2013). It was also recently reported that gastrointestinal UGT1A1 played a significant role in bilirubin metabolism (Aoshima et al., 2014).

In the brain, many endogenous compounds play important roles in the regulation of physiologic function, and are implicated to be a key factor of brain disorders. Some of these endogenous compounds have been identified as substrates for UGTs. For example, serotonin, which is a specific substrate for UGT1A6 (Krishnaswamy et al., 2003), is considered to be involved in the synchronization of locomotion in the cerebellum (Trouillas, 1993), memory information processing in the hippocampus (Sarkisyan and Hedlund, 2009), and mood regulation in the cerebral cortex and hippocampus (Gross et al., 2002). UGTs have been found in the brain. In rat cerebellum and cerebral cortex, mRNA of Ugt1a1, 1a3, 1a5, 1a6, 1a8, 2a1, 2b1, 2b2, 2b3, 2b6, 2b8, and 2b12 was detected (Shelby et al., 2003). Ugt1a1 was more highly expressed

ABBREVIATIONS: AS, antisense primer; CBZ, carbamazepine; CPH, cyclophilin; HPLC, high-performance liquid chromatography; *hUGT1*, humanized *UGT1*; PCN, pregnenolone-16α-carbonitrile; PXR, pregnane X receptor; UGT, UDP-glucuronosyltransferase.

in rat cerebellum than the cerebral cortex. King et al. (1999) demonstrated mRNA expressions of UGT1A6 and UGT2B7 in human brain using reverse-transcription polymerase chain reaction (RT-PCR) techniques. The glucuronidation activities of human brain microsomes toward morphine and naloxone, the probe substrates for UGT2B7, and 1-naphthol, which is glucuronidated by multiple UGT isoforms (Uchaipichat et al., 2004), have been reported (Wahlström et al., 1988; Viani et al., 1990). This suggests that functional UGTs were expressed in human brain. However, a complete expression pattern of human UGTs in total brain and in brain regions has not been clarified. In addition, as the number of studies on glucuronidation activity of UGTs in the brain was limited, the expression of functional UGTs in human brain remains to be investigated.

Mice and rats are often used for studies on UGTs in the brain because of the difficulty in obtaining human brains. However, it should be noted that there are species differences in UGT expressions between rodents and humans (Shelby et al., 2003; Buckley and Klaassen, 2007, Nakamura et al., 2008). Recently, humanized *UGT1* mice (*hUGT1* mice) in which the original *Ugt1* locus was disrupted and replaced with the human *UGT1* locus have been developed (Fujiwara et al., 2010, 2012). As UGT1A expression patterns in the liver and small intestine of *hUGT1* mice were similar to those of humans (Nakamura et al., 2008; Fujiwara et al., 2010), *hUGT1* mice have been used to evaluate pharmacological and toxicological species differences in UGTs (Kutsuno et al., 2013, 2014). Therefore, in this study, the expression pattern of UGT1As in brains from humans and *hUGT1* mice was examined. The expression of UGT1A1 and UGT1A6 in brain regions, such as the cerebellum, olfactory bulbs, midbrain, hippocampus, and cerebral cortex, of *hUGT1* mice was further quantitatively examined. Additionally, we investigated glucuronidation activity toward estradiol and serotonin in brain microsomes of *hUGT1* mice to examine whether UGTs in the brain were functional. The inducibility of UGT mRNAs and glucuronidation activities toward estradiol and serotonin in *hUGT1* mouse brain were also examined.

Materials and Methods

Chemicals and Reagents. The 36- to 51- years female human total brain (containing meningioma) RNA was purchased from OriGene (Rockville, MD). UDPGA, alamethicin, estradiol, and estradiol 3-*O*-glucuronide were purchased from Sigma-Aldrich (St. Louis, MO). Serotonin β-D-glucuronide was purchased from Toronto Research Chemicals (Toronto, ON, Canada). Primers were commercially synthesized at Life Technologies (Carlsbad, CA). KOD-Plus-Neo was purchased from Toyobo Co., Ltd. (Osaka, Japan). 5-Hydroxytryptamine hydrochloride (serotonin) was purchased from Wako Pure Chemical (Osaka, Japan). Human liver microsomes were obtained from BD Gentest (Woburn, MA). Protease inhibitor cocktails were from Roche Diagnostics (Mannheim, Germany). All other chemicals and solvents were of analytical grade or the highest grade commercially available.

Animals and Tissue Collection. *Tg (UGT1^{A1*28}) Ugt1$^{-/-}$* (*hUGT1*) mice were developed previously in a C57BL/6 background (Fujiwara et al., 2010). All animals received food and water ad libitum, and mouse handling and experimental procedures were conducted in accordance with the animal care protocol approved by Kitasato University. Adult male and female mice were anesthetized by diethyl ether inhalation and sacrificed by decapitation. Brains were removed and rinsed in cold phosphate-buffered saline, and were stored at −80°C. Carbamazepine (CBZ) suspended in 5% dimethylsulfoxide and pregnenolone-16α-carbonitrile (PCN) suspended in canola oil were administered to male *hUGT1* mice (16 weeks) for 7 consecutive days (100 mg/kg by mouth for CBZ and 100 mg/kg i.p. for PCN). Brains and livers perfused with ice-cold 1.15% KCl were collected 24 hours after the last administration and were used for the RNA extraction and preparation of microsomes. For RNA extraction of brain regions, young male mice (2 weeks) and adult male mice (6 months) were anesthetized by diethyl ether inhalation and sacrificed by decapitation. After brains were quickly removed and rinsed in phosphate-buffered saline, brain regions (cerebellum, cerebral cortex, midbrain, hippocampus, and olfactory bulb) were dissected on ice immediately and stored at −80°C.

Total RNA and Reverse Transcription. Total RNA of *hUGT1* mouse whole brain and brain regions was extracted with TRIzol reagent (Life Technologies). The cDNA was synthesized from 1 μg of total RNA of human and *hUGT1* mouse brain using ReverTra Ace (Toyobo, Osaka, Japan). After the reverse-transcription reaction at 37°C for 15 minutes and at 50°C for 5 minutes,

