Analysis of in vitro and in vivo metabolism of zidovudine and gemfibrozil in trans-chromosomal mouse line expressing human UGT2 enzymes

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Abstract

UDP-glucuronosyltransferases (UGTs) catalyze the conjugation of various substrates with sugars. Since the UGT2 family forms a large cluster spanning 1.5 Mb, transgenic mouse lines carrying the entire human UGT2 family have not been constructed because of limitations in conventional cloning techniques. Therefore, we made a humanized mouse model for UGT2 by chromosome engineering technologies. The results showed that six UGT2 isoforms examined were expressed in the liver of adult humanized UGT2 (hUGT2) mice. Thus, the functions of human UGT2B7 in the liver of hUGT2 mice were evaluated. Glucuronide of azidothymidine (AZT, zidovudine), a typical UGT2B7 substrate, was formed in the liver microsomes of hUGT2 mice but not in the liver microsomes of wild-type and Ugt2-knockout mice. When AZT was intravenously administered, AZT glucuronide was detected in the bile and urine of hUGT2 mice, but it was not detected in the bile and urine of wild-type and Ugt2-knockout mice. These results indicated that the hUGT2 mice express functional human UGT2B7 in the liver. This finding was also confirmed by using gemfibrozil as an alternative UGT2B7 substrate. Gemfibrozil glucuronide was formed in the liver microsomes of hUGT2 mice and was mainly excreted in the bile of hUGT2 mice after intravenous dosing of gemfibrozil. This hUGT2 mouse model will enable improved predictions of pharmacokinetics, urinary
INTRODUCTION

UDP-glucuronosyltransferases (UGTs) catalyze the conjugation of lipophilic molecules with sugars. The superfamily of mammalian UGTs comprises UGT1, UGT2, UGT3 and UGT8 families. UGT1 and UGT2 enzymes are phase II enzymes that transfer glucuronyl group from UDP-glucuronic acid (UDPGA) to various lipophilic substrates. The formed glucuronides are often pharmacologically inactive and soluble in water, and they can be excreted through bile or urine. The mammalian UGT1 and UGT2 families consist of UGT1A, UGT2A and UGT2B subfamilies.

Among the human UGT2 enzymes, UGT2B7 is one of the most significant isoforms in the human liver. Human UGT2B7 is involved in the metabolism of various therapeutic drugs including morphine, carbamazepine, mycophenolic acid, zidovudine, gemfibrozil, di-clofenac and chloramphenicol. Human liver microsomes and recombinant UGT isoforms are useful for clarifying the contribution of UGT2 enzymes to the metabolism of interest drug and also for examining the interactions with other substrates. A recent study suggested a bottom-up approach for predicting hepatic clearance in vivo by using protein contents and activity of UGT2B7 in human liver microsomes. However, predicting the involvement of human UGT2 enzymes in in vivo drug disposition from in vitro data remains challenging.

Previously, transgenic mice expressing the entire UGT2B7 gene were created, but human UGT2 genes other than UGT2B7 have not been introduced. In addition, mouse endogenous Ugt genes remain in the transgenic mice. Recently, Fay et al. made a mouse line in which the entire Ugt2 gene family is deleted. This mouse model is useful for investigating the contribution of mouse UGT2 enzymes to the drug metabolism in vivo. However, transgenic mice lines carrying the entire human UGT2 family encoded on about a 1-Mb locus on human chromosome 4 have not been constructed because of limitations in conventional cloning techniques. Recently, we developed a humanized UGT2 rat model by chromosome-engineering techniques with a mouse artificial chromosome (MAC) vector. Rats are often used as animal models for testing the efficacy and toxicity of drugs in preclinical studies of drug development, but large amounts of drugs are needed for studies using rats. Thus, a mouse model for predicting human UGT2 functions would be convenient when large amounts of drugs are unavailable.

