DMD Fast Forward. Published on May 13, 2009 as doi:10.1124/dmd.109.027227

Quantitative analysis of UGT1A and UGT2B expression levels in human livers

Takeshi Izukawa, Miki Nakajima, Ryoichi Fujiwara, Hiroyuki Yamanaka, Tatsuki Fukami, Masataka Takamiya, Yasuhiro Aoki, Shin-ichi Ikushiro, Toshiyuki Sakaki and Tsuyoshi Yokoi

Drug Metabolism and Toxicology, Faculty of Pharmaceutical Sciences, Kanazawa University, Kakuma-machi, Kanazawa 920-1192, Japan (T. I., M. N., R. F., H. Y., T. F., T. Y.), Department of Legal Medicine, Iwate Medical University School of Medicine, 19-1 Uchimaru, Morioka 020-8505, Japan (M. T., Y. A.) and Food Science and Technology, Biotechnology Research Center, Faculty of Engineering, Toyama Prefectural University, Toyama, Japan (S.I., T.S.)

DMD Fast Forward. Published on May 13, 2009 as DOI: 10.1124/dmd.109.027227
This article has not been copyedited and formatted. The final version may differ from this version.

Running title: Expression of UGT1A and UGT2B isoforms in human livers

To whom all correspondence should be sent:

Miki Nakajima, Ph.D.,

Drug Metabolism and Toxicology

Faculty of Pharmaceutical Sciences

Kanazawa University

Kakuma-machi, Kanazawa 920-1192, Japan

E-mail: nmiki@kenroku.kanazawa-u.ac.jp

Tel / Fax +81-76-234-4407

This manuscript consists of 15 pages of text, 4 table, 5 figures, and 37 references.

Abstracts: 248words

Introduction: 471 words

Discussion: 1095 words

Abbreviations: GAPDH, glyceraldehyde-3-phosphate dehydrogenase; RT-PCR, reverse transcriptase-polymerase chain reaction; UGT, UDP-glucuronosyltransferase

DMD Fast Forward. Published on May 13, 2009 as DOI: 10.1124/dmd.109.027227
This article has not been copyedited and formatted. The final version may differ from this version.

Abstract

UDP-glucuronosyltransferases (UGT) catalyze glucuronidation of a variety of xenobiotics and endobiotics. UGTs are divided into two families, UGT1 and UGT2. The purpose of this study was to estimate the absolute expression levels of each UGT isoform in human liver and to evaluate the interindividual variability. Real-time RT-PCR analysis was performed to determine the copy numbers of functional nine UGT1A isoforms and seven UGT2B isoforms. We noticed that not only primers but also templates as a standard for quantification should prudently be selected. Once we established appropriate conditions, the mRNA levels of each UGT isoform in 25 individual human livers were determined. UGT1A1 (0.9–138.5), UGT1A3 (0.1–66.6), UGT1A4 (0.1–143.3), UGT1A6 (1.0–70.4), UGT1A9 (0.3–132.4), UGT2B4 (0.3–615.0), UGT2B7 (0.2–97.4), UGT2B10 (0.7–253.2), UGT2B15 (0.3–107.8), and UGT2B17 (0.5–157.1) were substantially expressed ($x10^4$ copy/μg RNA) with large interindividual variability. Abundant isoforms were UGT2B4 and UGT2B10, followed by UGT1A1, UGT2B15, and UGT1A6. The sum of the UGT2B mRNA levels was higher than that of UGT1A mRNA levels. Interestingly, the mRNA levels normalized with GAPDH mRNA for almost UGT isoforms that are substantially expressed in liver showed significant correlations each other. Western blot analysis was performed using antibodies specific for UGT1A1, UGT1A4, UT1A6, or UGT2B7. Correlation between the protein and mRNA levels was observed in only UGT1A1 ($r = 0.488$, $p < 0.01$). In conclusion, this study comprehensively determined the absolute values of mRNA expression of each UGT isoform in human livers and found considerable interindividual variability.

Introduction

UDP-glucuronosyltransferase (UGT) enzymes catalyze glucuronidation of a variety of xenobiotics and endogenous compounds (Tukey and Strassburg, 2000). In human, UGTs are classified into UGT1 and UGT2 family, and the latter is divided into UGT2A and UGT2B subfamily, based on evolutionary divergence and homology (Mackenzie et al., 2005). The human *UGT1A* gene complex is located on chromosome 2q37 and consisted of multiple unique first exons and common exon 2 to 5 (Gong et al., 2001), encoding nine functional members of the UGT1A subfamily. The *UGT2* gene family is located on chromosome 4q13 and includes three members of the UGT2A subfamily and seven functional members of the UGT2B subfamily. Each *UGT2* gene comprises six exons that are not shared between the UGT2 family members, with an exception of UGT2A1 and UGT2A2, which are arisen by the differential splicing of a variable first exon to the same set of five downstream exons, similar to the UGT1A enzymes (Mackenzie et al., 2005).

Liver is the major organ for glucuronidation in the body as it is directly exposed to the influx of drugs from the hepatic portal vein during oral absorption. Earlier studies demonstrated that the mRNAs of all UGT isoforms except for UGT2A1 were expressed in the human liver at any level (Strassburg et al., 1997a; Tukey and Strassburg, 2000; Tukey and Strassburg, 2001; Fisher et al., 2001; Aueviriyavit et al., 2007; Nakamura et al., 2008a; Court et al., 2008). In almost studies, the mRNA expressions were qualitatively evaluated using reverse transcriptase-polymerase chain reaction (RT-PCR). Recent studies quantitatively evaluated using a real-time RT-PCR, but the UGT expression levels were evaluated as relative values (Aueviriyavit et al., 2007; Court et al., 2008). Therefore, we could not directly compare the expression levels of different UGT isoforms. If we could know the absolute expression levels of each UGT isoform, the information may be useful to estimate contribution of each UGT isoform in certain glucuronidation that is catalyzed by multiple UGT isoforms. Moreover, there is limited data on the interindividual variability in UGT expression levels in human livers. Since interindividual variability of UGT expression in livers plays an important role in drug efficacy, toxicity, and susceptibility to environmental

chemicals (Wells et al., 2004), the analysis of interindividual variability of each UGT expression is important. The aims of this study were to determine the absolute values of copy number of UGT isoforms in human liver and to evaluate their interindividual variability. Although it is possible to quantify UGT protein levels using selective antibodies, immunodetection of UGT proteins is plagued by uncertainty regarding antibody specificity because UGT families possess a high degree of protein sequence homology. Their use is, therefore, restricted to the few UGT enzymes for which selective antibodies exist. Another aim of this study was to investigate the relationship between mRNA levels and protein levels for certain UGT isoforms.

Materials and Methods

Materials

RNAiso, random hexamer, and SYBR Premix Ex Taq were from Takara Bio (Shiga, Japan). ROX was purchased from Stratagene (La Jolla, CA). Rever Tra Ace (Mononey Murine Leukemia Virus Reverse Transcriptase RNaseH Minus) was obtained from Toyobo (Tokyo, Japan). Primers were commercially synthesized at Hokkaido System Sciences (Sapporo, Japan). Rabbit anti-human UGT1A1 polyclonal antibody and anti-human UGT2B7 polyclonal antibody were purchased from BD Gentest (Woburn, MA). Rabbit anti-human UGT1A4 and UGT1A6 peptide polyclonal antibodies were prepared previously (Ikushiro et al., 2006). All other reagents were of the highest grade commercially available.

Human livers

Human liver samples from 16 donors (10 Caucasians, 4 Hispanic, 1 Black and 1 Asian) were obtained from Human and Animal Bridging (HAB) Research Organization (Chiba, Japan), and those from 9 Japanese were obtained from autopsy materials that were discarded after pathological investigation (Supplementary Table 1). The use of the human livers was approved by the Ethics Committees of Kanazawa University (Kanazawa, Japan)

and Iwate Medical University (Morioka, Japan)

Total RNA and reverse transcription

Total RNA was extracted from 25 individual human liver samples using RNAiso. The integrity of the RNA was assessed by estimating the ratio of 28S and 18S rRNA bands on ethidium bromide-stained 1% agarose gel. The cDNA was synthesized using Rever Tra Ace according to the manufacturer's protocols.

Isolation and subcloning of human UGT cDNAs

Human UGT1A3, UGT1A5, UGT1A7, UGT1A8, UGT1A10, UGT2B4, UGT2B7, UGT2B10, UGT2B11, UGT2B15, UGT2B17 and UGT2B28 cDNAs were prepared by a RT-PCR using total RNA from appropriate human tissues or various cell lines (Nakamura et al., 2008a). The used primers are shown in Table 1. After an initiate denaturation at 94°C for 5 min, amplification was performed by denaturation at 94°C for 25 s, annealing at an appropriate temperature for 25 s and extension at 72°C for 2 min for 40 cycles. The final extension step was performed at 72°C for 5 min. These PCR products were subcloned into pTARGET Mammalian Expression Vector. The plasmid DNA was purified by a QIAGEN Plasmid Midi kit (QIAGEN, Valencia, CA) and submitted to DNA sequences using a Thermo Sequenase Cy5.5 Dye Terminator Cycle Sequencing kit (GE Healthcare Bio-Sciences, Piscataway, NJ) with a Long-Read Tower DNA sequencer (GE Healthcare Bio-Sciences). The pTARGET plasmids containing human UGT1A1, UGT1A4, UGT1A6 and UGT1A9 were previously constructed (Fujiwara et al., 2007). These plasmids were digested with appropriate restriction enzymes to prepare the standards for real-time RT-PCR analysis. As a standard for real-time RT-PCR analysis, amplification efficiencies with the intact plasmid, linearized plasmid, full-length of cDNA, and the purified product of the real-time RT-PCR *per se* were compared.