TABLE 1

Sequence of primers used for RT-PCR and quantitative RT-PCR

Isoforms	Primers	Sequence	Position	Annealing Temperature	References
				°C	
UGT1A1	UGT1A1-S	5′-CCT TGC CTC AGA ATT CCT TC-3′	696–715	62	Nakamura et al. (2008)
	UGT1A-AS	5′-ATT GAT CCC AAA GAG AAA ACC AC-3′	907–929		Nakamura et al. (2008)
UGT1A3	UGT1A3-S	5′-TGT TGA ACA ATA TGT CTT TGG TCT-3′	347–370	62	Nakamura et al. (2008)
	UGT1A3-AS	5′-CAC AGG ACT GTC TGA GGG ATT TT-3′	985–1007		—
UGT1A4	UGT1A4-S	5′-CCT GCT GTG TTT TTT TGG AGG T-3′	502–523	54	Izukawa et al. (2009)
	UGT1A-AS	5′-ATT GAT CCC AAA GAG AAA ACC AC-3′	907–929		Nakamura et al. (2008)
UGT1A5	UGT1A5-S	5′-ACA ATA TGT CTT TGA TCA TA-3′	353–372	54	Nakamura et al. (2008)
	UGT1A5-AS	5′-AGA AAC AGC ATG GCA AAG-3′	667–684		Nakamura et al. (2008)
UGT1A6	UGT1A6-S	5′-AGA GAA TTT CTG CAG GGG TTT T-3′	26–47	54	Nakamura et al. (2008)
	UGT1A6-AS	5′-TTG GAT TCT TTC AAA AGC-3′	195–212		Nakamura et al. (2008)
UGT1A7	UGT1A7-S	5′-CCC CTA TTT TTT CAA AAA TGT CTT-3′	660–683	54	Izukawa et al. (2009)
	UGT1A-AS	5′-ATT GAT CCC AAA GAG AAA ACC AC-3′	907–929		Nakamura et al. (2008)
UGT1A8	UGT1A8-S	5′-GGT CTT CGC CAG GGG AAT AG-3′	498–517	62	Nakamura et al. (2008)
	UGT1A-AS	5′-ATT GAT CCC AAA GAG AAA ACC AC-3′	907–929		Nakamura et al. (2008)
UGT1A9	UGT1A9-S	5′-GAA CAT TTA TTA TGC CAC CG-3′	646–665	62	Nakamura et al. (2008)
	UGT1A-AS	5′-ATT GAT CCC AAA GAG AAA ACC AC-3′	907–929		Nakamura et al. (2008)
UGT1A10	UGT1A10-S	5′-CTC TTT CCT ATG TCC CCA ATG A-3′	557–578	62	Nakamura et al. (2008)
	UGT1A-AS	5′-ATT GAT CCC AAA GAG AAA ACC AC-3′	907–929		Nakamura et al. (2008)
Cyp2b10	Cyp2b10-S	5′-GCA AGC CAT GTT GCT CCT AA-3′	1405–1424	60	—
	Cyp2b10-AS	5′-CTT GGA GCC CTG GAG ATT TGG-3′	1604–1624		—
Cyp3a11	Cyp3a11-S	5′-CTC AAT GGT GTG TAT ATC CCC-3′	1228–1248	60	Fujiwara et al. (2012)
	Cyp3a11-AS	5′-CCG ATG TTC TTA GAC ACT GCC-3′	1630–1650		Fujiwara et al. (2012)
GAPDH	GAPDH-S	5′-CCA GGG CTG CTT TTA ACT C-3′	56–74	62	Nakamura et al. (2008)
	GAPDH-AS	5′-GCT CCC CCC TGC AAA TGA-3′	330–347		Nakamura et al. (2008)
CPH	CPH-S	5′-CAG ACG CCA CTG TCG CTT T-3′	5–23	62	Fujiwara et al. (2012)
	CPH-AS	5′-TGT CTT TGG AAC TTT GTC TGC AA-3′	115–137		Fujiwara et al. (2012)

GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

the reaction mixture was incubated at 95°C for 5 minutes for deactivation of the reverse transcriptase. RT mixture of human brain total RNA was diluted 10-fold in Tris-EDTA buffer.

RT-PCR. A 0.5-μl portion of RT mixture of *hUGT1* mouse brain or the diluted cDNA of human brain was added to PCR mixtures (25 μl). After an initial denaturation at 95°C for 2 minutes, the amplification was performed by denaturation at 98°C for 10 seconds, annealing at an appropriate temperature for 30 seconds, and extension at 72°C for 10 seconds for 45 cycles. The sequences and annealing temperature of primers are shown in Table 1. The PCR products (20 μl) were analyzed by electrophoresis with 2% agarose gel and visualized by ethidium bromide staining. Expression of glyceraldehyde 3-phosphate dehydrogenase or cyclophilin (CPH) mRNA was used as an internal control for the cDNA quantity and quality.

Quantitative RT-PCR. Quantitative RT-PCR was performed with THUNDERBIRD SYBR qPCR Mix (Toyobo), and the reactions were run in a CFX96 Real-Time PCR Detection System (Bio-Rad, Hercules, CA). Primer pairs were UGT1A1-sense primer (S) and UGT1A-antisense primer (AS), UGT1A6-S and UGT1A6-AS, Cyp2b10-S and Cyp2b10-AS, Cyp3a11-S and Cyp3a11-AS, and CPH-S and CPH-AS (Table 1). After an initial denaturation at 95°C for 30 seconds, the amplification was performed by denaturation at 95°C for 5 seconds, annealing at an appropriate temperature for 30 seconds, and extension at 72°C for 30 seconds for 45 cycles. UGT mRNA expression levels were normalized with mouse CPH mRNA level.

Preparation of Brain Microsomes. Brain was homogenized in 3 volumes of homogenization buffer (1.15% KCl/100 mM potassium phosphate buffer, pH 7.4, containing protease inhibitor cocktails). The homogenate was centrifuged at 9000 $\times$ *g* for 15 minutes at 4°C, and the supernatant was collected. The supernatant was centrifuged at 105,000 $\times$ *g* for 60 minutes at 4°C, and the pellet was suspended in a stock buffer (20% glycerol/1.15% KCl/100 mM potassium phosphate buffer, pH 7.4) and used as the microsomal fraction. All of these procedures were performed as quickly as possible. Protein concentrations of microsomal fractions were measured by the Bradford method using bovine serum albumin as a standard (Bradford, 1976).

Enzyme Assays. Estradiol and serotonin glucuronide formation was determined according to the reported method, with slight modifications (Krishnaswamy et al., 2003; Shibuya et al., 2013). In brief, a typical incubation mixture (200 μl of total volume) contained 100 mM phosphate buffer (pH 7.4), 4 mM $MgCl_2$, 5 mM UDPGA, 50 μg/ml alamethicin, 1.0 mg/ml brain microsomes, and 100 μM estradiol or 10 mM serotonin. The reaction was initiated by the addition of UDPGA after a 3-minute preincubation at 37°C. Incubation was performed for 12 hours for estradiol 3-*O*-glucuronidation in brain microsomes, 60 minutes for serotonin glucuronidation, and 30 minutes for estradiol 3-*O*-glucuronidation in liver microsomes. The reaction was terminated by the addition of 200 μl of cold methanol for estradiol 3-*O*-glucuronidations or acetonitrile for serotonin glucuronidation. After removal of the protein by centrifugation at 12,000*g* for 5 minutes, a 50-μl portion of the sample was subjected to high-performance liquid chromatography (HPLC).

HPLC Conditions. Glucuronides were determined by the HPLC system with an LC-10AD pump (Shimadzu, Kyoto, Japan), an FP-2020 fluorescence detector (JASCO, Tokyo Japan), a SIL-10A autosampler (Shimadzu), an SLC-10A system controller (Shimadzu), and a Mightysil RP-18 GP column (4.6 $\times$ 150 mm, 5 μm; Kanto Chemical, Tokyo, Japan). The mobile phases were 3% acetonitrile containing 20 mM phosphate potassium buffer (pH 7.4) for the serotonin glucuronide and 10 mM H_3PO_4-methanol (45:55, v/v) for estradiol *3-O*-glucuronide. The flow rate was 1.0 ml/min. Glucuronides were detected with a fluorescence detector at 280-nm excitation and 310-nm emission for the estradiol *3-O*-glucuronide and at 225-nm excitation and 330-nm emission for the serotonin glucuronide. Quantifications of estradiol 3-*O*-glucuronide and serotonin glucuronide were carried out by comparing the HPLC peak area to those of the authentic standards. The retention times of estradiol 3-*O*-glucuronide and serotonin glucuronide were 6.4 and 3.7 minutes, respectively.