In the present study, we created a new humanized mouse model for UGT2B7 by chromosome-engineering techniques with MAC vectors. The MAC vector is an ideal gene-delivery vector, having unique characters such as stable episomal maintenance and the capacity to carry large genomic loci with their regulatory elements. The gene delivery using the MAC vector allows physiological regulation of the introduced gene in a manner similar to that of the native chromosome. Furthermore, the expression of human UGT2 mRNA in several tissues of humanized UGT2 mice was evaluated. Since human UGT2B7 was expressed in the liver at the mRNA level, we investigated the function of human UGT2B7 in the liver of hUGT2 mice in vitro and in vivo by using zidovudine (azithromycin, AZT) and gemfibrozil as typical UGT2B7 substrates.

MATERIALS AND METHODS

2.1 Reagents

Azidothymidine (AZT, zidovudine) and gemfibrozil were obtained from FUJIFILM Wako Chemicals (Osaka, Japan) and LKT Laboratories (St. Paul, MN), respectively. AZT glucuronide, AZT-d3 glucuronide, gemfibrozil glucuronide and gemfibrozil-d6 glucuronide were purchased from Toronto Research Chemicals (Ontario, Canada). All other chemicals were of analytical grade.

2.2 Cell culture

Mouse A9 cells carrying an MAC vector that contains the human UGT2 gene cluster (UGT2-MAC) were maintained at 37°C in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% fetal bovine serum and 800μg/ml G418. TT2 and TT2F mouse embryonic stem (mES) cell lines were maintained on mitomycin C (Sigma Aldrich, St. Louis, MO)-treated neomycin-resistant MEFs (Oriental Yeast Co., Ltd., Tokyo, Japan) as a feeder layer in DMEM containing 18% fetal bovine serum (HyClone Laboratories, Logan, UT), 2 mM L-glutamine (Invitrogen), 1 mM sodium pyruvate (Invitrogen, Carlsbad, CA), 0.1 mM 2-mercaptoethanol (Sigma Aldrich), 0.1 mM thymidine, AZT-glucuronide, AZT-d3-glucuronide, thymidine, AZT-glucuronide, AZT-d3-glucuronide, gemfibrozil, gemfibrozil-d6 glucuronide and gemfibrozil-d6 glucuronide were purchased from Toronto Research Chemicals (Ontario, Canada). All other chemicals were of analytical grade.

Significance Statement

The present study provides the first generation of humanized UGT2 (hUGT2) mice by chromosome engineering technology. The results of in vitro and in vivo experiments clearly indicate that human UGT2B7 catalyzed the formation of azidothymidine glucuronide and gemfibrozil glucuronide in the hUGT2 mice. The hUGT2 mice will be powerful model mice for various applications in drug development research and basic research.
non-essential amino acids (Invitrogen), and 1000 U/ml leukemia inhibitory factor (Funakoshi, Tokyo, Japan).

2.3 Vector construction

Toward the elimination of the Ugt2 cluster by the Cre/loxP system, a control vector mimicking the resultant genomic sequence (Big deletion allele in Figure S1) was constructed. Homologous arms were amplified by using primers: YtUg_KpnI_5AF1/Up5AR1 for the 5’ homologous arm of the upstream site and Down3AF1/ UgSu_NotI_3AR1 for the 3’ homologous arm of the downstream site. The fragments were inserted into Acc65I/XhoI and Ascl/NotI sites of pCAGGS-loxP-Hyg. The PCR template was genomic DNA obtained amplified with primers, YtUg_KpnI_5AF1/Up5AR1 for the 5′ arm and Up3AF3/ Up3AR1 for the 3’ arm, into Acc65I/XhoI and Ascl/NotI sites of pCAGGS-loxP-NeoR plasmid. A vector targeting the site downstream of the Ugt2 cluster between Ythdc1 and Ugt2b34 (Knock-in vector in Figure S1) was constructed by inserting arms that were amplified with primers, YtUg_KpnI_5AF1/Up5AR1 for the 5′ arm and Down3AF3/ Up3AR1 for the 3′ arm, into Acc65I/XhoI and Ascl/NotI sites of pCAGGS-loxP-HygR fragment into pBlue-ScriptII. For the amplicon of loxP-BamHI-F/UgSu_NotI_3AR1, the pCAGGS-loxP-Hyg control vector was used as a template. Sequences of primers for genomic PCR are shown in Table S1.