Preliminary PCR analyses to validate the amplification with different primer pairs

DMD Fast Forward. Published on May 13, 2009 as DOI: 10.1124/dmd.109.027227
This article has not been copyedited and formatted. The final version may differ from this version.

An 1-μl portion of the reverse-transcribed mixture was added to PCR mixtures (25 μl) consisting of 1 × PCR buffer [67 mM Tris-HCl buffer (pH 8.8), 16.6 mM $(NH_4)_2SO_4$, 0.45% Triton X-100, 0.02% gelatin], 1.5 mM $MgCl_2$, 0.4 μM primers, 250 μM dNTPs, and 1 U of Taq DNA polymerase (Greiner Japan, Tokyo, Japan). The primer pairs are 1A4 ex1-S and 1A4 ex1-AS, int-AS, or 1A ex2-AS, 1A5 ex1-S and 1A5 ex1-AS, int-AS, or 1A ex2-AS, and int-S and 1A ex2-AS. The sequences of the primers are shown in Table 2. PCR reaction was performed with Takara PCR Thermal Cycler Dice TP600 (Takara). After an initial denaturation at 94°C for 5 min, amplification was performed by denaturation at 94°C for 25 sec, annealing at 58°C for 25 sec and extension at 72°C for 40 sec for 25 cycles. The final extension step was performed at 72°C for 5 min. The PCR products (8 μl) were analyzed by electrophoresis with 2% agarose gel and visualized by ethidium bromide staining.

Real-time RT-PCR analysis

The UGT mRNA levels in human livers were quantified by real-time RT-PCR using the Mx3000P™ (Stratagene, La Jolla, CA). PCR mixture contained 1-μl portion of the reverse-transcribed mixture, SYBR Premix Ex Taq solution, and 0.4 μM of primers. The sequences and position of the primers as well as annealing temperatures are shown in Table 3. After an initial denaturation at 95°C for 30 sec, amplification was performed by denaturation at 94°C for 4 sec, annealing at an appropriate temperature for 7 sec and extension at 72°C for 20 sec for 45 cycles. Amplified products were monitored directly by measuring the increase of the dye intensity of the SYBR Green (Takara Shiga, Japan) that binds to double-strand DNA amplified by PCR. Copy number in the samples was defined based on a standard curve using full-length UGT cDNA. The specificity of all primer pairs was confirmed by digestion of the PCR products with appropriate restriction enzymes and sequence analysis. Negative control samples were processed in the same manner, except that the template was omitted. Calibration curve was constructed by plotting the PCR threshold cycle (Ct) number at which the fluorescent signal generated during the replication process passes above a threshold value against known amounts of cDNA. UGT mRNA expression levels were normalized with

GAPDH mRNA level.

Western blot analysis

Microsomes from 25 human livers were prepared according to a method described previously (Tabata et al., 2004). The human liver microsomes (10 μg) were separated on 10% SDS-polyacrylamide gel and transferred electrophoretically to either PVDF or nitrocellulose membrane. PVDF membrane Immobilon-P (Millipore, Bedford, MA) was probed with anti-human UGT1A1 antibody or anti-human UGT2B7 antibody. In the data sheet provided by the manufacturer, it is described that the UGT1A1 antibody did not cross-reacted with UGT1A4, UGT1A6, UGT1A9, UGT1A10 and UGT2B15, and that the UGT2B7 antibody did not cross-reacted with UGT1A1, UGT1A4, UGT1A6, UGT1A9, UGT1A10 and UGT2B15. We confirmed that UGT2B7 antibody did not cross-react with UGT2B4, UGT2B15, UGT2B17 using the recombinant enzymes expressed in baculovirus-infected insect cells (Supersomes) (data not shown). Nitrocellulose membrane (Whatman, Germany) was probed with anti-human UGT1A4 antibody or anti-human UGT1A6 antibody. These antibodies have shown no cross-reactivity with the other UGT1A isoforms (Ikushiro et al., 2006). Biotinylated anti-rabbit IgG and a Vectastain ABC kit (Vector Laboratories, Burlingame, CA) were used for diaminobenzidine staining. The expression level of UGTs protein was defined based on a standard curve using recombinant human UGTs expressed in baculovirus-infected insect cells (BD Gentest). The quantitative analysis was performed using a GT-9800F scanner (Seiko Epson, Suwa, Japan) and ImageQuant TL Image Analysis software (GE Healthcare Bio-Sciences).

Statistical Analysis

Data are expressed as the mean ± SD. Correlation analysis was performed by Spearman rank method. A value of $p < 0.05$ was considered statistically significant.

Results

Primer design to quantify the UGT mRNA

For the specific amplification of each human UGT1A mRNA, many investigators used sense and anti-sense primers that are within exon 1 (Congiu et al., 2002; Finel et al., 2005; Kaivosaari et al., 2007; Itäaho et al., 2008; Ohno et al., 2009), because the exon 2 to 5 is common to all UGT1A isoforms. To avoid overestimation by contamination of genomic DNA, the sense and anti-sense primers are preferable to be located in different exons. In this study, we compared the amplification with different primer sets for UGT1As, targeting UGT1A4 and UGT1A5 that are highly and marginally expressed in liver, respectively. When the primer pairs were located in exon 1 of UGT1A4 (1A4 ex1-S and 1A4 ex1-AS) or in exon 1 of UGT1A5 (1A5 ex1-S and 1A5 ex1-AS), PCR amplicon was detected with the reverse-transcribed mixture, but not with the mixture excluding reverse transcriptase (Fig. 1A), suggesting that the contamination of genomic DNA was negligible. Nevertheless, when the sense and anti-sense primers were located in exon 1 and intron 1, respectively, for UGT1A4 (1A4 ex1-S and int-AS) and UGT1A5 (1A5 ex1-S and int-AS), obvious amplicon was detected (Fig. 1B), indicating the presence of pre-mRNA or excised intron in lariat shape (Fig. 1E). Since the primer set of int-S and 1A ex2-AS also show an obvious band (Fig. 1C), the presence of pre-mRNA was supported. This would not be specific for UGT1A family, since the primer sets located in exon 1 and intron 1 for UGT2B7, UGT2B10, and UGT2B28 showed obvious amplification with the reverse-transcribed mixture (data not shown). Collectively, if the primers were set in the same exon, the amplification of not only genomic DNA but also pre-mRNA would occur. The sense and antisense primers should properly be in different exons (Fig. 1D).

Standard curves using different length of cDNA fragments as a template

Plasmids containing full-length cDNA are frequently used as a template to calculate the copy numbers in RNA samples in real-time RT-PCR analysis. We examined the extent of amplification with the intact plasmid, the linearized plasmid, and the purified PCR product by

the real-time RT-PCR, comparing with that with the full-length cDNA. Fig. 2 shows Ct number with 10^4, 10^5, or 10^6 copies of these templates. In the case of UGT1A1, the standard curve with the linearized plasmid was overlaid with that with full-length cDNA. In contrast, Ct values with the intact plasmid or PCR product were greater than those with full-length cDNA (Fig. 2A). In the case of UGT1A4, the standard curves with the intact plasmid and linearized plasmid were shifted to lower Ct values, but that with the PCR product was shifted to higher Ct values, comparing with that with the full-length cDNA (Fig. 2B). In the case of UGT1A6, the standard curve with the intact plasmid was overlaid with that with full-length cDNA (Fig. 2C). In contrast, the Ct values with the linearized plasmid were lower than those with full-length cDNA. The Ct values with the PCR product were higher than those with full-length cDNA. In the case of UGT2B7, four kinds of standard curves were not overlaid at all (Fig. 2D). Taken together, these results suggest that the absolute values of copy number would be misjudged depending on the kinds of template. For the subsequent study, the full-length cDNAs were used as a template for the quantification, because it is exactly the reverse-transcribed product of intact mRNA. We confirmed that freezing and thawing of the full-length cDNAs did not affect the quantification (data not shown).

Expression level of UGTs mRNA in human livers

The expression levels of UGT1A and UGT2B mRNA in 25 human livers were determined by real-time RT-PCR (Fig. 3). UGT1A1 mRNA levels were 0.9 – 138.5 x 10^4 copy/μg. UGT1A3 mRNA levels were 0.1 – 66.6 x 10^4 copy/μg. UGT1A4 mRNA levels were 0.1 – 143.3 x 10^4 copy/μg. UGT1A6 mRNA levels were 1.0 – 70.4 x 10^4 copy/μg. UGT1A9 mRNA levels were 0.3 – 132.4 x 10^4 copy/μg. UGT1A5, UGT1A7, UGT1A8 and UGT1A10 mRNAs were marginally expressed. UGT2B4 mRNA levels were 0.3 – 615.0 x 10^4 copy/μg. UGT2B7 mRNA levels were 0.2 – 97.4 x 10^4 copy/μg. UGT2B10 mRNA levels were 0.7 – 253.2 x 10^4 copy/μg. UGT2B15 mRNA levels were 0.3 – 107.8 x 10^4 copy/μg. UGT2B17 mRNA levels were 0.5 – 157.1 x 10^4 copy/μg. UGT2B11 and UGT2B28 mRNAs were marginally expressed. After the normalization with GAPDH mRNA levels (0.9 – 12.3 x 10^7

DMD Fast Forward. Published on May 13, 2009 as DOI: 10.1124/dmd.109.027227
This article has not been copyedited and formatted. The final version may differ from this version.

copy/μg), the interindividual variation was estimated as follows: UGT1A1, 9-fold: UGT1A3, 37-fold: UGT1A4, 28-fold: UGT1A6, 22-fold: UGT1A9, 45-fold: UGT2B4, 72-fold: UGT2B7, 506-fold: UGT2B10, 223-fold: UGT2B15, 29-fold: UGT2B17, 223-fold. Thus, UGT2B subfamily showed relatively larger interindividual variability than UGT1A subfamily. Fig. 4 shows the percentage of each UGT mRNA level in sum of all UGT levels. Abundant isoforms were UGT2B4 (34.5 ± 12.1%, 6.5 – 59.7% of total UGT) and UGT2B10 (19.6 ± 13.4%, 2.0 – 52.3%), followed by UGT1A1 (11.3 ± 6.9%, 2.2 – 25.1%), UGT2B15 (8.0 ± 3.5%, 2.2 – 15.5%), and UGT1A6 (6.8 ± 4.3%, 1.8 – 21.6%). The sum of the UGT2B mRNA levels (70.1 ± 11.4%, 42.7 – 90.0%) was higher than that of UGT1A mRNA levels (29.9 ± 11.4%, 10.0 – 57.3%).