Statistical Analysis. Statistical differences in UGT1A1 and 1A6 expression levels between corresponding regions of 2-week-old and 6-month-old *hUGT1* mouse brains were analyzed by a two-way analysis of variance with post-hoc *t* test comparisons. Statistical differences in UGT1A1 and UGT1A6 expression levels between brain regions in 2-week-old and 6-month-old *hUGT1* mice were analyzed by a two-way analysis of variance with post-hoc Tukey test. $P < 0.05$ was considered significant.

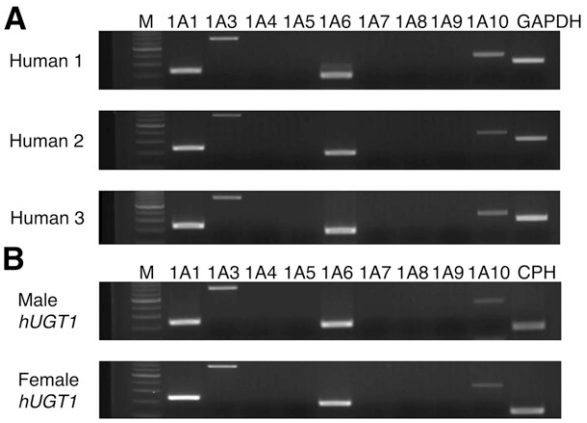


Fig. 1. RT-PCR analysis of UGT mRNA in brains of humans and *hUGT1* mice. Total RNA samples from three human brains containing meningioma (A) or male and female *hUGT1* mouse brains (B) were analyzed by RT-PCR using primer pairs specific for each UGT1A isoform. M, 100 bp DNA ladder marker.

Results

mRNA Expression of UGT1As in Human Brain and *hUGT1* Mouse Brain. Although it was reported that mRNA of UGT1A6 and UGT2B7 was expressed in human brain (King et al., 1999), expressions of the other UGTs in human brain have not been clarified yet. In this study, expressions of UGT1A in human and *hUGT1* mouse brain were determined. In human brain, UGT1A1, UGT1A3, UGT1A6, and UGT1A10 were expressed (Fig. 1A). As UGTs in brains from three different human donors were expressed similarly, large interindividual differences in the expression pattern of UGT1As were not observed. In the *hUGT1* mice, UGT1A1, 1A3, 1A6, and 1A10 were expressed in the brain (Fig. 1B), which was in agreement with our previous study (Sakamoto et al., 2015). The expression pattern of UGT1As in the brain was very similar between human and hUGT1 mice (Fig. 1, A and B), indicating that *hUGT1* mice might be useful to understand the expression and function of human UGT1As in the brain. As the expression patterns of UGT1As in female and male *hUGT1* mouse brains were similar to each other (Fig. 1B), sex differences in expression pattern of UGT1As were not observed.

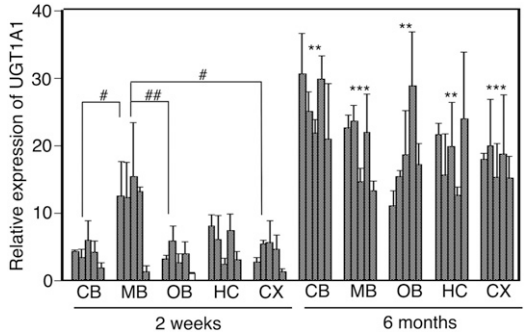


Fig. 2. The expression level of UGT1A1 in *hUGT1* mouse brain regions. Total RNA from brains of 2-week-old mice (n = 5) and 6-month-old mice (n = 5) was analyzed by quantitative RT-PCR. Each column is the mean ± S.D. of three independent determinations. Two-way analysis of variance with post-hoc *t* test and Tukey test was used to determine statistical significance. $^{**}P < 0.01$, $^{***}P < 0.001$ compared with the expression in each region of 2-week-old mice. $\#P < 0.05$, $\#\#P < 0.01$ when comparing brain regions between 2-week-old and 6-month-old *hUGT1* mice (two-way analysis of variance with post-hoc Tukey test). CB, cerebellum; HC, hippocampus; MB, midbrain; OB, olfactory bulbs; CX, cerebral cortex.

Expression Level of UGT1A1 and UGT1A6 in Brain Regions of *hUGT1* Mouse. The region- and age-dependent differences in the expression of CYP2D6 involved in the metabolism of endogenous compounds have been reported (Miksys et al., 2000). However, the expression of UGTs in human brain regions has not been examined. As UGT1A1, 1A3, 1A6, and 1A10 were expressed in *hUGT1* mouse brain (Fig. 1B), we investigated the expression levels of UGT1A1, 1A3, 1A6, and 1A10 in brain regions of young (2 weeks) and adult (6 months) *hUGT1* mice. Since threshold cycle values of UGT1A3 and UGT1A10 were high (threshold cycle > 35), we were not able to quantitatively analyze the expression of UGT1A3 and UGT1A10. ERV_{mch8} (murine leukemia virus type–endogenous retrovirus mapped on chromosome 8), GATA binding protein 3, and serine peptidase inhibitor Kazal type 8 have been known to be highly expressed in the cerebellum, midbrain, and hippocampus, respectively (Nozawa et al., 2009; Lee et al., 2011). It was confirmed that those marker genes were specifically expressed in the respective brain regions in our study (Supplemental Fig. 1).

In this study, relative expression levels were expressed as the ratio relative to the lowest expression sample in 2-week-old and 6-month-old *hUGT1* mice (white columns in Figs. 2 and 3). The relative expression of UGT1A1 in 2-week-old *hUGT1* mice was between 1.8 and 6.0 in the cerebellum, 1.4 and 15.0 in the midbrain, 1.0 and 5.9 in the olfactory bulb, 2.4 and 8.1 in the hippocampus, and 1.3 and 5.7 in the cerebral cortex, showing a 3.2- to 11.2-fold variability in each region. The expression level of UGT1A1 in the midbrain of 2-week-old *hUGT1* mice was statistically higher than that in the cerebellum, olfactory bulb, and cerebral cortex of 2-week-old *hUGT1* mice ($P < 0.05$). The relative expression of UGT1A1 in 6-month-old *hUGT1* mice was between 21.0 and 30.6 in the cerebellum, 13.3 and 23.6 in the midbrain, 11.1 and 28.9 in the olfactory bulb, 12.6 and 24.0 in the hippocampus, and 15.2 and 20.1 in the cerebral cortex, showing a 1.3- to 2.6-fold variability in each region. UGT1A1 in the cerebellum, midbrain, olfactory bulb, hippocampus, and cerebral cortex of 6-month-old *hUGT1* mice was expressed significantly higher than that of 2-week-old *hUGT1* mice ($P < 0.05$).