2.4 Targeting

The donor vector (32.4 μg/cuvette) targeting the site between Ythdc1 and Ugt2b34 and 5 μg each/cuvette of TALEN plasmid (left TALEN: 5’-TGA ACA CTA CTA TTC CA-3’, right TALEN: 5’-GCT CAT TTG GTA ATG TA-3’) were introduced into the TT2 mouse ES cell line (1 × 10^7 cells) by electroporation. The screening PCR primers were as follows: YtUg_KpnI_5AF5/Neo_108r for the 5′ junction and YtUg_NotI_3AR3/neo_MSO2 for the 3′ junction (Recombinant allele in Figure S1). The donor vector (12.5 μg/cuvette) targeting the site between Ugt2a1 and Sult1b1 and 5 μg each/cuvette of TALEN plasmid (left TALEN: 5’-ACA TAA AGC TTA ACA TT-3’, right TALEN: 5’-ATT GTG TTC AAT TTA AGG-3’) were introduced into the clone (1 x 10^7 cells) by electroporation. The screening PCR primers were as follows: sc_5AF4/puro_F4 for the 5′ junction and puro_R2/UgSu_NotI_3AR4 for the 3′ junction (Recombinant allele in Figure S1).

2.5 Deletion of the Ugt2 cluster by the Cre/loxP system

The Cre expression vector (20μg/cuvette) was introduced into the clones (1 x 10^7 cells) with a floxed Ugt2 cluster allele by electroporation and 24 drug-resistant clones were obtained under hygromycin (225 μg/ml) selection. The screening PCR primer set to detect the deletion junction was YtUg_KpnI_5AF1/UgSu_NotI_3AR1 (Big deletion allele in Figure S1). The primer sets YtUg_KpnI_5AF5/neor_108r and puro_R2/UgSu_NotI_3AR4 further confirmed the deletion. Absence of the Cre transgene was confirmed with Primer A/Primer B.

2.6 Microcell-mediated chromosome transfer

Microcell-mediated chromosome transfer (MMCT) was performed as described previously. TT2F mouse ES cells were fused with microcells prepared from mouse A9 cells carrying UGT2-MAC and selected with G418 (250 μg/ml). The transfer of UGT2-MAC was confirmed by PCR and FISH analyses.

2.7 Chimeric mouse production and germline transmission

Chimeric Ugt2-KO mice were produced as described previously. Briefly, Ugt2-KO ES cells were injected into eight-cell-stage embryos obtained from ICR mice (CLEA, Tokyo, Japan) and the embryos were transferred into pseudopregnant ICR females. Chimeric male mice showing 100% coat-color chimerism were generated. Germline transmission could successfully occur by mating chimeric mice with female ICR mice, and the offspring were further mated for production of Ugt2-KO mice. Ugt2-KO was further confirmed by PCR with primers: YtUg_KpnI_5AF5/UgSu_NotI_3AR4 for the deletion junction and primer sets for mouse Ugt2-KO target. The animals were kept in a temperature-controlled environment with a 12-h light/dark cycle.

2.8 FISH analysis

Samples were treated for 15 min in 0.075 M KCI and fixed with methanol and acetic acid (3:1). Metaphase spreads were prepared using standard methods. FISH analysis was performed using digoxigenin-labeled mouse minor satellite DNA and biotin-labeled UGT2-BAC DNA (RP11-643N16), essentially as described previously. Chromosomal DNA was counterstained with DAPI (Sigma Aldrich). Images were
captured using an AxiolmageZ2 fluorescence microscope (Carl Zeiss, Jena, Germany) and processed using Isis software provided with the microscope (MetaSystems GmbH, Altlussheim, Germany).

