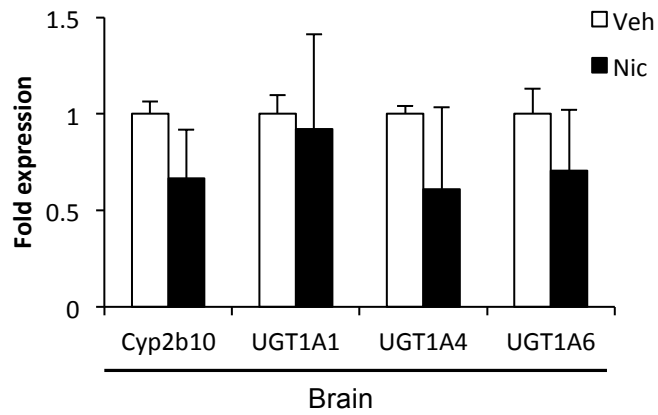


Supplementary materials

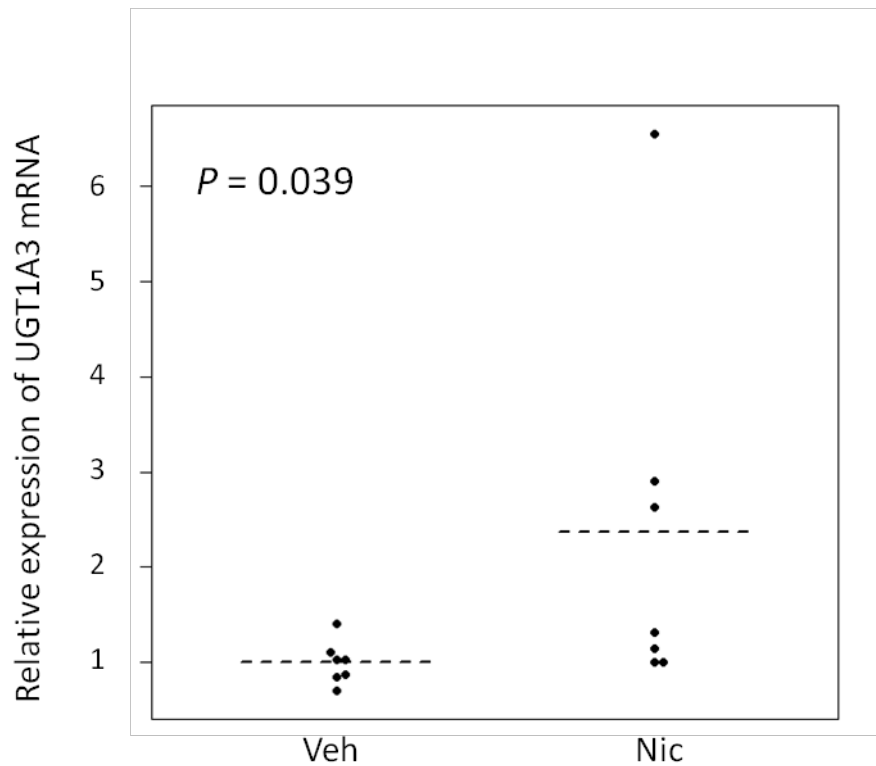
Journal title: Drug Metabolism and Pharmacokinetics

Article title: Nicotine regulates the expression of UDP-glucuronosyltransferase (UGT) in humanized *UGT1* mouse brain

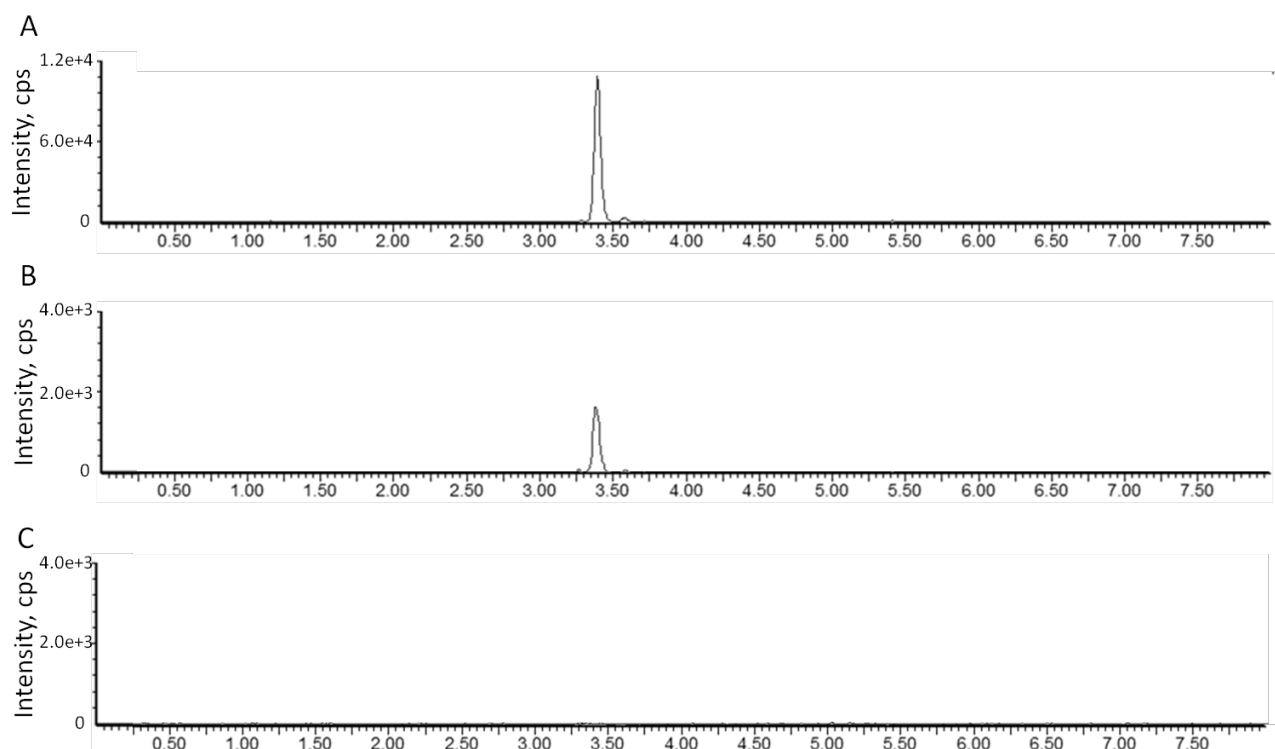
Article authors: Masaya Sakamoto, Tomoo Itoh, Robert H. Tukey, and Ryoichi Fujiwara



