

## **Multiplexed targeted quantitative proteomics predicts hepatic glucuronidation potential.**

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**List of non-standard abbreviations:**

AZT: zidovudine; HLM: human liver microsome; MRM: multiple reaction monitoring; MS:  
mass spectrometry; SNP: single nucleotide polymorphism; UGT: UDP-  
glucuronosyltransferase

## Abstract

Phase II metabolism is prominently governed by UDP-glucuronosyltransferases (UGTs) in humans. These enzymes regulate bioactivity of many drugs and endogenous small molecules in many organs, including the liver, a major site of regulation by the glucuronidation pathway. This study determined the expression of hepatic UGTs by targeted proteomics in 48 liver samples and by measuring the glucuronidation activity using probe substrates. It demonstrates the sensitivity and accuracy of nanoUPLC-MS/MS to establish the complex expression profiles of 14 hepatic UGTs in a single analysis. UGT2B7 is the most abundant UGT in our collection of livers, expressed at 69 pmoles/mg microsomal proteins, whereas UGT1A1, UGT1A4, UGT2B4 and UGT2B15 are similarly abundant, averaging 30-34 pmoles/mg proteins. The average relative abundance of these five UGTs represents 81% of the measured hepatic UGTs. Our data further highlights strong relationships in the expression of several UGTs. Most notably, UGT1A4 correlates with most measured UGTs, whereas expression levels of UGT2B4/UGT2B7 displayed the strongest correlation. Significant interindividual variability is however observed for all UGTs, both at the level of enzyme concentrations and activity (coefficient of variation: 45-184%). The reliability of targeted proteomics quantification is supported by the high correlation between UGT concentration and activity. Collectively, these findings expand our understanding of hepatic UGT profiles by establishing absolute hepatic concentrations of 14 UGTs and further suggest coregulated expression between most abundant hepatic UGTs. Data support the value of multiplexed targeted quantitative proteomics to accurately assess specific UGT concentrations in liver samples and hepatic glucuronidation potential.

## Introduction

Liver is a key organ for the metabolism of drugs and endogenous compounds such as hormones and bile acids. Through the expression of a complex cocktail of enzymes such as uridine 5'-diphosphoglucuronosyltransferases (UGTs), the biochemical properties and bioactivity of these compounds is highly regulated by the liver. Glucuronidation contributes 35% of the phase II drug metabolic pathways and is involved in the clearance of 55% of the 200 most prescribed drugs (Guillemette et al., 2014). Liver expresses a diversified array of UGTs with 12 of the 16 UGT1A and UGT2B proteins (Guillemette et al., 2014). The expression profile of UGTs was first described using various techniques based on mRNA and immunochemical quantification (Court et al., 2012). However, significant quantitative inaccuracy arises from the lack of correlation between mRNA and protein expression levels and the high sequence similarity among UGTs (Margaillan et al., 2015). Recently, a number of research groups including ours developed mass spectrometry-based methods to quantify UGTs in human tissues (**Table 1**) (Harbourt et al., 2012; Ohtsuki et al., 2012; Fallon et al., 2013b; Achour et al., 2014; Groer et al., 2014; Sato et al., 2014; Yan et al., 2015). Despite the higher precision and specificity in UGT quantification, a strong interindividual variability in the expression profile of all UGTs is highlighted by all studies, and consequently, substantial differences in UGT quantification arise from the different studies. Genetic variation importantly influences UGT expression (Guillemette et al., 2010) such as the well characterized variant *UGT1A1*\*28 (rs8175347; [TA]<sub>6>7</sub>) associated with lower hepatic conjugation of UGT1A1 substrates (Innocenti et al., 2004).

With the aim to expand our understanding of hepatic UGT profiles, UGT protein expression was determined by targeted proteomics quantification in 48 liver specimens. The reliability of this approach as a predictor of hepatic drug metabolizing capacity was then established by correlating UGT expression levels with glucuronidation activity using probe substrates and, for UGT1A1, to promoter genotype. Our quantification reveals important diversity and variability in the UGT protein expression profiles, and highlights UGT2B7, UGT1A1, UGT2B4, UGT1A4 and UGT2B15 as the most expressed in the liver, and significant correlations with probe substrate activities.