The relative expression of UGT1A6 in 2-week-old *hUGT1* mice was between 1.2 and 3.6 in the cerebellum, 1.0 and 4.0 in the midbrain, and 1.3 and 2.3 in the cerebral cortex, showing small variability in each region. The relative expression of UGT1A6 in 2-week-old *hUGT1* mice was between 1.1 and 6.9 in the olfactory bulb and 1.0 and 19.2 in the hippocampus, showing greater variability in each region. The relative expression of UGT1A6 in 6-month-old *hUGT1* mice was between 2.4 and 4.0 in the hippocampus and 3.0 and 5.2 in the cerebral cortex, showing small variability in each region. The relative expression of UGT1A6 in 6-month-old *hUGT1* mice was between 4.3 and 19.3 in the cerebellum, 1.3 and 5.5 in the midbrain, and 2.5 and 10.4 in the olfactory bulb, showing 4-fold variability in each region. The expression level of UGT1A6 in the cerebellum of 6-month-old *hUGT1* mice was statistically higher than that in the midbrain, hippocampus, and cerebral cortex of 6-month-old *hUGT1* mice ($P < 0.05$). Although the expression levels of UGT1A6 in the midbrain, olfactory bulb, and hippocampus of 6-month-old *hUGT1* mice were similar to those of 2-week-old *hUGT1* mice, the expression levels of UGT1A6 in the cerebellum and cerebral cortex of 6-month-old *hUGT1* mice were statistically higher than those of 2-week-old *hUGT1* mice ($P < 0.05$).

Effect of CBZ and PCN on mRNA Expression Levels of UGT1A1 and UGT1A6 in the Brain and Liver of *hUGT1* Mice. Whereas CBZ can induce UGTs, CYP2B6, and CYP3A4 in the human liver through constitutive androstane receptor (Hewitt et al., 2007), PCN can induce hepatic Ugts and Cyp3a11 through the pregnane X receptor (PXR) (Buckley and Klaassen, 2007). Since it has not been reported whether UGTs could be induced in brains, we investigated the effect of CBZ and PCN on expression levels of UGT1A1 and UGT1A6 in the brain and liver of *hUGT1* mice. CBZ and PCN significantly induced UGT1A1 in the *hUGT1* mouse liver (Fig. 4A). In the brain of *hUGT1* mice, UGT1A1 was slightly induced by CBZ, but not by PCN (Fig. 4B). UGT1A6 was not significantly induced by CBZ in the liver, whereas it was induced by PCN (Fig. 4C). Although there was a considerable interindividual difference in expression levels of UGT1A6 in brains of *hUGT1* mice treated with CBZ, one of the CBZ-treated mice had a significantly higher expression of UGT1A6 in the brain than nontreated *hUGT1* mice, although UGT1A6 was not induced in all of the PCN-treated mouse brains (Fig. 4D).

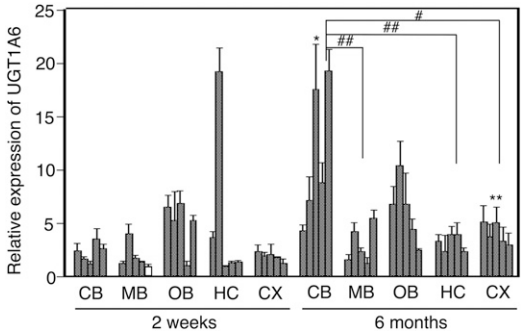


Fig. 3. The expression level of UGT1A1 in *hUGT1* mouse brain regions. Total RNA from brains of 2-week-old *hUGT1* mice ($n = 5$) and 6-month-old *hUGT1* mice ($n = 5$) was analyzed by quantitative RT-PCR. Two-way analysis of variance with post-hoc *t* test and Tukey test was used to determine statistical significance. $*P < 0.05$, $**P < 0.01$ compared with the expression in each region of 2-week-old mice (two-way analysis of variance with post-hoc *t* test). $\#P < 0.05$, $\#\#P < 0.01$ when comparing brain regions between 2-week-old and 6-month-old *hUGT1* mice (two-way analysis of variance with post-hoc Tukey test). CB, cerebellum; HC, hippocampus; MB, midbrain; OB, olfactory bulbs; CX, cerebral cortex.

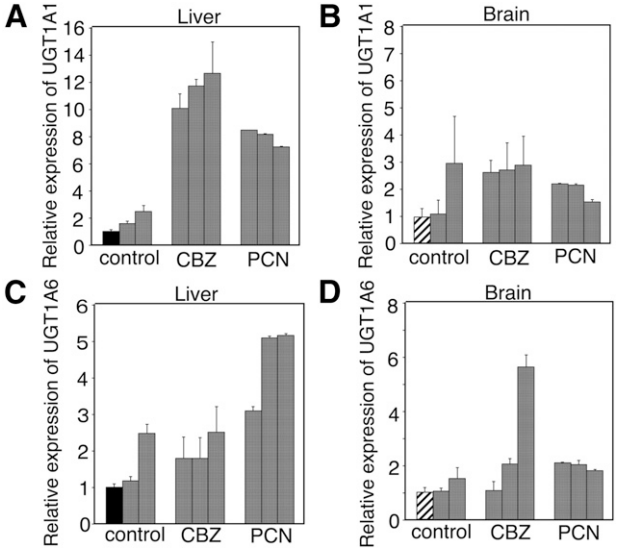


Fig. 4. The effect of inducers treatment on UGT1A1 expression in brains and livers of *hUGT1* mice. Total RNA was isolated from nontreated *hUGT1* mice ($n = 3$) and inducers-treated *hUGT1* mice ($n = 3$). Complementary DNA was synthesized, and then quantitative RT-PCR was carried out for human UGT1A1 in the *hUGT1* mouse liver (A) and brain (B) and UGT1A6 in the *hUGT1* mouse liver (C) and brain (D). Each column is the mean ± S.D. of three independent determinations.

The relative levels of UGT1A1 and UGT1A6 mRNA expressions in brain regions, total brain, and liver were compared (Supplemental Fig. 2). We found that the expression levels of UGT1A1 and UGT1A6 in the brain were much lower than those in the liver.

CBZ also increased the expressions of Cyp2b10 and 3a11, orthologs of human CYP2B6 and 3A4, in the brain of *hUGT1* mice (Fig. 5, A and B). Although PCN induced the expression of Cyp3a11 in the liver, it did not induce the gene in the brain (Fig. 5B).

Glucuronidation of Estradiol and Serotonin in *hUGT1* Mouse Brain Microsomes and Human Liver Microsomes. To examine the expression of functional UGTs in *hUGT1* mouse brains, glucuronidation activities toward estradiol, which is a probe substrate for UGT1A1, and serotonin, which is a probe substrate for UGT1A6 (Court, 2005), were examined using brain microsomes prepared from *hUGT1* mice. Furthermore, the effect of CBZ and PCN on glucuronidation activity toward estradiol and serotonin in *hUGT1* mouse brain microsomes was also investigated in this study. Although the activity toward estradiol 3-*O*-glucuronidation was not detected in more than half of nontreated *hUGT1* mouse brain microsomes, a few samples of brain microsomes exhibited activity toward estradiol 3-*O*-glucuronidation (Fig. 6, left). In contrast, all of the CBZ-treated *hUGT1* mice exhibited estradiol 3-*O*-glucuronidation activity (Fig. 6). The estradiol 3-*O*-glucuronidation activity in brain microsomes of CBZ-treated *hUGT1* mice was higher than that of nontreated *hUGT1* mice, with mean values of 0.13 pmol/min/mg for brain microsomes of nontreated *hUGT1* mice and 1.52 pmol/min/mg for brain microsomes of CBZ-treated *hUGT1* mice (Fig. 6). PCN-treated *hUGT1* mice exhibited no estradiol 3-*O*-glucuronidation activity (Fig. 6).