Expression level of UGTs protein in human livers and relationship with the mRNA levels

The expression levels of UGT1A1, UGT1A4, UGT1A6 and UGT2B7 protein in 25 human liver microsomes were determined by Western blot analysis (Fig. 5). Although the specific antibody against UGT1A9 is available (Ikushiro et al., 2006), it showed a large number of non-specific bands with the human liver microsomes (data not shown). Another antibodies against UGT1A9 obtained Abnova (Taiwan) cross-reacted with other UGT1As. Therefore, we could not determine the UGT1A9 levels in human liver microsomes. The interindividual variabilities of UGT1A1, UGT1A4, UGT1A6 and UGT2B7 proteins were 6-fold, 13-fold, 2-fold, and 4-fold, respectively. The UGT1A1 protein levels were significantly correlated with the UGT1A1 mRNA levels ($Rs = 0.488$, $P < 0.01$). However, no correlation between the protein and mRNA levels was observed for UGT1A4, UGT1A6 and UGT2B7.

Correlation analyses of the expression levels of UGT mRNA or protein in human livers between different isoforms

As for UGT isoforms that are substantially expressed in human livers, correlations analyses of the expression levels of UGT mRNA between isoforms were performed. UGT

DMD Fast Forward. Published on May 13, 2009 as DOI: 10.1124/dmd.109.027227
This article has not been copyedited and formatted. The final version may differ from this version.

mRNA levels normalized with GAPDH mRNA in 25 human livers were used for the analysis. Surprisingly, almost isoforms showed significant correlations each other (Table 4). An exception was UGT2B17, which was hardly correlated with other isoforms. In contrast to the results of mRNA levels, at the protein levels, a significant correlation was observed only between UGT1A4 and UGT1A6 ($Rs = 0.441$, $P < 0.05$).

Discussion

In this study, we found that UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A9, UGT2B4, UGT2B7, UGT2B10, UGT2B15 and UGT2B17 were substantially expressed in human liver in agreement with previous studies (Strassburg et al., 1997b, 2000; Tukey and Strassburg, 2001). In addition, UGT1A5, UGT1A7, UGT1A8, UGT1A10, UGT2B11 were detected in agreement with previous studies (Beaulie et al., 1998; Zheng et al., 2002; Finel et al., 2005; Nakamura et al., 2008a), although their levels were extremely low. We also examined the expression of UGT2A1, UGT2A2, and UGT2A3 in human livers. Although UGT2A2 and UGT2A3 were detected (data not shown), their Ct values were as high as those for latter isoforms such as UGT1A5. Therefore, we did not determine the copy numbers of UGT2As.

One of advantages of our study was that the UGT mRNA levels were quantitatively assessed, which allow us to compare directly the expression levels between different isoforms. We found that the abundant isoforms were UGT2B4 and UGT2B10, followed by UGT1A1, UGT2B15, and UGT1A6. Roughly describing, our results were compatible with recent works reporting the quantitative data of UGT mRNA levels in human liver, although they used pooled samples (Kaivosaar et al., 2007; Ohno et al., 2008; Itäaho et al., 2009). Finel and co-workers (Kaivosaar et al., 2007; Itäaho et al., 2009) reported using a pooled human liver from 47 donors that the expression levels (copies/10^9 copies 18S rRNA) are as follows: UGT1A9 (3239 ± 42, mean ± SE) > UGT2B10 level (1980, mean) ≈ UGT1A4 (1321, mean) ≈ UGT2B7 (1309, mean) >>> UGT1A10 (6 ± 0, mean ± SE). Ohno et al (2009) reported

DMD Fast Forward. Published on May 13, 2009 as DOI: 10.1124/dmd.109.027227
This article has not been copyedited and formatted. The final version may differ from this version.

using a pooled human liver from 3 donors that the abundant isoforms (copies /10^4 copies GAPDH) are as follows: UGT2B4 (37900 ± 711, mean ± SD) > UGT2B15 (18500 ± 285) > UGT2B7 (4220 ± 13) > UGT2B10 (3380 ± 93). In dissecting the relative expression levels of UGTs, however, there is discordance between the previous and our studies. One possibility of the inconsistency might be due to the interindividual variability. Another possibility is that the differences in primers and the template to construct calibration curve. In the previous studies, sense and antisense primers were both localized in the same exon, and intact plasmids containing cDNA or purified PCR product were used as the template. Earlier studies underappreciated the selection of template, but the present study clearly demonstrated that the different length templates lead to different results. The full-length cDNA would be preferable as the template, because it is just the reverse-transcribed product of intact mRNA. In the last, we found that the sum of the UGT2B mRNA levels (70.1 ± 11.4%, 42.7 – 90.0%) was higher than that of UGT1A mRNA levels (29.9 ± 11.4%, 10.0 – 57.3%).

Another advantage of this study was that the interindividual variability of mRNA levels was assessed for all UGT isoforms. The interindividual variability of UGT1A mRNA levels was largely consistent with previous studies. Aueviriyavit et al (2007) have reported that the interindividual variability in 18 human livers were as follows: UGT1A1 (8.6-fold), UGT1A3 (6.5-fold), UGT1A4 (2.5-fold), UGT1A6 (4.9-fold), and UGT1A9 (5.1-fold) mRNA levels. Krishnaswamy et al. (2005) reported that the interindividual variability of UGT1A6 mRNA was 7-fold in 50 human livers. Our study demonstrated that the UGT2B isoforms had a great interindividual variability. Of particular interest finding is that the mRNA levels for almost UGT isoforms showed significant correlations each other. Supporting our study, Ramirez et al. (2008) reported that the UGT1A1 mRNA levels were significantly correlated with the UGT1A9 (r^2 = 0.49, P < 0.0001, n = 44) and UGT2B7 (r^2 = 0.39, P < 0.0001, n = 54) mRNA levels, and that the UGT1A9 mRNA levels were significantly correlated with the UGT2B7 mRNA levels (r^2 = 0.54, P < 0.0001, n = 44). They concluded that the results would be due to the common regulation by HNF1α, since these UGT mRNA levels were significantly correlated with the HNF1α mRNA levels. In addition, Aueviriyavit et al (2007) also reported

that UGT1A6 and UGT1A9 mRNA levels were significantly correlated with the HNF1α and HNF4α mRNA levels. In fact, there is literature demonstrating that HNF1α and HNF4α can bind and activate their promoters (Bernard et al., 1999; Gregory et al., 2004; Gardner-Stephen et al., 2007; Barbier et al., 2005; Ishii et al., 2000). Thus, these liver-enriched transcriptional factors would be the major determinant of the expression of hepatic UGTs. Beside, hormones, bile acids, drugs and other xenobiotics may modulate the UGT expression through interaction with receptors such as pregnane X receptor, constitutive androstane receptor, peroxisome proliferators-activated receptor α, farnesoid X receptor, aryl hydrocarbon receptor, and Nrf2 (Zhou et al., 2005; Nakamura et al., 2008b). These factors may also contribute the interindividual variability.

Genetic polymorphisms might be another factor determining the variability of UGT expression. *UGT1A1*28* possessing TA-inserted promoter $(TA)_7TA$ as well as *UGT1A1*60* possessing a single nucleotide polymorphism (SNP) -3263T>G show a reduced transcriptional activity (Guillemette et al., 2001; Sugatani et al., 2002). Two SNPs in the promoter region of *UGT1A4* gene have been reported to be associated with the reduced transcriptional activity (Erichsen et al., 2008). For *UGT2B17* gene, a polymorphism of entire gene deletion is known (Wilson et al., 2004; Karypidis et al., 2008). Although we did not investigate whether our samples have such variants, it is possible that the variation in UGT mRNA may partly be explained by the genetic polymorphisms.

We performed Western blot analysis to determine the UGT protein levels in human liver microsomes. Our results demonstrated a significant correlation between mRNA and protein levels for UGT1A1 ($Rs = 0.488$, $P < 0.01$), but not for UGT1A4, UGT1A6, and UGT2B7. Previously, Krishnaswamy et al. (2005) have reported that the UGT1A6 mRNA levels in 54 human livers were significantly ($Rs = 0.53$ $P < 0.001$) correlated with UGT1A6 protein levels. The inconsistency may be due to the differences in the sample numbers, experimental conditions including antibodies and/or donors. Although we did confirm the linearity of assay response through use of a standard curves constructed using different amounts of recombinant UGTs, the semiquantitative immunoblotting may be difficult to show

the true range of values. The fact may be one of factors of less correlation between mRNA and protein levels. Alternatively, post-transcriptional and/or post-translational regulation might be feasible, but such mechanisms largely remain to be studied for UGTs. Further studies will be required to draw definitive conclusion.