### 2.9 | Gene expression assays

Total RNA from tissue specimens was prepared using ISOGEN (Nippon Gene, Tokyo, Japan), purified using RNeasy columns (Qiagen, Hilden, Germany) in accordance with manufacturer’s instructions, and then treated with DNase I (Wako Pure Chemicals, Osaka, Japan). Total RNA was converted to cDNA by using random hexamers and SuperScript III reverse transcriptase (Invitrogen). The primer sets for detecting the expression of human UGT2 genes were described previously.\(^\text{15}\)

### 2.10 | In vitro glucuronidation assays

Liver microsomes were prepared from ICR (wild type, WT), Ugt2-KO and hUGT2 (UGT2-MAC/Ugt2-KO) mice according to a previous report.\(^\text{18}\) An incubation mixture (total volume of 100 μl) was prepared using UGT Reaction Mix Solution A and UGT Reaction Mix Solution B (Corning, Corning, NY, USA) and 0.5 mg/ml microsomes. After pre-incubation at 37°C for 5 min, the reaction was initiated by adding 200 μM AZT or 5 μM gemfibrozil to the mixture. After incubation at 37°C, 20 μl of the reaction mixture was added to 200 μl of ice-cold acetonitrile containing 200 nM of an internal standard (AZT-d3 glucuronide for AZT, gemfibrozil-d6 glucuronide for gemfibrozil) to terminate the reaction. After the removal of protein by filtration, a 3-μl portion of the sample was subjected to liquid chromatography tandem mass spectrometry (LC–MS/MS) analysis.

### 2.11 | In vivo glucuronidation analysis

Mice were anesthetized with isoflurane and treated with buprenorphine as an analgesic, and then the gallbladder of each mouse was cannulated. AZT and gemfibrozil were dissolved in 10% sulfobutylether-β-cyclodextrin (CyDex Pharmaceuticals, San Diego, CA) and administered to mice intravenously at the dosage of 1 mg/kg via the tail vein under an overnight fasted condition. Then the mice were individually placed in metabolic cages with a free moving system to collect bile and urine up to 24 h. Excretion in bile and excretion in urine were calculated as cumulative excretion by the following equation:

\[
\text{Biliary excretion (% of dose)} = \frac{C_{\text{bile}} \times V_{\text{bile}}}{\text{dose}} \times 100 \\
\text{Urinary excretion (% of dose)} = \frac{C_{\text{urine}} \times V_{\text{urine}}}{\text{dose}} \times 100
\]

where \(C_{\text{bile}}, C_{\text{urine}}, V_{\text{bile}}\) and \(V_{\text{urine}}\) are the concentrations of compounds excreted in bile and urine (C, nmol/ml) and the volumes of bile and urine (V, ml), respectively.

### 3 | LC–MS/MS

Preparation methods of collected urine and bile for LC–MS/MS analysis were described in Appendix S1. The conditions of LC–MS/MS analysis are shown in Appendix S1 and Tables S2–S6.

### 3.1 | Statistical analysis

Statistical analyses were performed using Statcel (OMS, Tokyo, Japan). Comparisons of multiple groups were performed by one-way ANOVA with Scheffe’s F-test. \(p < .05\) was considered statistically significant.

### 3.2 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY,\(^\text{19}\) and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20.\(^\text{20}\)

### 4 | RESULTS

#### 4.1 | Generation of a fully humanized mouse line for UGT2

hUGT2 mice were generated by crossing endogenous Ugt2-KO mice with Tc mice carrying UGT2-MAC (Figure 1A). Ugt2-KO mice were generated with Cre/loxP-mediated deletion of the mouse Ugt2 cluster (Figure S1). The upstream site of the Ugt2 cluster was targeted with a donor vector containing the loxP site and a neomycin-resistant gene in TT2 mES cells. Following G418 screening, 264 drug-resistant clones were obtained and analyzed. Two clones were positive for PCR and one clone was used for the next targeting. The downstream site of the Ugt2 cluster was targeted with a donor vector containing loxP and a puromycin-resistant gene in the mES clone. Following puromycin selection, 276 drug-resistant clones were obtained and analyzed. Three clones were positive for PCR and two of them were used for deletion of the Ugt2 cluster by the Cre/loxP system. To delete the Ugt2 cluster, a Cre expression vector was introduced into mES cells with floxed Ugt2 cluster alleles. Since the deletion of Ugt2 induces the expression of a hygromycin-resistant gene, cells were selected with hygromycin. Twenty-four drug-resistant clones from one parental clone were obtained and analyzed by PCR. Twenty-two of the 24 clones were positive for PCR screening and four Ugt2 single KO clones were selected for chimeric mouse production. Two of the four mES clones gave chimeric mice with 100% coat-color chimerism and germline transmission successfully occurred. Further mating generated offspring in which the Ugt2 cluster is completely knocked-out. The genome
structure by the deletion does not affect the neighboring gene expression and the selection marker does not affect phenotype of the mouse strain.\cite{14}