## Materials and Methods

Human liver microsomes (HLM) were obtained from non-tumorous liver samples (n=48) and were from an equal number of males and females of Caucasian origin as described (Sumida et al., 1999; Gomes et al., 2009). The institutional boards approved the study and written consent was obtained from all subjects concerning the use of their tissues for research purposes. Total protein concentration was measured using a bicinchoninic acid assay (ThermoFisher Scientific, Ottawa, ON, Canada). UGT1A1 (rs8175347; [TA]<sub>6>7</sub>) was genotyped by sequencing PCR products, as described (Thibaudeau et al., 2006). Fourteen UGT1A and UGT2B proteins were quantified (Fallon et al., 2013a; Fallon et al., 2013b; Margailan et al., 2015), not including UGT2B11, UGT2B28 and UGT2As, for which specific signature peptides had not been obtained. Before quantification, each HLM was diluted to 1 mg/ml with 50 mM ammonium bicarbonate. For all samples, 20 µg of microsomal protein was denatured, reduced, carbamidomethylated, and then digested with trypsin. A mixture of stable isotope

labeled standard peptides (Thermo Biopolymers, Ulm, Germany) corresponding to the selected signature peptides of each UGT was added to each sample. Quantitative analysis was achieved on a nanoACQUITY binary pump system coupled to a QTRAP 5500 mass spectrometer (AB SCIEX, Framingham, MA), using two multiple reaction monitoring (MRM) transitions to quantify each UGT, as described recently (Fallon et al., 2013a; Fallon et al., 2013b). Limit of detection was 0.2 pmol/mg proteins for all UGTs with the exception of UGT1A9, for which it was 1.0 pmol/mg protein. Glucuronidation assays were conducted on HLMs (4 µg) in a final volume of 100 µl with a panel of UGT probe substrates: bilirubin (10 µM; 10 min), deferiprone (20 mM, 60 min), propofol (50 µM, 30 min), and zidovudine (AZT; 500 µM, 60 min) obtained from Sigma-Aldrich (St-Louis, MO), estradiol (100 µM, 180 min; from Steraloids (Newport, RI)), tacrolimus (200 µM, 60 min, from Cell Signaling Technologies (Danvers, MA)). Glucuronides were measured by MS-based methods (Lepine et al., 2004; Benoit-Biancamano et al., 2009). Statistical correlation scores were determined by a Spearman correlation test using XLSTAT (Addinsoft, New York, NY). Statistical significance between rs8175347 and UGT1A1 expression and activity was determined by a Kruskal-Wallis analysis of variance using GraphPad Prism Software (La Jolla, CA).

## Results and Discussion

### *Quantification of hepatic UGTs by targeted proteomics*

Absolute quantification of UGT1As and UGT2Bs in liver microsomes was conducted by acquiring MRMs for specific signature peptides and their heavy labeled standards in a collection of 48 adult livers. UGT2B7 was the most abundant UGT, with an average

concentration of 69.4 pmol/mg protein (**Fig.1A**). Average concentrations of UGT1A1, UGT1A4, UGT2B4, and UGT2B15 were in a similar range, of about half that of UGT2B7, at 30.2-34.3 pmol/mg proteins. The relative abundance of these five UGTs represents over 80% of the measured hepatic UGTs (**Fig.1B**). Moderate levels of UGT1A9 (22.7 pmol/mg proteins) and UGT1A6 (11.3 pmol/mg proteins) were measured, while average concentrations of other measurable UGTs were below 7 pmol/mg protein. The average concentration of UGT2B10 (6.9 pmol/mg proteins) is however likely underestimated because the signature peptide lies in a polymorphic region. Of note, UGT2B15 was on average five to six times more abundant than UGT2B17 in the liver. This is in part due to undetectable levels of UGT2B17 in 25% of the liver samples, reflected by the high coefficient of variation (CV = 183%) for this protein (**Fig.1A**), and likely caused by the common deletion of the gene (McCarroll et al., 2006). A wide variability in the expression level of the other expressed UGTs was also observed, with CVs of 45-89%. Consistent with previous reports, UGT1A5, UGT1A7, UGT1A8 and UGT1A10 were not detected in HLMs.