Serotonin glucuronidation was detected in all but one of the nontreated *hUGT1* mouse brain microsomes (Fig. 7, left). All of the CBZ- and PCN-treated *hUGT1* mice exhibited serotonin glucuronidation activity (Fig. 7). The serotonin glucuronidation activities in brain microsomes of *hUGT1* mice treated with CBZ and PCN were higher than that of nontreated *hUGT1* mice, with 5.17 pmol/min/mg in nontreated *hUGT1* mouse brain microsomes, 12.7 pmol/min/mg in brain microsomes of *hUGT1* mice treated with PCN, and 31.7 pmol/min/mg in brain microsomes of *hUGT1* mice treated with CBZ.

Discussion

CYP2D6, which is expressed in the human brain, can catalyze *O*-demethylation of 5-methoxytryptamine to generate serotonin (Yu et al., 2003). CYP2D6 has many polymorphisms associated with personality traits and neurologic or psychiatric disorders. A previous study showed that female CYP2D6 ultra-rapid metabolizers were more frequent among patients with eating disorders due to the alteration of brain serotonin concentration (Peñas-Lledó et al., 2012). This study indicated that enzymes capable of catalyzing the generation and elimination of endogenous compounds possibly regulate the local concentration and function of the endogenous compounds in the brain. In the present study, UGT1A1, 1A3, 1A6, and 1A10 were detected in human brain (Fig. 1). The brain microsomes of *hUGT1* mice exhibited glucuronidation activities (Figs. 6 and 7), suggesting that the endogenous compounds that are substrates of these UGTs may be glucuronidated in human brain. Dopamine, which is highly glucuronidated by UGT1A10 (Itäaho et al., 2009), and thyroxine, which is highly glucuronidated by UGT1A1, 1A3, 1A8, and 1A10 (Tong et al., 2007), might be glucuronidated by brain UGTs. Moreover, as the region- and age-dependent differences in expression levels of brain UGT1A1 and 1A6 were observed in *hUGT1* mice (Figs. 2 and 3), UGTs in the brain may be involved in the regulation of the concentration and functions of endogenous compounds in a region- and age-dependent manner.

PCN significantly induced UGT1A1 and UGT1A6 mRNA in the liver (Fig. 4, A and C). In the brain, however, such induction was not observed (Fig. 4, B and D). The glucuronidation activities of UGT1A1 and UGT1A6 were not induced by the PCN treatment (Figs. 6 and 7),

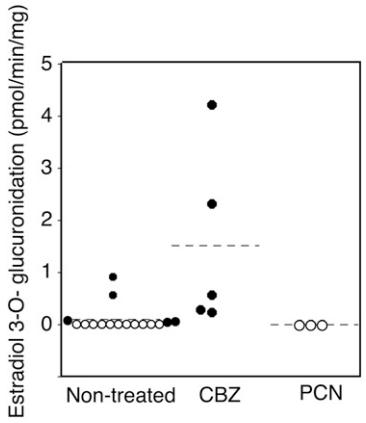


Fig. 6. Effects of inducers treatments on UGT1A1 activity in the brain microsomes of *hUGT1* mice. Microsomes from nontreated *hUGT1* mice (n = 12) and inducers-treated *hUGT1* mice (n > 3) were prepared, and estradiol 3-*O*-glucuronide formation was measured. Open circles indicate the microsome samples that did not exhibit glucuronidation activity toward estradiol. Filled circles indicate the microsome samples that exhibited glucuronidation activity toward estradiol.

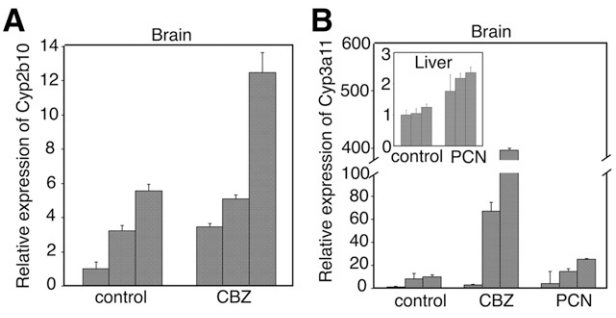


Fig. 5. The effect of inducers treatment on Cyp expression in *hUGT1* mice. Total RNA was isolated from tissues of nontreated *hUGT1* mice (n = 3) and inducers-treated *hUGT1* mice (n = 3). Complementary DNA was synthesized, and then quantitative RT-PCR was carried out for mouse Cyp2b10 (A) and Cyp3a11 (B) in the *hUGT1* mice. Each column is the mean ± S.D. of three indepedent determinations.

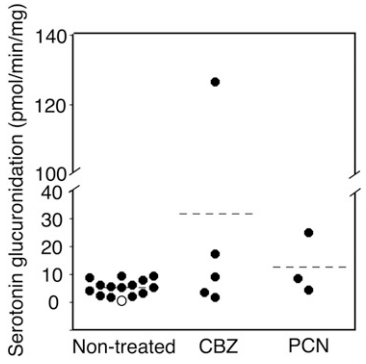


Fig. 7. Effects of inducers treatments on UGT1A6 activity in the brain microsomes of *hUGT1* mice. Microsomes from nontreated *hUGT1* mice (n = 12) and inducers-treated *hUGT1* mice (n > 3) were prepared, and serotonin glucuronide formation was measured. Open circle indicates the microsome samples that did not exhibit glucuronidation activity toward serotonin. Filled circles indicate the microsome samples that exhibited glucuronidation activity toward serotonin.

which was in agreement with our quantitative RT-PCR data (Fig. 4). Although PCN induced the expression of Cyp3a11 in the liver, it did not induce the gene in the brain (Fig. 5B). Previously, Lamba et al. (2004) reported that PXR was not expressed in human brain regions such as the cerebral cortex and cerebellum, except for the thalamus. These data indicate that PXR might not be functional in the brain. In contrast, CBZ-treated mice showed slightly higher UGT1A1 and UGT1A6 activities in brain microsomes compared with the control *hUGT1* mice (Figs. 6 and 7), although this was not statistically significant. This finding indicates that constitutive androstane receptor activators such as CBZ might increase UGT expressions and activities to affect the function of endogenous compounds in the brain. Developmental disorders have been reported in infants who were born from CBZ-administered mothers (Ornoy and Cohen, 1996). Thyroxine is very important in the development of the brain (Dussault and Ruel, 1987). Administered CBZ could be distributed to the infant's brain to induce brain UGTs. Therefore, the reported developmental disorders might have been attributed to a reduced brain thyroxine level, which could be caused by induced UGT functions.