UGT2-MAC that was previously constructed in CHO cells,\cite{15} was transferred to TT2F mouse ES cells through mouse A9 cells by MMCT. Under G418 selection, 28 GFP-expressing and drug-resistant clones were obtained. FISH analysis revealed independent maintenance of UGT2-MAC without integration into the host genome. The mouse ES cells containing UGT2-MAC were used for chimeric mouse production. Chimeric mice with 100% coat-color chimerism were obtained and UGT2-MAC was successfully transmitted through the germline. Further mating of Tc mice carrying UGT2-MAC with Ugt2-KO mice resulted in the generation of fully humanized mice for UGT2. The UGT2-MAC was stably maintained without integrating into host genome in hUGT2 mouse (Figure 1B).

### 4.2 Gene expression

To confirm tissue-specific expression of human UGT2 isoforms, various tissues of an hUGT2 mouse were used for RT-PCR analyses (Figure 1C). UGT2A3, UGT2B7, UGT2B11, UGT2B15 and UGT2B28 were mainly expressed in the liver, kidney and intestine. Transcription of UGT2B4 was detected in the heart, liver and kidney. UGT2B10 was detected in the liver. UGT2B17 was not detected in any tissues. The expression profile of human UGT2 genes in the hUGT2 mouse was comparable with that in humans. Therefore, these data suggested that the transcription dynamics of UGT2 isoforms in hUGT2 mice is tightly regulated as in humans. Expression of mouse Ugt2 isoforms was completely absent in various tissues of hUGT2 mouse due to Ugt2-KO (Figure S2).

### 4.3 In vitro glucuronidation

To analyze the function of human UGT2 expressed in the liver of hUGT2 mice, glucuronidation of AZT and gemfibrozil, UGT2B7-selective substrates,\cite{8,9} was investigated in WT, Ugt2-KO, and hUGT2 mice. As shown in Figure 2A, AZT glucuronide was not formed in liver microsomes of WT and Ugt2-KO mice, indicating that mouse endogenous Ugt enzymes do not catalyze AZT glucuronidation in the liver. In contrast, AZT glucuronide was formed in an incubation time-dependent manner in the liver microsomes of hUGT2 mice. On the other hand, gemfibrozil glucuronide was formed in the
liver microsomes of WT and hUGT2 mice, but the formation was negligible in liver microsomes of Ugt2-KO mice (Figure 2B), suggesting that both mouse Ugt2 enzymes and human UGT2 enzymes catalyze gemfibrozil glucuronidation in the liver. Taken together, the results indicated that the introduced human UGT2 gene produced functional UGT2B7 protein in the liver.

Although UGT2B7 mRNA was detected in the kidney of hUGT2 mice (Figure 1C), AZT glucuronide was not detected in samples in which AZT was incubated with kidney microsomes of hUGT2 mice as in the case of WT and KO mice (data not shown).

4.4 | In vivo glucuronidation of AZT

To determine whether UGT2B7 expressed in hUGT2 mice is functional in vivo, we examined the excretion of AZT and its glucuronide in the urine and bile of WT, Ugt2-KO and hUGT2 mice. In agreement with the results from the in vitro study, AZT glucuronide was detected in the bile and urine of hUGT2 mice (14.7% and 53.1%, respectively), whereas it was not detected in the bile and urine of WT and Ugt2-KO mice (Figure 3A). As shown in Figure 3B, AZT was detected in the urine of WT and Ugt2-KO mice (48.4% and 18.8%, respectively). The levels of AZT excreted in the urine of hUGT2 mice was low (3.5%). AZT levels in the bile were less than 6% in all lines of mice.