Several recent studies have addressed the absolute concentration of hepatic UGTs by a targeted mass spectrometry approach, enabling comparisons of UGT concentrations among the different liver sample collections (**Table 1**). The measured concentrations of UGTs in the current study are well in line with those measured in another collection comprising 60 livers, using the same signature peptides and a similar approach (Fallon et al., 2013a; Fallon et al., 2013b). Both studies revealed UGT2B7 as the most abundant, and UGT1A1, UGT1A4, UGT2B4, and UGT2B15 as the next most abundant UGTs. Although their rank order differs slightly, the concentrations determined for these UGTs are in similar ranges, clearly indicating the reproducibility of the approach. All

other studies but one (Achour et al., 2014) revealed the predominant expression of UGT2B7 in the liver, despite some important differences in absolute concentrations determined, as was also the case for other main liver UGTs. Discrepancies in absolute concentrations may arise from interindividual variability, the ethnic origins of the human samples, and the choice of signature peptides can also certainly influence quantification. The quantitative approach based on the use of a multiplexed peptide concatamer strategy (QconCAT) produced significantly different quantitative data than the use of spiked labeled peptides and will deserve further investigations (Achour et al., 2014).

Comparisons of hepatic UGT concentrations revealed high and significant correlations for several UGTs (**Table S1**). The strongest correlation was found between UGT2B7 and UGT2B15 ( $r=0.88$ ,  $p<0.001$ ). UGT2B7 expression was also highly correlated with UGT2B4 ( $r=0.73$ ,  $p<0.001$ ). Notably, UGT1A4 expression correlated well with all measured UGTs besides UGT2B17, with highest correlations with UGT1A6 and UGT2B4 ( $r\geq 0.70$ ,  $p<0.001$ ). UGT1A6 expression correlated well with UGT1A9, UGT2B4, UGT2B7 and UGT2B15 ( $r=0.65-0.70$ ,  $p<0.001$ ). In contrast, UGT1A1 expression moderately correlated only with UGT1A4 and UGT2B4 ( $r=0.50$  and  $r=0.56$  respectively,  $p<0.001$ ), and the expression of UGT2B17 did not correlate with any of the measured UGTs. These observations support the co-regulated expression of several UGTs by hepatic transcription factors including the farnesoid X receptor (UGT2B4 and UGT2B7) and the hepatic nuclear factor 1 $\alpha$  (most UGT1As and UGT2Bs) (reviewed by (Hu et al., 2014)).

#### *Quantification of hepatic glucuronidation activity using probe substrates*

The reliability of targeted proteomics quantification was further established by correlating



absolute concentrations with the glucuronidation activity of selected probe substrates by the HLMs (**Fig.2**). Inter-individual differences in hepatic glucuronidation capacity ranged between 48 and 79%, in line with that of UGT protein concentrations (**Fig.1**). UGT1A1 activity was established based on the formation of bilirubin glucuronide and correlated well with UGT1A1 protein concentrations measured by MRM ( $r=0.88$ ,  $p<0.001$ ; **Fig.2A**). Furthermore, UGT1A1-dependent bilirubin-G and estradiol-3G formation were significantly reduced by 45% and 37% respectively in carriers of the variant *UGT1A1\*28* allele, as a consequence of the reduced expression of UGT1A1 by 35% in carriers (**Fig.S1**). The concentrations of UGT1A4 and UGT1A6 were also highly correlated with the glucuronidation of their respective probe substrates, tacrolimus and deferiprone (**Fig.2B-C**). The correlation between UGT2B7 activity and the glucuronidation of its probe substrate AZT was similarly strong ( $r=0.82$ ,  $p<0.001$ ; **Fig.2E**). Furthermore, estradiol-3G formation strongly correlated with UGT1A1 concentrations ( $r=0.93$ ,  $p<0.001$ ; **Fig.2A**) whereas as expected, estradiol-17G correlated with UGT2B7 concentration ( $r=0.84$ ,  $p<0.001$ ; **Fig.2E**) (Lepine et al., 2004). UGT1A9-dependent conjugation of propofol was the least but nonetheless well correlated with measured protein concentrations ( $r=0.72$ ,  $p<0.001$ ; **Fig.2D**).