A significant interindividual variability was observed in the responsiveness to a CYP3A inducer (Fig. 5B). Previously, liver biopsies were collected from 14 patients before and after rifampicin treatment (600 mg/day for 4 days) (Ged et al., 1989). After the rifampicin treatment, there was an 18-fold difference in the induction of CYP3A4 protein. The extent of increase ranged from 160 to 2900% among these patients, indicating that there could be a significant variability in the responsiveness to CYP3A inducers.

Additional discussion can be found online (Supplemental Discussion; Supplemental Fig. 3; Supplemental Table 1).

In the present study, we demonstrated that UGT1A1, 1A3, 1A6, and 1A10 were expressed in human brains. The expression pattern of UGT1As in human brains was similar to that in *hUGT1* mouse brain, indicating that *hUGT1* mice might be useful to study the importance of brain UGTs in vivo. The expression levels of UGT1A1 and 1A6 in *hUGT1* mouse brains were different in a region- and age-dependent manner. Therefore, UGTs might play an important role in function regulation of endogenous compounds in a region- and age-dependent manner.

Authorship Contributions

Participated in research design: Kutsuno, Itoh, Tukey, Fujiwara.

Conducted experiments: Kutsuno, Hirashima, Sakamoto, Ushikubo, Fujiwara.

Performed data analysis: Kutsuno, Hirashima, Michimae, Fujiwara.

Wrote or contributed to the writing of the manuscript: Kutsuno, Itoh, Tukey, Fujiwara.

References

Al-Jahdari WS, Yamamoto K, Hiraoka H, Nakamura K, Goto F, and Horiuchi R (2006) Prediction of total propofol clearance based on enzyme activities in microsomes from human kidney and liver. *Eur J Clin Pharmacol* **62**:527–533.

Aoshima N, Fujie Y, Itoh T, Tukey RH, and Fujiwara R (2014) Glucose induces intestinal human UDP-glucuronosyltransferase (UGT) 1A1 to prevent neonatal hyperbilirubinemia. *Sci Rep* **4**:6343.

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **72**:248–254.

Buckley DB and Klaassen CD (2007) Tissue- and gender-specific mRNA expression of UDP-glucuronosyltransferases (UGTs) in mice. *Drug Metab Dispos* **35**:121–127.

Court MH (2005) Isoform-selective probe substrates for in vitro studies of human UDP-glucuronosyltransferases. *Methods Enzymol* **400**:104–116.

Court MH, Zhang X, Ding X, Yee KK, Hesse LM, and Finel M (2012) Quantitative distribution of mRNAs encoding the 19 human UDP-glucuronosyltransferase enzymes in 26 adult and 3 fetal tissues. *Xenobiotica* **42**:266–277.

de Leon J (2003) Glucuronidation enzymes, genes and psychiatry. *Int J Neuropsychopharmacol* **6**:57–72.

Dussault JH and Ruel J (1987) Thyroid hormones and brain development. *Annu Rev Physiol* **49**: 321–334.

Dutton GJ (1980) Acceptor substrates of UDP glucuronosyltransferase and their assay, in *Glucuronidation of Drugs and Other Compounds* (Dutton GJ ed) pp 69–78, CRC Press, Boca Raton, FL.

Fujiwara R, Chen S, Karin M, and Tukey RH (2012) Reduced expression of UGT1A1 in intestines of humanized UGT1 mice via inactivation of NF-κB leads to hyperbilirubinemia. *Gastroenterology* **142**:109–118, e2.

Fujiwara R, Nguyen N, Chen S, and Tukey RH (2010) Developmental hyperbilirubinemia and CNS toxicity in mice humanized with the UDP glucuronosyltransferase 1 (UGT1) locus. *Proc Natl Acad Sci USA* **107**:5024–5029.

Ged C, Rouillon JM, Pichard L, Combalbert J, Bressot N, Bories P, Michel H, Beaune P, and Maurel P (1989) The increase in urinary excretion of 6 beta-hydroxycortisol as a marker of human hepatic cytochrome P450IIIA induction. *Br J Clin Pharmacol* **28**:373–387.

Gross C, Zhuang X, Stark K, Ramboz S, Oosting R, Kirby L, Santarelli L, Beck S, and Hen R (2002) Serotonin1A receptor acts during development to establish normal anxiety-like behaviour in the adult. *Nature* **416**:396–400.

Hewitt NJ, Lecluyse EL, and Ferguson SS (2007) Induction of hepatic cytochrome P450 enzymes: methods, mechanisms, recommendations, and in vitro-in vivo correlations. *Xenobiotica* **37**: 1196–1224.

Itäaho K, Court MH, Uutela P, Kostiainen R, Radominska-Pandya A, and Finel M (2009) Dopamine is a low-affinity and high-specificity substrate for the human UDP-glucuronosyltransferase 1A10. *Drug Metab Dispos* **37**:768–775.

Izukawa T, Nakajima M, Fujiwara R, Yamanaka H, Fukami T, Takamiya M, Aoki Y, Ikushiro S, Sakaki T, and Yokoi T (2009) Quantitative analysis of UDP-glucuronosyltransferase (UGT) 1A and UGT2B expression levels in human livers. *Drug Metab Dispos* **37**:1759–1768.

King CD, Rios GR, Assouline JA, and Tephly TR (1999) Expression of UDP-glucuronosyltransferases (UGTs) 2B7 and 1A6 in the human brain and identification of 5-hydroxytryptamine as a substrate. *Arch Biochem Biophys* **365**:156–162.

Krishnaswamy S, Duan SX, Von Moltke LL, Greenblatt DJ, and Court MH (2003) Validation of serotonin (5-hydroxtryptamine) as an in vitro substrate probe for human UDP-glucuronosyltransferase (UGT) 1A6. *Drug Metab Dispos* **31**:133–139.

Kutsuno Y, Itoh T, Tukey RH, and Fujiwara R (2014) Glucuronidation of drugs and drug-induced toxicity in humanized UDP-glucuronosyltransferase 1 mice. *Drug Metab Dispos* **42**:1146–1152.

Kutsuno Y, Sumida K, Itoh T, Tukey RH, and Fujiwara R (2013) Glucuronidation of drugs in humanized UDP-glucuronosyltransferase 1 mice: Similarity with glucuronidation in human liver microsomes. *Pharmacol Res Perspect* **1**:e00002.

Lamba V, Yasuda K, Lamba JK, Assem M, Davila J, Strom S, and Schuetz EG (2004) PXR (NR1I2): splice variants in human tissues, including brain, and identification of neurosteroids and nicotine as PXR activators. *Toxicol Appl Pharmacol* **199**:251–265.

Lee KH, Horiuchi M, Itoh T, Greenhalgh DG, and Cho K (2011) Cerebellum-specific and age-dependent expression of an endogenous retrovirus with intact coding potential. *Retrovirology* **8**:82.

Mackenzie PI, Bock KW, Burchell B, Guillemette C, Ikushiro S, Iyanagi T, Miners JO, Owens IS, and Nebert DW (2005) Nomenclature update for the mammalian UDP glycosyltransferase (UGT) gene superfamily. *Pharmacogenet Genomics* **15**:677–685.