4.5 | In vivo glucuronidation of gemfibrozil

We also monitored gemfibrozil and its glucuronide in urine and bile to examine the function of UGT2B7 in vivo. In WT mice, gemfibrozil glucuronide was detected at a higher level in bile than in urine (Figure 4A). The levels of gemfibrozil glucuronide in bile and urine of Ugt2-KO mice were lower than those in bile and urine of WT mice, indicating that glucuronidation of gemfibrozil was mainly mediated via mouse endogenous Ugt2 enzymes. In hUGT2 mice, gemfibrozil glucuronide was highly detected in bile, but it was negligible in urine. On the other hand, gemfibrozil levels in bile and urine were less than 5% in all lines of mice (Figure 4B).

5 | DISCUSSION

In the present study, endogenous Ugt2 single KO was successfully performed in mES cells by sequential targeted insertion of floxed loxP followed by Cre-mediated deletion of the floxed Ugt2 cluster. UGT2-MAC was transferred into mouse ES cells via MMCT and transmitted through the germline. Tc mice carrying UGT2-MAC were crossed with Ugt2-KO mice to generate fully humanized mice for UGT2. The transmission rate of UGT2-MAC was comparable to the previous observation. The humanized mice were phenotypically normal and gave progeny without any problem. UGT2-MAC was stably maintained in individuals as checked by FCM analysis of blood lymphocytes and was transmitted to further generations as previously reported.

Some gene affects the outcome of studies of drug metabolism as haploinsufficiency because of a single copy on the MAC in the mice. In this case, we can load two copies of gene cluster to the MAC or produce mice carrying two copies of the MAC with gene of interest by crossing Tc mice.

The mRNA expression of six UGT2 isoforms was detected in the liver of adult hUGT2 mice (Figure 1C). UGT2A3 is also expressed in the kidney and intestine, being in agreement with the expression profile in human tissues, although the expression in adipose tissue and the pancreas was not examined. UGT2B7 and UGT2B11 were also expressed in the kidney as reported in human tissues. UGT2B28 was also detected in the kidney and intestine, but the expression levels in human tissues are low. The results of the present study indicated that multiple UGT2 isoforms are mainly expressed in the liver and kidney. Absence of UGT2B17 expression in hUGT2 mouse may be attributed to whole-gene deletion polymorphism.

Preclinical and clinical studies on the disposition of AZT revealed significant species differences in metabolism and elimination of the drug. AZT was extensively metabolized to glucuronide...
in humans. On the other hand, mice excreted the drug largely unchanged, and decreased metabolism was compensated by increased renal elimination. A previous clinical study showed that AIDS patients excreted 60% of the i.v. dose of AZT in urine as the glucuronide metabolite. Only 18% of the i.v. dose was recovered in urine as unchanged drug. Consistent with the previous findings, the percentage of urinary excretion of AZT glucuronide (53.1%) was much more than that of AZT (3.5%) in our hUGT2 mice (Figure 3). WT mice did not excrete AZT glucuronide in urine and bile. These results suggest the hUGT2 mice likely reflect the metabolism and urinary excretion of AZT in humans.

In the present study, gemfibrozil was mainly excreted as glucuronide in the bile of WT mice (Figure 4). The biliary excretion of gemfibrozil glucuronide was decreased by KO of mouse Ugt2 genes, suggesting that mouse Ugt2 enzymes catalyze the glucuronidation of gemfibrozil in vivo. However, the biliary excretion of gemfibrozil glucuronide was not completely diminished in Ugt2-KO mice. Since previous studies using recombinant human UGT isozymes indicated that UGT1A1, UGT1A3 and UGT1A9 also catalyze the glucuronidation of gemfibrozil, endogenous mouse Ugt1 enzymes may be involved in the formation of gemfibrozil glucuronide in Ugt2-KO mice. In addition, hUGT2 mice also excreted gemfibrozil glucuronide in bile. The percentage of biliary excretion of gemfibrozil glucuronide was significantly higher than that in Ugt2-KO mice. Therefore, our results suggest that hUGT2 mice express functional human UGT2 that can catalyze the glucuronidation of gemfibrozil in vivo.