In conclusion, this is the first study to comprehensively assess the expression levels of each UGT isoform in human livers, evaluating their interindividual variability. The findings presented here provide important insight to understand the interindividual variability of glucuronidations.

Acknowledgements

We acknowledge Brent Bell for reviewing the manuscript.

References

Aueviriyavit S, Furihata T, Morimoto K, Kobayashi K, and Chiba K (2007) Hepatocyte nuclear factor 1 alpha and 4 alpha are factors involved in interindividual variability in the expression of UGT1A6 and UGT1A9 but not UGT1A1, UGT1A3 and UGT1A4 mRNA in human livers. *Drug Metab Pharmacokinet* **22:**391-398.

Barbier O, Girard H, Inoue Y, Duez H, Villeneuve L, Kamiya A, Fruchart JC, Guillemette C, Gonzalez FJ, Staels B (2005) Hepatic expression of the UGT1A9 gene is governed by hepatocyte nuclear factor 4α. *Mol Pharmacol* **67:**241-249.

Beaulieu M, Lévesque É, Hum DW, and Bélanger A (1998) Isolation and characterization of human orphan UDP-glucuronosyltransferase, UGT2B11. *Biochem Biophys Res Commun* **248:**44-50.

Bernard P, Goudonnet H, Artur Y, Desvergne B, Wahli W (1999) Activation of the mouse TATA-less and human TATA-containing UDP-glucuronosyltransferase 1A1 promoters by hepatocyte nuclear factor 1. *Mol Pharmacol* **56:**526-536.

Congiu M, Mashford ML, Slavin JL, and Desmond PV (2002) UDP glucuronosyltranferase mRNA levels in human liver disease. *Drug Metab Dispos* **30:**129-134.

Court MH, Hazarika S, Krishnaswamy S, Finel M, and Williams JA (2008) Novel polymorphic human UDP-glucuronosyltransferase 2A3: cloning, functional characterization of enzyme variants, comparative tissue expression, and gene induction. *Mol Pharmacol* **74:**744-754.

Erichsen TJ, Ehmer U, Kalthoff S, Lankisch TO, Müller TM, Munzel PA, Manns MP, and Strassburg CP (2008) Genetic variability of aryl hydrocarbon receptor (AhR)-mediated regulation of the human UDP glucuronosyltransferase (UGT) 1A4 gene. *Toxicol Appl Pharmacol* **230:**252-260.

Finel M, Li X, Gardner-Stephen D, Bratton S, Mackenzie PI, and Radominska-Pandya A (2005) Human UDP-glucuronosyltransferase 1A5: identification, expression, and activity. *J Pharmacol Exp Ther* **315:**1143-1149.

Fisher MB, Paine MF, Strelevitz TJ, and Wrighton SA (2001) The role of hepatic and

extrahepatic UDP-glucuronosyltransferases in human drug metabolism. *Drug Metab Rev* **33:**273-297.

Fujiwara R, Nakajima M, Yamanaka H, Nakamura A, Katoh M, Ikushiro S, Sakaki T, and Yokoi T (2007) Effects of coexpression of UGT1A9 on enzymatic activities of human UGT1A isoforms. *Drug Metab Dispos* **35:**747-757.

Gardner-Stephen DA and Mackenzie PI (2007) Hepatocyte nuclear factor1 transcription factors are essential for the UDP-glucuronosyltransferase 1A9 promoter response to hepatocyte nuclear factor 4α. *Pharmacogenet Genomics* **17:**25-36.

Gong QH, Cho JW, Huang T, Potter C, Gholami N, Basu NK, Kubota S, Carvalho S, Pennington MW, Owens IS, and Popescu NC (2001) Thirteen UDP glucuronosyltransferase genes are encoded at the human UGT1 gene complex locus. *Pharmacogenetics* **11:**357-368.

Gregory PA, Lewinsky RH, Gardner-Stephen DA, Mackenzie PI (2004) Coordinate regulation of the human *UDP-glucuronosyltransferase 1A8*, *1A9*, and *1A10* genes by hepatocyte nuclear factor 1α and the caudal-related homeodomain protein 2. *Mol Pharmacol* **65:**953-963.

Guillemette C, De Vivo I, Hankinson SE, Haiman CA, Spiegelman D, Housman DE, and Hunter DJ (2001) Association of genetic polymorphisms in UGT1A1 with breast cancer and plasma hormone levels. *Cancer Epidemiol Biomarkers Prev* **10:**711-714.

Ikushiro S, Emi Y, Kato Y, Yamada S, and Sakaki T (2006) Monospecific antipeptide antibodies against human hepatic UDP-glucuronosyltransferase 1A subfamily (UGT1A) isoforms. *Drug Metab Pharmacokinet* **21:**70-74.

Ishii Y, Hansen AJ, Mackenzie PI (2000) Octamer transcription factor-1 enhances hepatic nuclear factor-1α-mediated activation of the human UDP glucuronosyltransferase 2B7 promoter. *Mol Pharmacol* **57:**940-947.

Itäaho K, Court M, 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* in press.

Kaivosaari S, Toivonen P, Hesse LM, Koskinen M, Court MH, and Finel M (2007) Nicotine glucuronidation and the human UDP-glucuronosyltransferase UGT2B10. *Mol Pharmacol* **72:**761-768.

Karypidis A-H, Olsson A, Andersson S-O, Rane A, and Ekström L (2008) Deletion polymorphism of the UGT2B17 gene is associated with increased risk for prostate cancer and correlated to gene expression in the prostate. *Pharmacogenomics J* **8:**147-151.

Krishnaswamy S, Hao Q, Al-Rohaimi A, Hesse LM, von Moltke LL, Greenblatt DJ, and Court MH (2005) UDP glucuronosyltransferase (UGT) 1A6 pharmacogenetics: I. Identification of polymorphisms in the 5'-regulatory and exon 1 regions, and association with human liver UGT1A6 gene expression and glucuronidation. *J Pharmacol Exp Ther.* **313:**1331-1339.

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.

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

Nakamura A, Nakajima M, Higashi E, Yamanaka H, and Yokoi T (2008b) Genetic polymorphisms in the 5'-flanking region of human UDP-glucuronosyltransferase 2B7 affect the Nrf2-dependent transcriptional regulation. *Pharmacogenet Genomics* **18:**709-720.

Ohno S and Nakajin S (2009) Determination of mRNA expression of human UGTs and application for localization in various human tissues by real time RT-PCR. *Drug Metab Dispos* **37:**32-40.

Ramírez J, Mirkov S, Zhang W, Chen P, Das S, Liu W, Ratain MJ, and Innocenti F (2008) Hepatocyte nuclear factor-1 alpha is associated with UGT1A1, UGT1A9 and UGT2B7 mRNA expression in human liver. *Pharmacogenomics J* **8:**152-161.

Strassburg CP, Manns MP, and Tukey RH (1997a) Differential down-regulation of the UDP-glucuronosyltransferase 1A locus is an early event in human liver and biliary cancer. *Cancer Res* **57:**2979-2985.

Strassburg CP, Oldhafer K, Manns MP, and Tukey RH (1997b) Differential expression of the UGT1A locus in human liver, biliary, and gastric tissue: identification of UGT1A7 and UGT1A10 transcripts in extrahepatic tissue. *Mol Pharmacol* **52:**212-220.

Strassburg CP, Kneip S, Topp J, Obermayer-Straub P, Barut A, Tukey RH, and Manns MP (2000) Polymorphic gene regulation and interindividual variation of UDP-glucuronosyltransferase activity in human small intestine. *J Biol Chem* **275:**36164-36171.

Sugatani J, Yamakawa K, Yoshinari K, Machida T, Takagi H, Mori M, Kakizaki S, Sueyoshi T, Negishi M, and Miwa M (2002) Identification of a defect in the UGT1A1 gene promoter and its association with hyperbilirubinemia. *Biochem Biophys Res Commun* **292:**492-497.

Tabata T, Katoh M, Tokudome S, Hosokawa M, Chiba K, Nakajima M, and Yokoi T (2004) Bioactivation of capecitabine in human liver: involvement of the cytosolic enzyme on 5'-deoxy-5-fluorocytidine formation. *Drug Metab Dispos* **32:**762-767.

Toide K, Takahashi Y, Yamazaki H, Terauchi Y, Fujii T, Parkinson A, and Kamataki T (2002) Hepatocyte nuclear factor-1α is a causal factor responsible for interindividual differences in the expression of UDP-glucuronosyltransferase 2B7 mRNA in human livers. *Drug Metab Dispos* **30:**613-615.

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

Tukey RH and Strassburg CP (2001) Genetic multiplicity of the human UDP-glucuronosyltransferases and regulation in the gastrointestinal tract. *Mol Pharmacol* **59:**405-414.

Wells PG, Mackenzie PI, Chowdhury JR, Guillemette C, Gregory PA, Ishii Y, Hansen AJ, Kessler FK, Kim PM, Chowdhury NR, and Ritter JK (2004) Glucuronidation and the

UDP-glucuronosyltransferases in health and disease. *Drug Metab Dispos* **32:**281-290.