Supplemental Fig. 1. Relative expression levels of human UGT1A mRNA and mouse Cyp2b10 in brains of *hUGT1* mice that were treated with 1.0 mg/kg nicotine (Nic) or vehicle (Veh). *hUGT1* mice were treated 1.0 mg/kg nicotine, once daily for 7 days. Data were mean \pm SD of 3 mice in each group. The expression levels in vehicle-treated groups were defined as 1.



Supplemental Fig. 2. Relative expression levels of human UGT1A3 mRNAs in brains of *hUGT1* mice that were treated with nicotine (Nic) or vehicle (Veh). Seven mice each were treated with Nic or Veh as described in the manuscript (Group B). The mean expression level in the vehicle treated group was defined as 1. Brunner-Munzel test with significance set at 5% was used to examine the significance. Nicotine significantly induced the expression of UGT1A3 mRNA in the brain ($P = 0.039$).



Supplemental Fig. 3. Multiple reaction monitoring chromatograms of chenodeoxycholic acid (CDCA) glucuronide in the reaction mixtures. CDCA was incubated with human liver microsomes (A) or brain microsomes from *hUGT1* mouse treated with nicotine (B) in the presence of UDPGA. CDCA was also incubated with brain microsomes from *hUGT1* mouse treated with nicotine in the absence of UDPGA (C). Peaks in A and B were CDCA glucuronides, as no peak was obtained in C. cps, counts per second.

Supplemental Table 1. The detailed information of the RT-qPCR analysis

Nucleic acid extraction

Procedure and/or instrumentation	Brain was homogenized in Traizol. The homogenate was added to fifth volume of chloroform and shaken severely for 15 seconds. After 3 min, the solution was centrifuged at 12,000 g for 15 min at 4°C. The supernatant was added to two volumes of isopropanol and then mixed moderately. After 10 min, the solution was centrifuged at 12,000 g for 10 min at 4°C. The pellet was washed with 80% ethanol and centrifuged at 12,000 g for 10 min at 4°C. The pellet was incubated in TE buffer for 10 min at 55 °C.
Nucleic acid quantification and Purity (A_{260}/A_{280})	The extracted RNA was quantitated for spectrophotometry using NanoDrop (Thermo Scientific). The A_{260}/A_{280} ratio of the RNA sample was more than 1.8.

Reverse transcription

Complete reaction conditions and amount of RNA	A typical incubation mixture (10 μ L) contained 2 μ L of 5 \times RT Master Mix, 1 μ g RNA, and nuclease-free water.
Temperature and time	The reaction was performed in TaKaRa PCR Thermal Cycler Dice Gradient (Takara). The cycling parameters consisted of reverse transcription at 37°C for 15 minutes followed by 50°C for 5 minutes, and deactivation reaction at 98°C for 5 minutes.

qPCR oligonucleotides

Reaction volume and amount of cDNA	A typical incubation mixture (20 μ L) contained 10 μ L THUNDERBIRD SYBR qPCR Mix, 6 pmol forward primer, 6 pmol reverse primer, and 1 μ L cDNA.
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qPCR validation

Specificity (melt, digest)	The melt peak analysis for each gene showed a single peak.
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Supplemental Table 2. The ΔC_q and C_q values of the samples from brains and livers of *hUGT1* mice in group A.