### Conclusion

This study establishes the concentration of human liver UGT enzymes by targeted proteomics. We observed that the proteomics quantification correlates well with the enzymatic activity of main enzymes for a probe substrate, supporting the value of multiplexed targeted quantitative proteomics to accurately assess specific UGT concentrations in liver samples and glucuronidation potential.

## Authorship Contributions

*Participated in research design:* Guillemette

*Conducted experiments:* Fallon, Caron, Villeneuve

*Contributed new reagents or analytic tools:* Klein, Fallon, Smith, Zanger

*Performed data analysis:* Margaillan, Rouleau, Fallon, Caron, Villeneuve, Smith,

Guillemette

*Manuscript drafting:* Margaillan, Rouleau, Guillemette

*Critical revision of the manuscript.* All authors

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## Footnotes

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## Figure legends

**Figure 1: Hepatic expression profiles of UGT proteins.** A. Absolute concentration of UGTs was established by targeted quantitative proteomics analysis of 48 human liver microsomal fractions. Mean concentration (pmol/mg proteins) and coefficient of variation (%) are provided for each measured UGT. B. Relative abundance of hepatic UGTs based on average concentrations.

**Figure 2:** Relationship between UGT protein concentrations and glucuronidation activity. Glucuronidation activity in liver microsomal fractions (n=39) was correlated with the absolute concentrations of the relevant UGT. A. UGT1A1 protein concentration and glucuronidation of estradiol 3-hydroxyl group (coefficient of variation (CV): 79%), and bilirubin (CV: 61%); B. UGT1A4 protein concentration and glucuronidation of tacrolimus (CV: 48%); C. UGT1A6 protein concentration and glucuronidation of deferiprone (CV: 49%); D. UGT1A9 protein concentration and glucuronidation of propofol (CV:48%); E. UGT2B7 protein concentration and glucuronidation of zidovudine (CV: 49%) and

estradiol 17-hydroxyl group (CV: 50%). Spearman correlation coefficients ( $r$ ) and corresponding  $p$  values are given for each correlation.



**Table 1.** UGT quantification by targeted mass spectrometry (MRM) in this and previous studies.

Reported mean UGT concentrations (pmol/mg protein)								
	Ohtsuki	Harbourt	Sato	Yan	Groer	Achour	Fallon	This study <sup>a</sup>
	n=17	n=33	n=50	n=22	n=25	n=24	n=60	n=48
UGT	Number of livers							
<b>1A1</b>	33.2 (4) <sup>b</sup>	18.3 (2)	124.0 (2)	21.7 (2)	20.2 (3)	33.6 (8)	31.7 (4)	<b>34.3</b> <b>(2)</b>
<b>1A3</b>	17.3 (6)	9.9 (3)	20.6 (9)	-	0.4 (4)	123.1 (1)	8.2 (7)	<b>6.3</b> <b>(9)</b>
<b>1A4</b>	n.d.	4.6 (5)	84.0 (5)	28.2 (1)	-	58.0 (6)	41.8 (2)	<b>33.0</b> <b>(4)</b>
<b>1A5</b>	-	-	-	-	-	-	n.d.	n.d.
<b>1A6</b>	114.0 (1)	5.2 (4)	22.6 (8)	12.0 (5)	-	107.1 (2)	7.8 (8)	<b>11.3</b> <b>(7)</b>
<b>1A7</b>	-	n.d.	n.d.	-	-	-	n.d.	n.d.
<b>1A8</b>	-	n.d.	n.d.	-	-	-	n.d.	n.d.
<b>1A9</b>	25.9 (5)	26.7 (1)	61.1 (6)	12.6 (4)	-	40.0 (7)	21.8 (6)	<b>22.7</b> <b>(6)</b>
<b>1A10</b>	n.d.	n.d.	n.d.	-	-	-	n.d.	n.d.
<b>2B4</b>	-	-	102.0 (3)	-	-	70.8 (4)	35.1 (3)	<b>33.9</b> <b>(3)</b>
<b>2B7</b>	84.3 (2)	-	200.0 (1)	21.0 (3)	24.1 (2)	82.9 (3)	67.7 (1)	<b>69.4</b> <b>(1)</b>
<b>2B10</b>	-	-	69.3 (8)	-	-	-	5.4 <sup>c</sup> (10)	<b>6.9<sup>c</sup></b> <b>(8)</b>
<b>2B15</b>	61.8 (3)	-	99.7 (4)	-	27.9 (1)	62.1 (5)	28.8 (5)	<b>30.2</b> <b>(5)</b>
<b>2B17</b>	-	-	60.8 (7)	-	-	-	7.3 (9)	<b>4.9</b> <b>(10)</b>

