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

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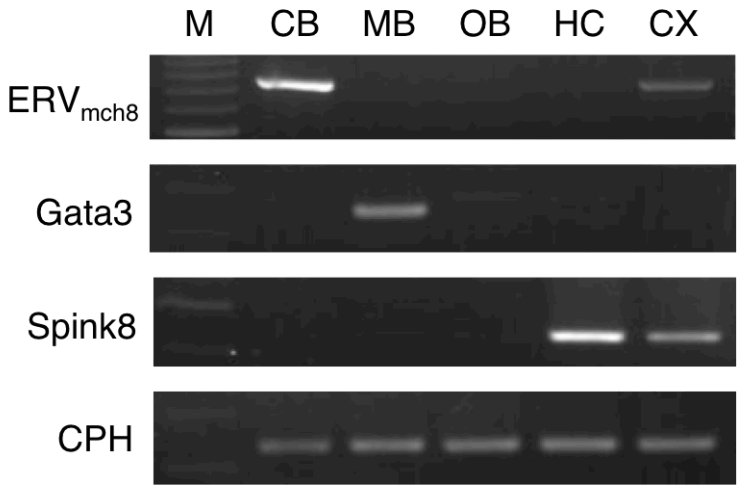
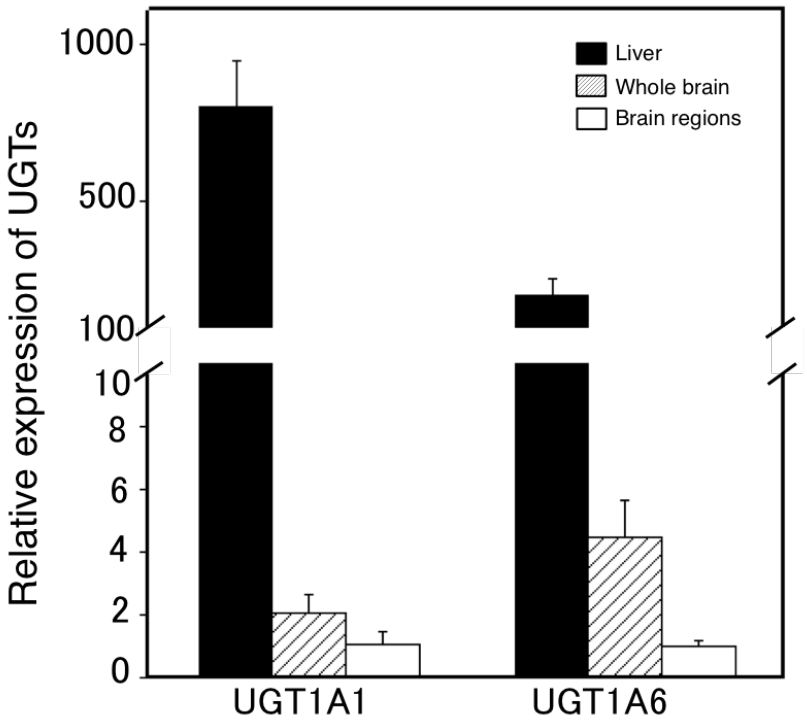
Supplemental Fig. 1



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.

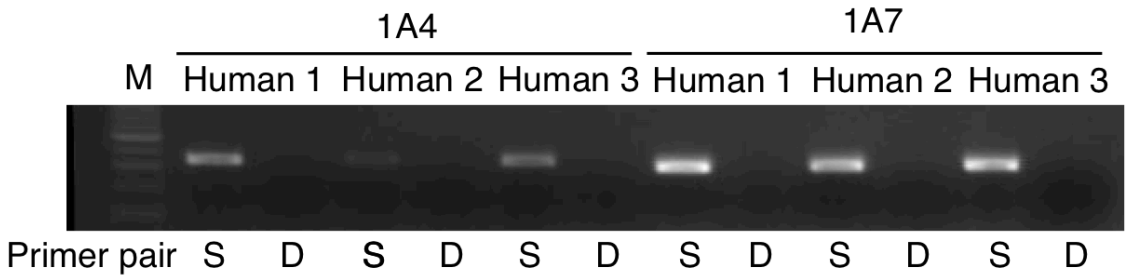
Supplemental Fig.2



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