Wilson W III, Pardo-Manuel de Villena F, Lyn-Cook BD, Chatterjee PK, Bell TA, Detwiler DA, Gilmore RC, Valladeras IC, Wright CC, Threadgill DW, and Grant DJ (2004) Characterization of a common deletion polymorphism of the *UGT2B17* gene linked to *UGT2B15*. *Genomics* **84:**707-714.

Zheng Z, Fang J-L, and Lazarus P (2002) Glucuronidation: an important mechanism for detoxification of benzo[*a*]pyrene metabolites in aerodigestive tract tissues. *Drug Metab Dispos* **30:**397-403.

Zhou J, Zhang J, and Xie W (2005) Xenobiotic nuclear receptor-mediated regulation of UDP-glucuronosyltransferases. *Curr Drug Metab* **6:**289-298.

Figure legends

Fig. 1. Preliminary RT-PCR analyses to determine the specificity targeting UGT1A4 or UGT1A5 with different primer sets. RT-PCR was performed using the sense and antisense primers that are in the exon 1 of UGT1A4 or UGT1A5 (A). RT-PCR was performed using the sense primer in exon 1 and antisense primer in intron 1 of UGT1A4 or UGT1A5 (B). RT-PCR was performed using the sense primer in intron 1 of UGT1A4 or UGT1A5 and antisense primer in exon 2 (C). RT-PCR was performed using the sense primer in exon 1 of UGT1A4 or UGT1A5 and antisense primer in exon 2 (D). The numbers under each photograph represent the sample numbers. Schematic representation of *UGT1A* gene, pre-mRNA, mRNA, and excised intron (E). Boxes represent exons, and lines represent introns. The location and direction of primers were shown as arrows. PCR was performed with various primer sets.

Fig. 2. The standard curves for UGTs by real-time RT-PCR. The intact or linearized plasmid containing cDNA, full-length cDNA, and purified PCR product for UGT1A1 (A), UGT1A4 (B), UGT1A6 (C), and UGT2B7 (D) were used as the template. The standard curves were generated by plotting the Ct of the crossing points versus the copy number of template.

Fig. 3. The expression levels of UGT1A and UGT2B mRNA in 25 individual livers. The expression levels of UGT1A (A) and UGT2B (B) mRNA in human livers were measured by real-time RT-PCR. No. 1, 2, 6-8, 11-13, 15, and 16 were from Caucasian subjects, No. 3-5 and 10 were from Hispanic subjects, No. 9 was from black subject, No. 14 was from Asian subject, and No, 17-25 were from Japanese subjects. Each column represents the mean ± SD of triplicate data. ND: Not detectable.

Fig. 4. The percentage of mRNA levels of each UGT isoform in sum of all UGT levels. The mean values of triplicate determinations in Fig. 3 were adopted. The mRNA levels of each UGT isoform normalized with GAPDH mRNA levels were used for this analysis.

DMD Fast Forward. Published on May 13, 2009 as DOI: 10.1124/dmd.109.027227
This article has not been copyedited and formatted. The final version may differ from this version.

Fig. 5. Western blot analyses of UGT1A1, UGT1A4, UGT1A6, and UGT2B7 and the relationship between the UGT protein and mRNA levels. Western blot analyses were performed for 25 human liver microsomes using specific antibodies against human UGT1A1, UGT1A4, UGT1A6, and UGT2B7, and typical results are shown for 12 microsomes (A). M: Marker. The correlation analyses were performed between the protein and mRNA levels for UGT1A1 (B), UGT1A4 (C), UGT1A6, (D), and UGT2B7 (E) by Spearman's rank method.

DMD Fast Forward. Published on May 13, 2009 as DOI: 10.1124/dmd.109.027227
This article has not been copyedited and formatted. The final version may differ from this version.

Table 1. Sequence of primers used for amplification of UGT cDNAs.

Isoform	Primer	Sequence	Position[c]
UGT1A3	1A3-S[a]	5'-ATG GCA ATG TTG AAC AAT ATG T-3'	340 – 361
	1A ex5-AS[b]	5'-GCA CTC TGG GGC TGA TTA AT-3'	1720 – 1739
UGT1A5	1A5-S[a]	5'-ACA ATA TGT CTT TGA TCA TA-3'	353 – 372
	1A ex5-AS[b]	5'-GCA CTC TGG GGC TGA TTA AT-3'	1720 – 1739
UGT1A7	1A7 ex1-S	5'-GAA GTT CTC TGA TGG CTC GT-3'	-10 – 9
	1A ex5-AS[b]	5'-GCA CTC TGG GGC TGA TTA AT-3'	1708 – 1727
UGT1A8	1A8 ex1-S	5'-CCC ATT CCC CTA TGT GTT TC-3'	498 – 518
	1A ex5-AS[b]	5'-GCA CTC TGG GGC TGA TTA AT-3'	1708 – 1727
UGT1A10	1A10 ex1-S	5'-CCC AGC TGC TGG CTC G-3'	557 – 578
	1A ex5-AS[b]	5'-GCA CTC TGG GGC TGA TTA AT-3'	1708 – 1727
UGT2B4	2B4-S[a]	5'-CAT CTT CAG CTT CCA TTT C-3'	170 – 188
	2B4 ex6-AS	5'-TAT CTG GTT TTC CAG CTT C-3'	1603 – 1621
UGT2B7	2B7 ex1-S	5'-ATT GCA CCA GGA TGT CTG-3'	-10 – 7
	2B7 ex6-AS	5'-CTT GCA TCA CAA TCT TTC TTG CTG-3'	1648 – 1671
UGT2B10	2B10 ex1-S	5'-GGC TCT GAA ATG GAC TA-3'	-32 – -19
	2B10 ex6-AS	5'-TGA TGA TAA ATA GCA CG-3'	1506 – 1522
UGT2B11	2B11-S[a]	5'-CTT CCA TTC TTT TTG ATC CCA ATG AT-3'	179 – 204
	2B11 ex6-AS	5'-AGC AAT GTT ATC AGG TTG ATC AA-3'	1190 – 1212
UGT2B15	2B15 ex1-S	5'-GAA AAG AAG CAT TGC ATA AG-3'	-25 – -6
	2B15 ex6-AS	5'-GAG GAG TCC CAT CTT TCA-3'	1622 – 1639
UGT2B17	2B17 ex1-S	5'-GAA AAG AAG CAT TGC ATA AG-3'	-25 – -6
	2B17 ex6-AS	5'-GAG GAG TCC CAT CTT TTG-3'	1622 – 1639
UGT2B28	2B28-S[a]	5'-ATC CCA ATG ACG CAT TCA CTC TTA AAC TC-3'	194 – 222
	2B28 ex6-AS	5'-AAT GTT ATC AGG TTG ATC CCA-3'	1189 – 1209

[a]From Nakamura et al., (2008a)
[b]From Fujiwara et al., (2007)
[c]Nucleotide position on cDNA when the A in the start codon is 1.

DMD Fast Forward. Published on May 13, 2009 as DOI: 10.1124/dmd.109.027227
This article has not been copyedited and formatted. The final version may differ from this version.

Table 2. Sequence of primers used for RT-PCR analyses.

Primer	Sequence	Position
1A4 ex1-S[a]	5'-ACG CTG GGC TAC ACT CAA GG-3'	277 – 296[b]
1A4 ex1-AS[a]	5'-TCT GAA TTG GTC GTT AGT AAC T-3'	587 – 608[b]
1A5 ex1-S[a]	5'-ACA ATA TGT CTT TGA TCA TA-3'	353 – 372[b]
1A5 ex1-AS[a]	5'-AGA AAC AGC ATG GCA AAG-3'	667 – 684[b]
1A ex2-AS[a]	5'-ATT GAT CCC AAA GAG AAA ACC AC-3'	910 – 932[b]
int-S	5'-CAA GGA TAA TTC TGT AAG GCA-3'	IVS2-221 – IVS2-201[c]
int-AS [d]	5'-CAT TGA TTG GAT GAA GGC AC-3'	IVS1+11 – IVS1+30[c]

[a]From Nakamura et al., (2008a)

[b]Nucleotide position on cDNA when the A in the start codon is 1.

[c]Nucleotide position from the nearest exon on genome DNA.

[d]This primer can anneal both UGT1A4 and UGT1A5.

DMD Fast Forward. Published on May 13, 2009 as DOI: 10.1124/dmd.109.027227
This article has not been copyedited and formatted. The final version may differ from this version.

Table 3. Sequence of primers used for real-time RT-PCR analyses.