n.d.: UGT not detected or below detection limit

- : UGT not measured

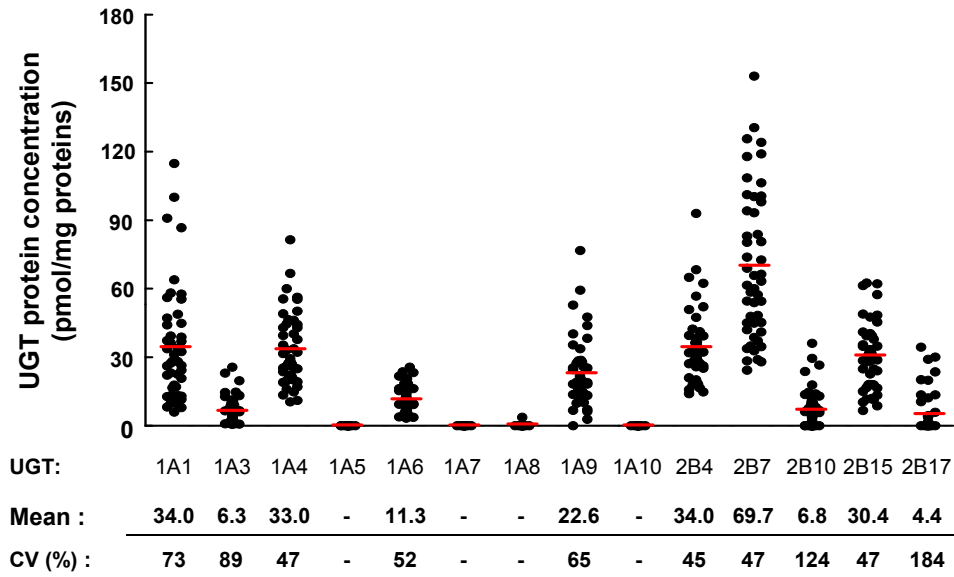
<sup>a</sup> Measured concentrations for each UGT in each individual is given in Table S2

<sup>b</sup> UGT rank order based on abundance

<sup>c</sup> UGT2B10 concentration is underestimated because a coding SNP is located in the sequence targeted by the signature peptide.

Figure 1

A.



B.