	Brain						Liver					
	Veh 1	Veh 2	Veh 3	Nic 1	Nic 2	Nic 3	Veh 1	Veh 2	Veh 3	Nic 1	Nic 2	Nic 3
Human UGT1A												
UGT1A1	17.48	18.12	18.75	18.61	19.63	19.4	7.96	6.96	7.14	7.92	9.83	7.37
UGT1A3	12.74	12.62	12.99	12.28	12.19	11.67	3.79	2.97	3.26	3.61	4.72	3.75
UGT1A4	Tr	Tr	Tr	Tr	Tr	Tr	5.23	4.59	4.73	4.59	5.62	5.05
UGT1A5	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr
UGT1A6	17.85	16.12	17.71	17.18	17.77	17.82	4.05	3.16	3.94	2.74	4.52	3.55
UGT1A7	Tr	Tr	Tr	Tr	Tr	Tr	12.8	13.44	12.22	11.65	13.25	12.04
UGT1A8	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr
UGT1A9	Tr	Tr	Tr	Tr	Tr	Tr	11.14	10.56	10.50	9.20	10.93	9.42
UGT1A10	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr
Mouse Ugt2b												
Ugt2b1	14.27	13.98	14.54	15.83	15.99	14.76	-0.21	-0.1	0.4	-0.39	-0.01	-0.09
Ugt2b5	14.58	14.29	14.57	15.62	15.98	14.98	0.12	0.19	0.47	-0.21	0.39	-0.12
Ugt2b34	16.64	17.06	19.85	18.87	19.47	18.46	0.59	0.55	1.05	0.11	0.73	0.49
Ugt2b35	14.63	15.4	13.85	15.5	16.05	14.19	2.92	3.59	3.91	2.92	3.59	3.43
Ugt2b36	17.02	18.03	17.18	18.03	18.97	16.79	2.94	2.74	2.71	2.49	2.79	2.76
Ugt2b37	14.37	15.05	13.85	15.16	15.36	14.03	7.48	9.1	3.11	2.43	7.54	7.71
Mouse Cyp2b												
Cyp2b10	16.32	17.51	19.11	18.68	18.37	18.71	0.37	0.23	0.46	-0.28	0.42	0.02
CPH (C_q)	15.86	15.36	15.45	15.58	15.63	15.58	17.60	17.37	17.44	17.26	17.66	17.19

ΔC_q values of the UGT and CYP gene, and the quantification cycle (C_q) value of the CPH gene were listed, respectively. ΔC_q value of the sample was determined by subtracting the C_q value of CPH gene from the C_q value of the UGT gene or CYP gene. Trace level (Tr) indicates that mRNA levels were below the detection limits. Veh = vehicle, Nic = nicotine.

Supplemental Table 3. The ΔC_q and C_q values of the samples from brains and livers of *hUGT1* mice in group B.

	Brain						Liver					
	Veh 1	Veh 2	Veh 3	Nic 1	Nic 2	Nic 3	Veh 1	Veh 2	Veh 3	Nic 1	Nic 2	Nic 3
Human UGT1A												
UGT1A1	19.02	18.1	19.16	18.27	17.75	19.03	11.2	9.7	5.58	9.59	8.13	6.22
UGT1A3	12.33	12.66	12.44	11.73	12.09	11.65	3.34	5.15	2.54	5.41	4.37	3.08
UGT1A4	Tr	Tr	Tr	Tr	Tr	Tr	4.51	6.35	4.33	6.64	4.39	4.19
UGT1A5	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr
UGT1A6	17.14	18.95	17.87	19.3	18.5	17.72	3.63	5.12	3.48	5.46	4.31	3.5
UGT1A7	Tr	Tr	Tr	Tr	Tr	Tr	12.57	13.22	11.58	13.09	12.54	11.58
UGT1A8	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr
UGT1A9	Tr	Tr	Tr	Tr	Tr	Tr	4.64	6.14	3.72	6.46	4.35	3.99
UGT1A10	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr
Mouse Ugt2b												
Ugt2b1	14.91	14.39	14.92	13.57	13.52	13.35	0.41	0.59	1.06	1.1	1.31	0.88
Ugt2b5	15.27	15.28	16.14	15.23	15.13	15.08	0.8	0.79	1.11	1.24	1.48	1.68
Ugt2b34	15.85	18.33	19.78	19.05	18.27	18.29	1.01	1.34	1.4	1.5	1.87	1.22
Ugt2b35	16.27	15.25	16.41	14.9	14.73	14.55	3.51	4.24	4.18	4.41	4.31	4.46
Ugt2b36	16.21	15.56	16.74	15.35	14.93	15.05	1.19	2.01	1.73	2.75	2.1	2.37
Ugt2b37	15.36	14.25	15.33	13.73	13.91	13.87	10.35	9.62	10.28	9.24	10.67	8.98
Mouse Cyp2b												
Cyp2b10	15.45	14.73	14.07	13.75	13.74	13.83	8.02	10.26	8.51	9.56	9.57	9.33
CPH (C_q)	15.68	15.17	15.66	15.22	15.27	15.54	17.13	16.10	16.19	16.95	16.88	16.51

ΔC_q values of the UGT and CYP gene, and the quantification cycle (C_q) value of the CPH gene were listed, respectively. ΔC_q value of the sample was determined by subtracting the C_q value of CPH gene from the C_q value of the UGT gene or CYP gene. Trace level (Tr) indicates that mRNA levels were below the detection limits. Veh = vehicle, Nic = nicotine.