Isoforms	Primer	Sequence	Position[a]	Annealing temperature (°C)
UGT1A1	1A1-S[b]	5'-CCT TGC CTC AGA ATT CCT TC-3'	696 - 715	64
	1A ex2-AS[b]	5'-ATT GAT CCC AAA GAG AAA ACC AC-3'	907 - 929	
UGT1A3	1A3-S1	5'-GTT GAA CAA TAT GTC TTT GGT CT-3'	348 - 370	58
	1A ex2-AS[b]	5'-ATT GAT CCC AAA GAG AAA ACC AC-3'	910 - 932	
UGT1A4	1A4-S1	5'-CCT GCT GTG TTT TTT TGG AGG T-3'	502 - 523	64
	1A ex2-AS[b]	5'-ATT GAT CCC AAA GAG AAA ACC AC-3'	910 - 932	
UGT1A5	1A5-S1	5'-TGT CCT ACC TTT GCC ATG CTG-3'	659 - 679	58
	1A ex2-AS[b]	5'-ATT GAT CCC AAA GAG AAA ACC AC-3'	910 - 932	
UGT1A6	1A6-S1	5'-CAA CTG TAA GAA GAG GAA AGA C-3'	831 - 851	58
	1A ex2-AS[b]	5'-ATT GAT CCC AAA GAG AAA ACC AC-3'	910 - 932	
UGT1A7	1A7-S1	5'-CCC CTA TTT TTT CAA AAA TGT CTT-3'	660 - 683	64
	1A ex2-AS[b]	5'-ATT GAT CCC AAA GAG AAA ACC AC-3'	898 - 920	
UGT1A8	1A8-S1	5'-GGT CTT CGC CAG GGG AAT AG-3'	498 - 517	64
	1A ex2-AS[b]	5'-ATT GAT CCC AAA GAG AAA ACC AC-3'	898 - 920	
UGT1A9	1A9-S1	5'-GAA CAT TTA TTA TGC CAC CG-3'	646 - 665	64
	1A ex2-AS[b]	5'-ATT GAT CCC AAA GAG AAA ACC AC-3'	898 - 920	
UGT1A10	1A10-S[b]	5'-CTC TTT CCT ATG TCC CCA ATG A-3'	557 - 578	64
	1A ex2-AS[b]	5'-ATT GAT CCC AAA GAG AAA ACC AC-3'	898 - 920	
UGT2B4	2B4-S1	5'-TGG TGA GCT GCT GGC CGA GT-3'	468 - 487	58
	2B ex2-AS	5'-CAT TGT CTC AAA TAA TGT AGT G-3'	729 - 750	
UGT2B7	2B7-S1	5'-GGA AAT CAT GTC AAT ATT TGG-3'	339 - 359	58
	2B ex2-AS	5'-CAT TGT CTC AAA TAA TGT AGT G-3'	729 - 750	
UGT2B10	2B10-S[c]	5'-TGA CAT CGT TTT TGC AGA TGC TTA-3'	432 - 455	58
	2B ex2-AS	5'-CAT TGT CTC AAA TAA TGT AGT G-3'	726 - 747	
UGT2B11	2B11-S1	5'-AGA AAT CCT GTG GGA ATT AT-3'	339 - 358	54
	2B ex2-AS	5'-CAT TGT CTC AAA TAA TGT AGT G-3'	729 - 750	
UGT2B15	2B15-S[c]	5'-GTG TTG GGA ATA TTA TGA CTA CAG TAA C-3'	348 - 375	58
	2B ex2-AS	5'-CAT TGT CTC AAA TAA TGT AGT G-3'	732 - 753	
UGT2B17	2B17-S[c]	5'-GTG TTG GGA ATA TTC TGA CTA TAA TAT A-3'	348 - 375	58
	2B ex2-AS	5'-CAT TGT CTC AAA TAA TGT AGT G-3'	732 - 753	
UGT2B28	2B28-S1	5'-ACC GTT TGT GTA CAG TCT CT-3'	498 - 517	58
	2B ex2-AS	5'-CAT TGT CTC AAA TAA TGT AGT G-3'	729 - 750	
GAPDH	GAPDH-S[b]	5'-CCA GGG CTG CTT TTA ACT C-3'	56 - 74	68
	GAPDH-AS[b]	5'-GCT CCC CCC TGC AAA TGA-3'	330 - 347	

[a]Nucleotide position on cDNA when the A in the start codon is 1.
[b]From Nakamura et al. (2008a).
[c]From Congiu et al. (2002).

DMD Fast Forward. Published on May 13, 2009 as DOI: 10.1124/dmd.109.027227
This article has not been copyedited and formatted. The final version may differ from this version.

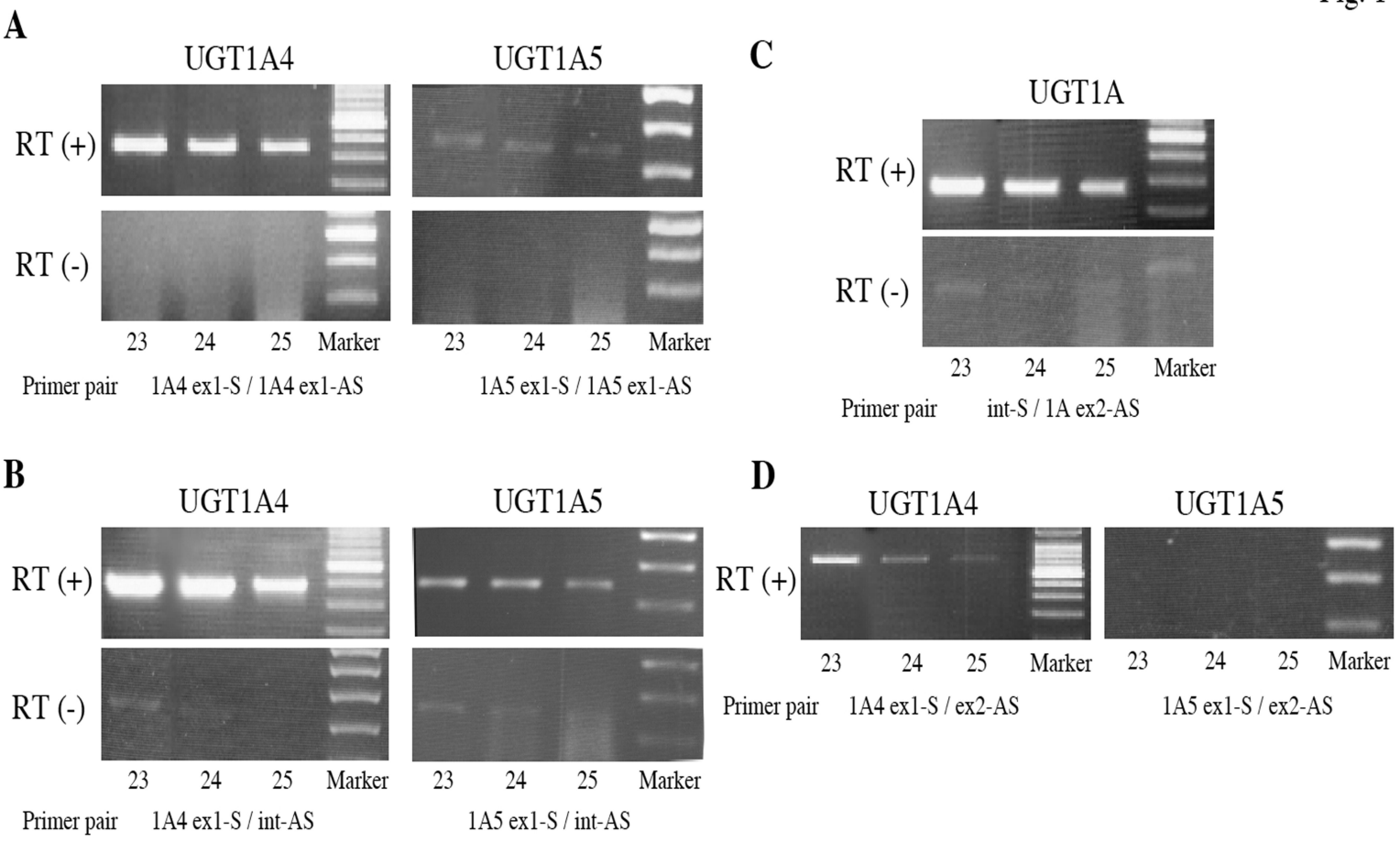
Table 4. Correlations of the expression levels of UGT mRNA or protein between different isoforms in 25 human livers.

	1A1	1A3	1A4	1A6	1A9	2B4	2B7	2B10	2B15	2B17
1A1		0.453 *	0.618 ***	0.445 *	0.492 **	0.511 **	0.452 *	0.389 *	0.397 *	0.433 *
1A3			0.704 ***	0.650 ***	0.596 ***	0.546 **	0.434 *	0.439 *	0.451 *	0.143 NS
1A4	0.302 NS			0.699 ***	0.788 ***	0.804 ***	0.486 **	0.419 *	0.481 *	0.407 NS
1A6	0.399 NS		0.441 *		0.840 ***	0.768 ***	0.379 NS	0.609 ***	0.563 ***	0.372 NS
1A9						0.796 ***	0.575 ***	0.730 ***	0.689 ***	0.264 NS
2B4							0.597 ***	0.695 ***	0.735 ***	0.308 NS
2B7	0.178 NS		0.137 NS	0.100 NS				0.809 ***	0.840 ***	0.024 NS
2B10									0.935 ***	0.015 NS
2B15										0.122 NS
2B17										

Upper half shows the correlation between expression levels of UGT mRNAs and lower half shows the correlation between expression levels of UGT protein levels. The correlation analyses were performed for UGT isoforms that are substantially expressed in human livers by Spearman's rank method. The Spearman correlation coefficients (*Rs*) are shown. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.005$. NS: not significant.

DMD Fast Forward. Published on May 13, 2009 as DOI: 10.1124/dmd.109.027227
This article has not been copyedited and formatted. The final version may differ from this version.