More than 70% sequence homology exists between the UGT2 enzymes, making it difficult to identify orthologs across species. In addition, which human UGT enzyme corresponds to the UGT enzyme in mice and rats is controversial. Since hUGT2 mice carry the human UGT2 gene cluster with a Ugt2-KO background, the hUGT2 mice should be a useful tool for predicting human drug glucuronidation via UGT2 enzymes.

In this study, we clarified that hepatic UGT2B7 was functional in hUGT2 mice. UGT2B7 is a major human UGT2B isoform that is involved in the glucuronidation of a many endobiotics and

FIGURE 3 In vivo glucuronidation of AZT in WT, Ugt2-KO and hUGT2 mice. Cumulative excretion rates of AZT glucuronide (A) and AZT (B) in bile and urine up to 24 after dosing were determined as % of dose. Data are expressed as means with S.D. of three mice. *p < .05 and **p < .01 versus Ugt2-KO mice. Statistical analysis was performed by one-way ANOVA, followed by Scheffe’s F-test.

FIGURE 4 In vivo glucuronidation of gemfibrozil in WT, Ugt2-KO and hUGT2 mice. Cumulative excretion rates of gemfibrozil glucuronide (A) and gemfibrozil (B) in bile and urine up to 24 after dosing were determined as % of dose. Data are expressed as means with S.D. of three mice. *p < .05 and **p < .01 versus Ugt2-KO mice. Statistical analysis was performed by one-way ANOVA, followed by Scheffe’s F-test.
xenobiotics. However, UGT2 enzymes other than UGT2B7 also catalyze the metabolism of drugs including tricyclic antidepressants (UGT2B10,33) and lorazepam (UGT2B15,34). It is needed to clarify the functions of other UGT2 isoforms in hUGT2 mice.

Humanized UGT1 mice, which express the human UGT1 locus in a Ugt1-null background, were created by transgenic technologies.35 On the other hand, transgenic mouse lines carrying the entire human UGT2 family have not been constructed because of large size of the human UGT2 locus. Here, we succeeded in developing hUGT2 mice by chromosome-engineering techniques. The hUGT2 mice are firstly generated as a mouse model bearing multiple human UGT2 genes. Thus, it will be possible to take advantage of the expression of not only UGT2B7 but also other human UGT2 isoforms for pharmacological and pharmacokinetic studies. In addition, the introduced UGT2 gene cluster contains not only coding regions but also regulatory regions such as promoters, enhancers and introns. This mouse model will be also used for studying hormonal regulation, post-transcriptional regulation, tissue-specific expression of human UGT2 isoforms.

In conclusion, the results of this study clearly indicate that human UGT2B7 catalyzed the formation of AZT glucuronide and gemfibrozil glucuronide in hUGT2 mice in vitro and in vivo. The hUGT2 mice will be powerful model for various applications in drug development research and basic research.

AUTHOR CONTRIBUTIONS
Participated in research design: Deguchi, Kurihara, Kazuki Y. Conducted experiments: Deguchi, Abe, Kajitani, Kazuki K, Takehara, Nakamura. Contributed new reagents or analytic tools: Oshimura, Kazuki Y. Performed data analysis: Kobayashi, Deguchi, Kazuki Y. Wrote or contributed to the writing of the manuscript: Kobayashi, Kazuki Y. Deguchi.

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CONFLICT OF INTEREST
T.D. and A.K. are employees of Daiichi Sankyo Co., Ltd. The other authors declare no conflicts of interest.

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SUPPORTING INFORMATION
Additional supporting information can be found online in the Supporting Information section at the end of this article.