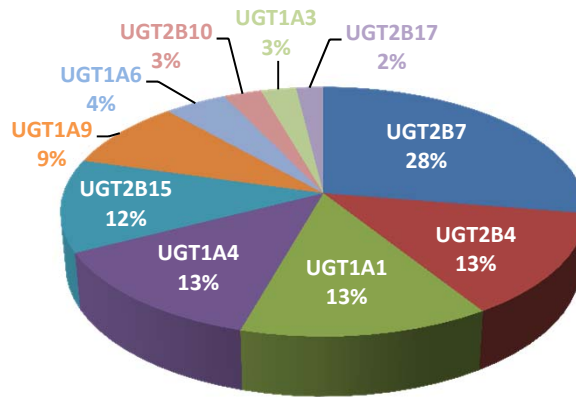
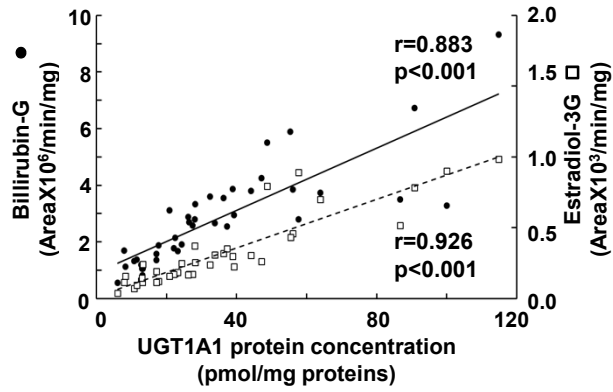
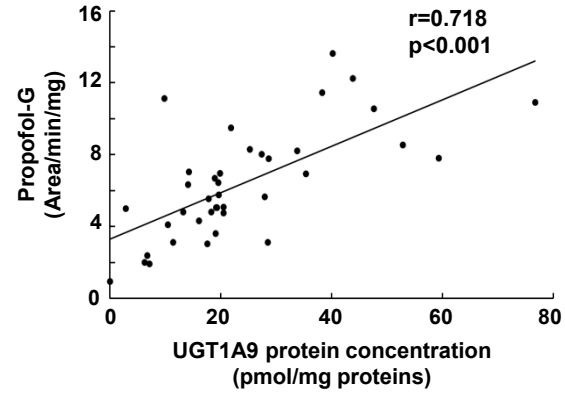


Figure 2

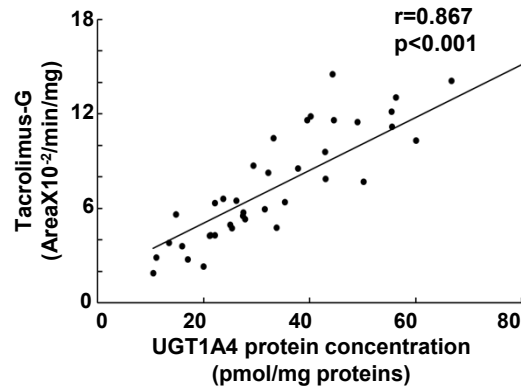
A. Bilirubin and estradiol (UGT1A1)



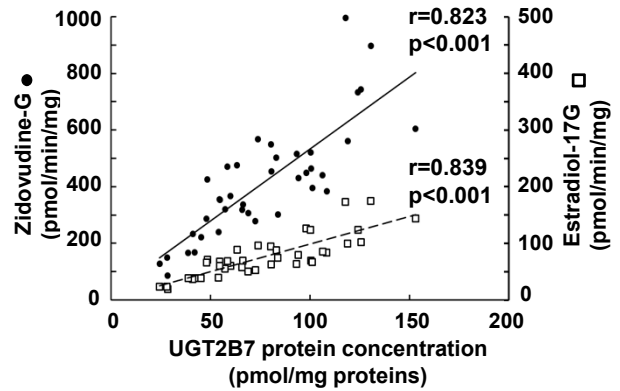
D. Propofol (UGT1A9)



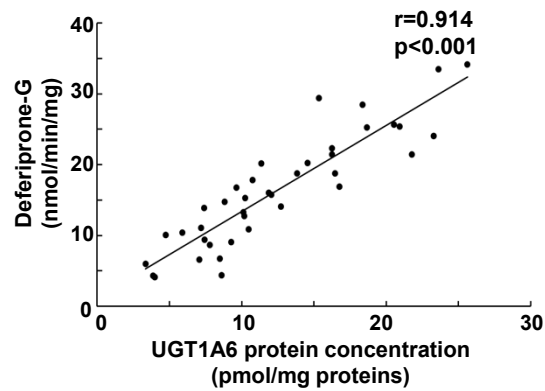
B. Tacrolimus (UGT1A4)



E. Zidovudine and estradiol (UGT2B7)



C. Deferiprone (UGT1A6)



**Supplementary material**




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**Table S1.** Correlations between UGT protein concentrations established by MRM