Fig. 1



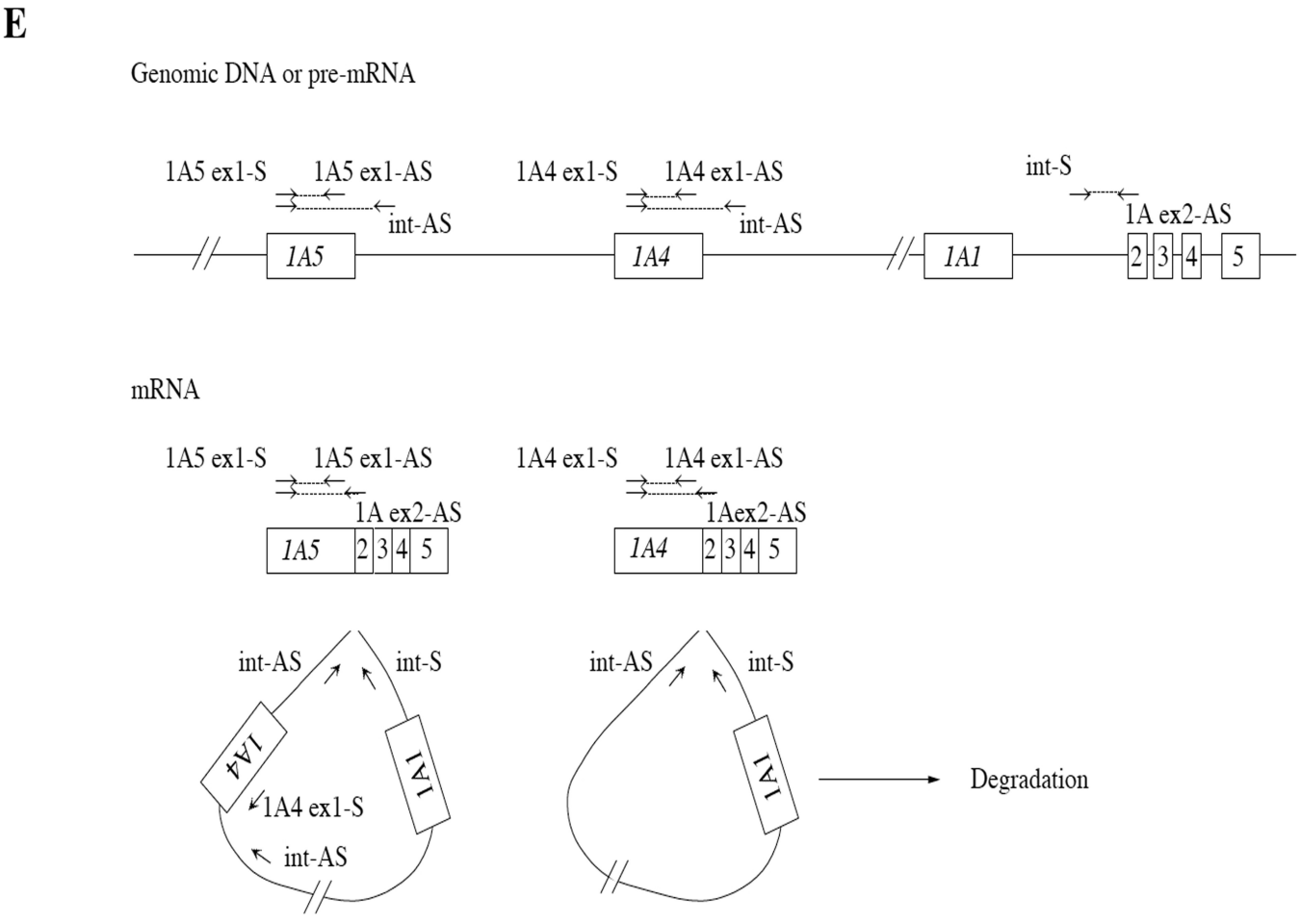
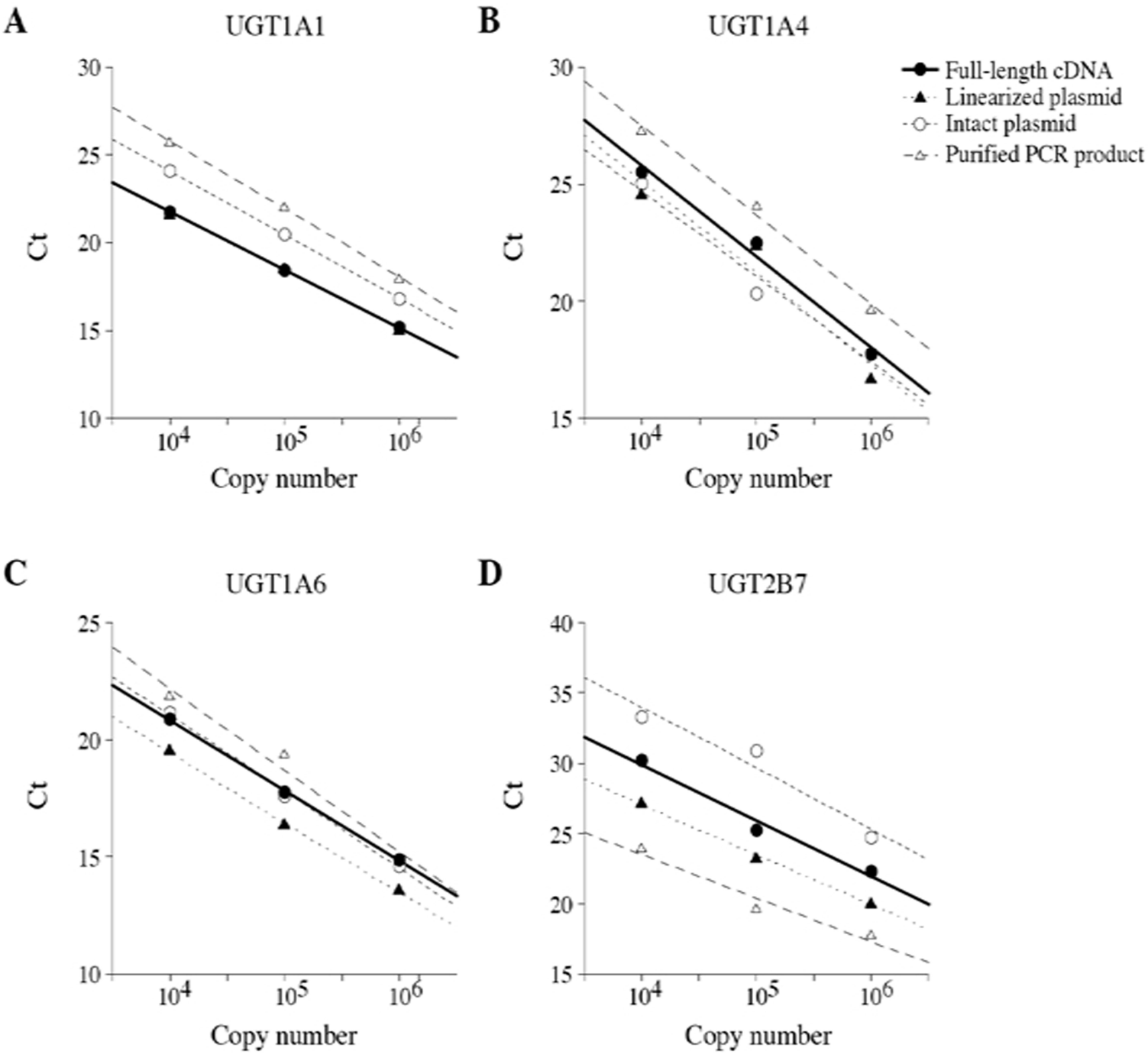


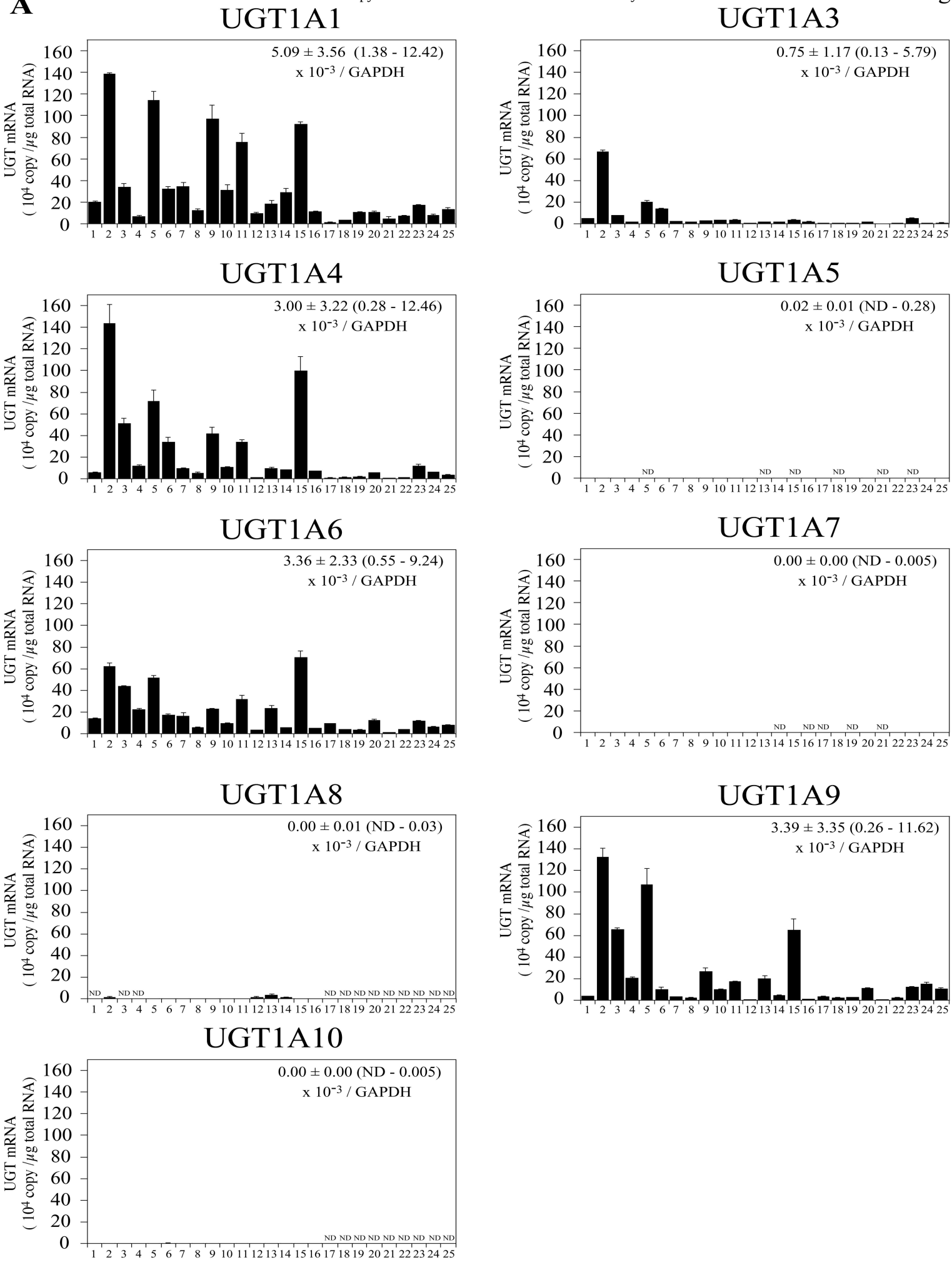
Fig. 2.