Miksys S, Rao Y, Sellers EM, Kwan M, Mendis D, and Tyndale RF (2000) Regional and cellular distribution of CYP2D subfamily members in rat brain. *Xenobiotica* **30**:547–564.

Nakamura A, Nakajima M, Yamanaka H, Fujiwara R, and Yokoi T (2008) Expression of UGT1A and UGT2B mRNA in human normal tissues and various cell lines. *Drug Metab Dispos* **36**:1461–1464.

Nozawa D, Suzuki N, Kobayashi-Osaki M, Pan X, Engel JD, and Yamamoto M (2009) GATA2-dependent and region-specific regulation of Gata2 transcription in the mouse midbrain. *Genes Cells* **14**:569–582.

Ornoy A and Cohen E (1996) Outcome of children born to epileptic mothers treated with carbamazepine during pregnancy. *Arch Dis Child* **75**:517–520.

Peñas-Lledó EM, Dorado P, Agüera Z, Gratacós M, Estivill X, Fernández-Aranda F, and Llerena A (2012) CYP2D6 polymorphism in patients with eating disorders. *Pharmacogenomics J* **12**:173–175.

Ritter JK, Chen F, Sheen YY, Tran HM, Kimura S, Yeatman MT, and Owens IS (1992) A novel complex locus UGT1 encodes human bilirubin, phenol, and other UDP-glucuronosyltransferase isozymes with identical carboxyl termini. *J Biol Chem* **267**:3257–3261.

Sarkisyan G and Hedlund PB (2009) The 5-HT_7 receptor is involved in allocentric spatial memory information processing. *Behav Brain Res* **202**:26–31.

Shelby MK, Cherrington NJ, Vansell NR, and Klaassen CD (2003) Tissue mRNA expression of the rat UDP-glucuronosyltransferase gene family. *Drug Metab Dispos* **31**:326–333.

Shibuya A, Itoh T, Tukey RH, and Fujiwara R (2013) Impact of fatty acids on human UDP-glucuronosyltransferase 1A1 activity and its expression in neonatal hyperbilirubinemia. *Sci Rep* **3**:2903.

Sakamoto M, Itoh T, Tukey RH, and Fujiwara R (2015) Nicotine regulates the expression of UDP-glucuronosyltransferase (UGT) in humanized UGT1 mouse brain. *Drug Metab Pharmacokinet* (in press). doi:10.1016/j.dmpk.2015.04.004

Sumida K, Kawana M, Kouno E, Itoh T, Takano S, Narawa T, Tukey RH, and Fujiwara R (2013) Importance of UDP-glucuronosyltransferase 1A1 expression in skin and its induction by UVB in neonatal hyperbilirubinemia. *Mol Pharmacol* **84**:679–686.

Tong Z, Li H, Goljer I, McConnell O, and Chandrasekaran A (2007) In vitro glucuronidation of thyroxine and triiodothyronine by liver microsomes and recombinant human UDP-glucuronosyltransferases. *Drug Metab Dispos* **35**:2203–2210.

Trouillas P (1993) The cerebellar serotoninergic system and its possible involvement in cerebellar ataxia. *Can J Neurol Sci* **20** (Suppl 3):S78–S82.

Tukey RH and Strassburg CP (2000) Human UDP-glucuronosyltransferases: metabolism, expression, and disease. *Annu Rev Pharmacol Toxicol* **40**:581–616.

Uchaipichat V, Mackenzie PI, Guo XH, Gardner-Stephen D, Galetin A, Houston JB, and Miners JO (2004) Human udp-glucuronosyltransferases: isoform selectivity and kinetics of 4-methylumbelliferone and 1-naphthol glucuronidation, effects of organic solvents, and inhibition by diclofenac and probenecid. *Drug Metab Dispos* **32**:413–423.

Viani A, Temellini A, Tusini G, and Pacifici GM (1990) Human brain sulphotransferase and glucuronyltransferase. *Hum Exp Toxicol* **9**:65–69.

Wahlström A, Winblad B, Bixo M, and Rane A (1988) Human brain metabolism of morphine and naloxone. *Pain* **35**:121–127.

Yu AM, Idle JR, Byrd LG, Krausz KW, Küpfer A, and Gonzalez FJ (2003) Regeneration of serotonin from 5-methoxytryptamine by polymorphic human CYP2D6. *Pharmacogenetics* **13**:173–181.

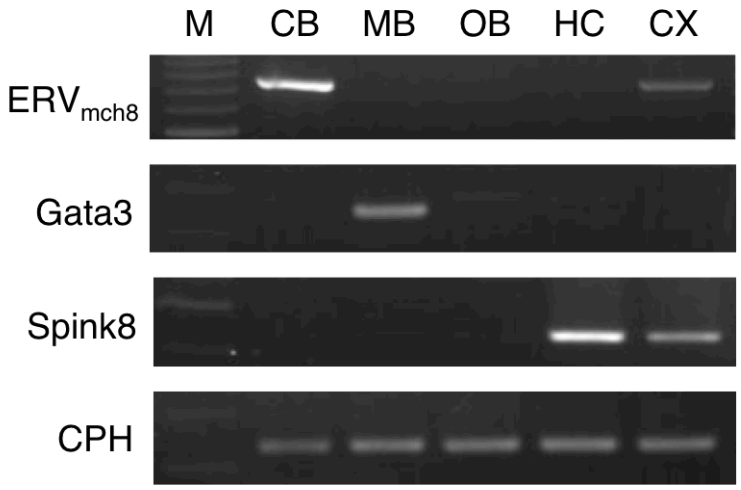
Address correspondence to: Dr. Ryoichi Fujiwara, School of Pharmacy, Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8641, Japan. E-mail: fujiwarar@pharm.kitasato-u.ac.jp

Journal: Drug Metabolism and Disposition

Expression of *UDP-glucuronosyltransferase 1* (*UGT1*) and glucuronidation activity toward endogenous substances in humanized *UGT1* mouse brain

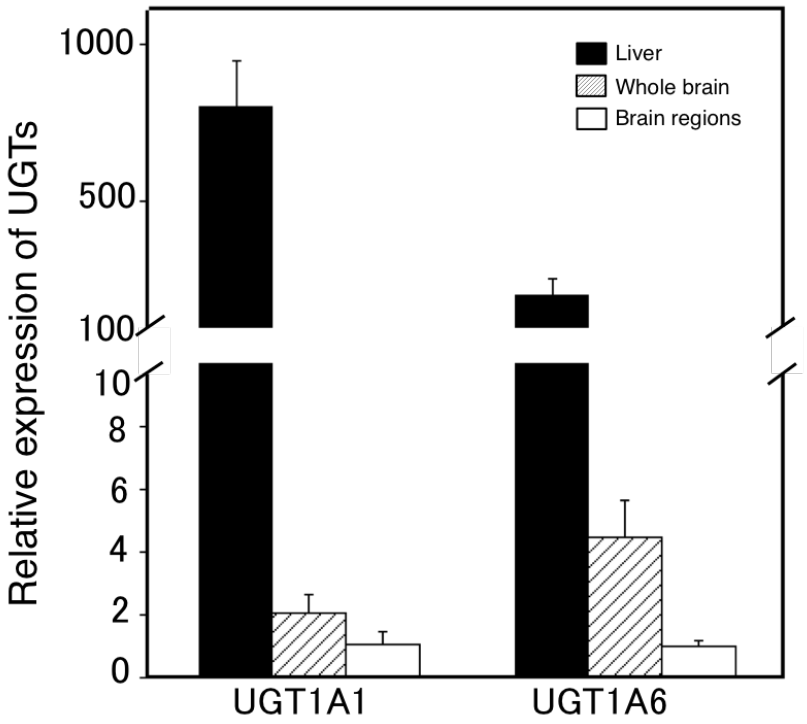
Yuki Kutsuno, Rika Hirashima, Masaya Sakamoto, Hiroko Ushikubo, Hirofumi Michimae, Tomoo Itoh, Robert H. Tukey, and Ryoichi Fujiwara