DMD Fast Forward. Published on May 13, 2009 as DOI: 10.1124/dmd.109.027227
This article has not been copyedited and formatted. The final version may differ from this version.

Fig. 3.

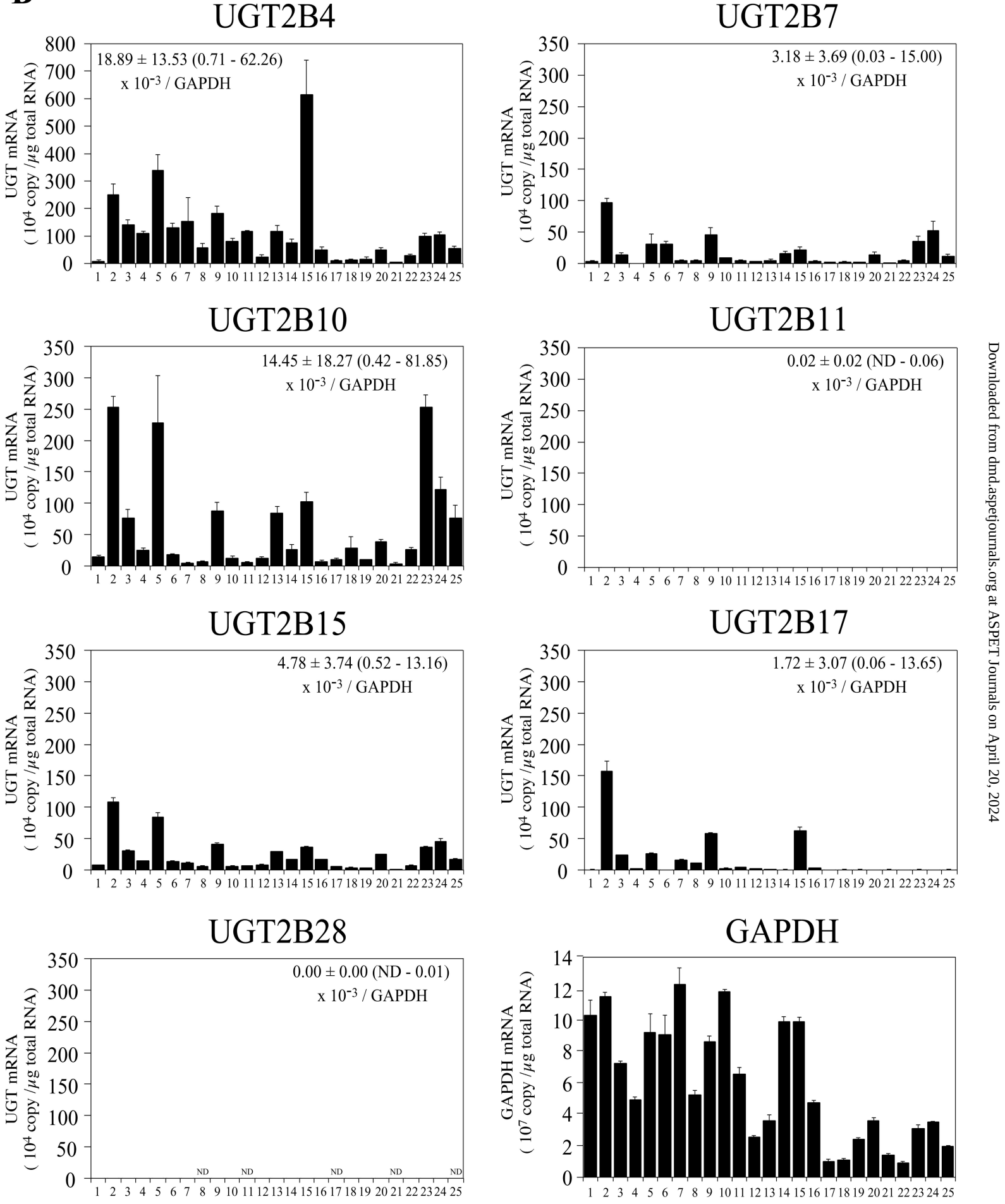
A

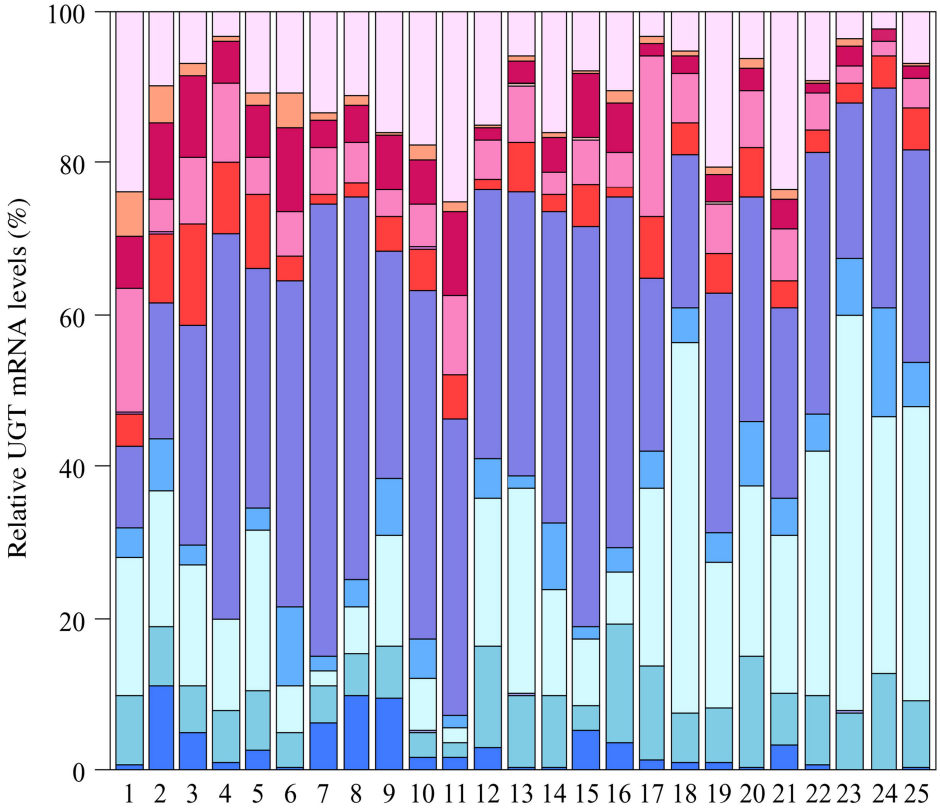


DMD Fast Forward. Published on May 13, 2009 as DOI: 10.1124/dmd.109.027227
This article has not been copyedited and formatted. The final version may differ from this version.

Fig. 3. continued

B





Isoforms	Percentage	(range)
UGT1A1	11.3 ± 6.8	(2.2 - 25.1)
UGT1A3	1.4 ± 1.4	(0.2 - 5.9)
UGT1A4	5.5 ± 3.2	(1.4 - 11.2)
UGT1A5	0.0 ± 0.1	(ND - 0.3)
UGT1A6	6.8 ± 4.3	(1.8 - 21.6)
UGT1A7	0.0 ± 0.0	(ND - 0.0)
UGT1A8	0.0 ± 0.0	(ND - 0.0)
UGT1A9	5.1 ± 3.1	(1.2 - 13.4)
UGT1A10	0.0 ± 0.0	(ND - 0.0)
UGT2B4	34.5 ± 12.1	(6.5 - 59.7)
UGT2B7	5.1 ± 3.2	(0.1 - 14.4)
UGT2B10	19.6 ± 13.4	(2.0 - 52.3)
UGT2B11	0.1 ± 0.1	(ND - 0.2)
UGT2B15	8.0 ± 3.5	(2.2 - 15.5)
UGT2B17	2.8 ± 3.3	(0.1 - 16.8)
UGT2B28	0.0 ± 0.0	(ND - 0.1)

DMD Fast Forward. Published on May 13, 2009 as DOI: 10.1124/dmd.109.027227
This article has not been copyedited and formatted. The final version may differ from this version.

Fig. 5