Supplemental Fig. 1



Supplemental Figure 1. Expression of brain region-specific marker genes in the brain.
Total RNA was prepared from brain regions and was used for the RT-PCR using primer pairs specific for brain region-specific markers, ERV_{mch8} (murine leukemia virus type-endogenous retrovirus mapped on chromosome 8), GATA binding protein 3 (Gata3), and serine peptidase inhibitor Kazal type 8 (Spink8). Forward and reverse primers used were: ERV_{mch8}-S, 5'-GAA GTT GAA AAG TCC ATC ACT AA -3' and ERVmch8-AS, 5'-TCT GGG TCT CTT GAA ACT GT-3'; Gata3-S, 5'-TCT CAC TCT CGA GGC AGC ATG T -3' and Gata3-AS, 5'- GTA CCA TCT CGC CGC CAC AG -3'; Spink8-S, 5'-CTG CTG TCC TTC TGA CGT CTT -3' and Spink8-AS, 5'-GCT CCC CGG TCA TGT GAA A-3'. M, 100 bp DNA ladder marker; CB, cerebellum; MB, midbrain; OB, olfactory bulbs; HC, hippocampus; CX, cerebral cortex.

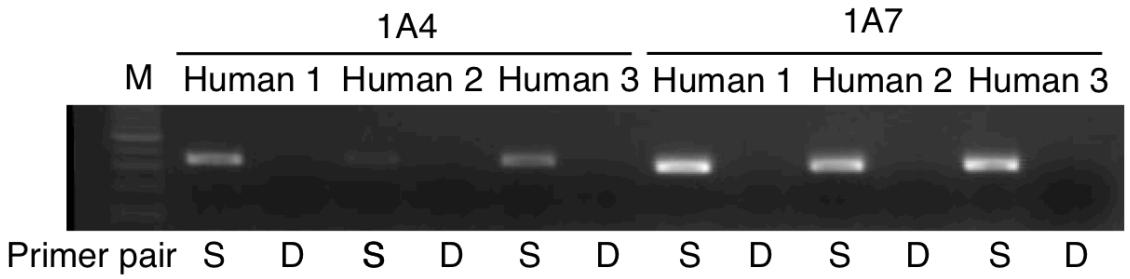
Supplemental Fig.2



Supplemental Figure 2. Comparison of mRNA expression levels of UGT1A1 and 1A6 between liver and brain in *hUGT1* mice.

Relative expression levels of UGT1A1 and UGT1A6 in the liver (black columns in Fig. 4), the total brain (shaded columns in Fig. 4), and the brain regions (white columns in Fig. 2 and 3) were shown.

Supplemental Fig. 3



Supplemental Figure 3. Location of primer pairs and detection of UGT1A4 and UGT1A7 by RT-PCR

Total RNA samples from three human brains containing meningioma were analyzed by RT-PCR using primer pairs specific to UGT1A4 and UGT1A7. Forward and reverse primers used were: UGT1A4 ex1-S, 5'-ACG CTG GGC TAC ACT CAA GG-3' and UGT1A4 ex1-AS, 5'-TCT GAA TTG GTC GTT AGT AAC T-3'; UGT1A4-S and UGT1A-AS (Table 1); UGT1A7 ex1-S, 5'-TGG CTC GTG CAG GGT GGA CTG-3' and UGT1A7 ex1-AS, 5'-TTC GCA ATG GTG CCG TCC AGC-3'; UGT1A7-S and UGT1A-AS (Table 1). The location of primers is indicated at the bottom of each gel. S and D indicate that the used primer pairs were located in the same exon and different exons, respectively. M, 100bp DNA ladder marker.

Supplemental discussion

A previous study reported that RT-PCR using the sense and antisense primers located in the same exon could overestimate the mRNA expression due to amplification of genomic DNA and pre-mRNA (Izukawa et al., 2009). Thus, RT-PCR using the primer pair located in the different exons provides more reliable data than that using the primer pair located in the same exon. A limited number of studies on UGT expression in human brain have been carried out by RT-PCR (Supplemental Table 1). King et al. demonstrated that UGT1A6 and UGT2B7 were detected in human brain by using RT-PCR techniques with the sense and antisense primers located in the different exons. In a following study, it was reported by using quantitative RT-PCR techniques with primer pairs located in the same exon that mRNAs of UGT1A1, 1A3, 1A4, 1A6, 1A7, 1A10, 2A2, 2A3, 2B7, and 2B11 were expressed in human brain (Court et al., 2012). The latter study was partly not consistent with the present study. As the interindividual difference in the expression pattern of UGT1As in human was not observed (Fig. 1A), the discrepancy of these results might be due to the difference of experimental condition such as primers. Actually, UGT1A4 and UGT1A7 mRNA were detected by using primer pairs located in the same exon in the present study (Supplemental Figure 3), while they were not detected by

using primer pairs located in different exons (Fig. 1A). Because we examined the expression of UGT isoforms in human brain by using the primer pairs located in different exons, our data could be more reliable than past data. Thus, we demonstrated that UGT1A1, 1A3, 1A6, and 1A10 were expressed in human brain in the present study.

It was reported that CYP activity was lost during the preparation of intestinal microsomes due to degradation by proteases (Kaminsky and Fasco, 1992). In another study, Abe et al. demonstrated that UGT proteins in UGT-expressing cells treated with protein kinase C inhibitors were degraded during the preparation of cell homogenates in the absence of a detergent. In the present study, several brain microsomes of *hUGT1* mouse brains did not exhibit glucuronidation activities toward estradiol and serotonin (Fig. 6 and 7). Therefore, the difference of the glucuronidation activity toward estradiol and serotonin in each microsome might have been partially caused by degradation during microsomes preparation. The preparation methods of brain microsomes should be improved to assess the glucuronidation activity of brain UGTs.

Supplemental Table 1. UGT1A mRNA expression in human brain determined by RT-PCR and location of primer pairs

UGT1A Isoforms	Location of primer pairs		References
	Forward	Reverse	
UGT1A1, 1A3, 1A4, 1A6, 1A7, and 1A10	Exon 1	Exon 1	Court et al. (2012)
UGT1A6	Exon 1	Exon 2	King et al. (1999)
UGT1A1, 1A3, 1A6, and 1A10	Exon 1	Exon 2	This study