	UGT1A3	UGT1A4	UGT1A6	UGT1A9	UGT2B4	UGT2B7	UGT2B15	UGT2B17
UGT1A1	0.14	<b>0.50</b> ***	<b>0.43</b> **	<b>0.32</b> *	<b>0.56</b> ***	0.23	0.24	-0.04
UGT1A3		<b>0.64</b> ***	<b>0.49</b> ***	<b>0.43</b> **	<b>0.37</b> *	0.26	0.16	-0.11
UGT1A4			<b>0.73</b> ***	<b>0.68</b> ***	<b>0.72</b> ***	<b>0.57</b> ***	<b>0.49</b> ***	0.02
UGT1A6				<b>0.70</b> ***	<b>0.69</b> ***	<b>0.65</b> ***	<b>0.66</b> ***	0.08
UGT1A9					<b>0.51</b> ***	<b>0.63</b> ***	<b>0.63</b> ***	0.17
UGT2B4						<b>0.73</b> ***	<b>0.68</b> ***	0.02
UGT2B7							<b>0.88</b> ***	<b>0.28</b> *
UGT2B15								0.26

**r (Spearman)<sup>a</sup>**

	< 0.5
	0.5 - 0.8
	> 0.8

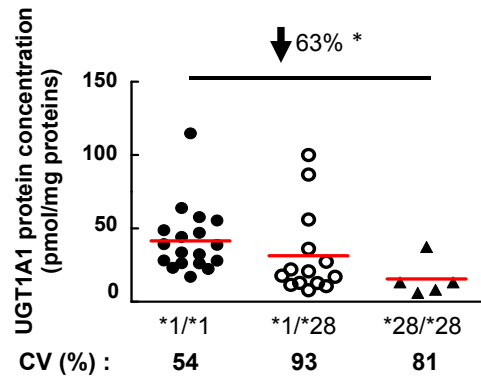
<sup>a</sup> Significant correlations are in bold (\*p<0.05; \*\*p<0.01; \*\*\*p<0.001)

**Table S2:** UGT protein expression of each isoform in each individual

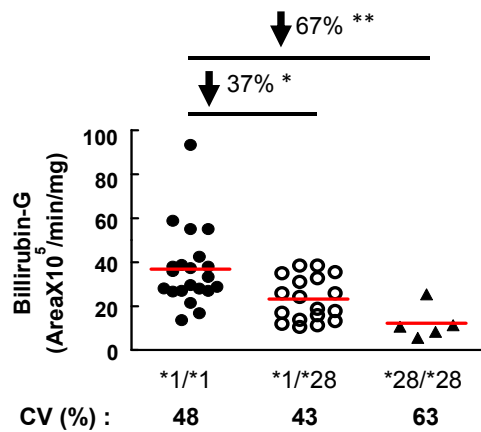
Liver Samples	UGT protein concentration (pmol/mg of proteins)													
	1A1	1A3	1A4	1A5	1A6	1A7	1A8	1A9	1A10	2B4	2B7	2B10	2B15	2B17
#1	26.4	0.6	22.1	0.0	10.2	0.0	0.0	9.8	0.0	39.6	93.3	0.0	29.4	0.1
#2	44.1	2.5	11.1	0.0	4.0	0.0	0.0	0.0	0.0	14.9	28.4	0.0	10.4	0.1
#3	56.1	23.0	33.2	0.0	14.6	0.0	0.0	13.3	0.0	36.6	73.8	3.5	27.9	0.0
#4	8.2	11.2	29.4	0.0	11.3	0.0	0.0	20.5	0.0	30.4	63.3	17.9	33.2	0.0
#5	48.8	5.2	66.7	0.0	18.4	0.0	0.0	40.2	0.0	52.1	130.5	0.0	61.4	0.5
#6	24.3	4.6	26.1	0.0	5.9	0.0	0.0	2.9	0.0	31.2	48.4	0.0	28.5	0.3
#7	63.9	4.2	55.6	0.0	16.2	0.0	0.0	25.2	0.0	50.8	153.1	0.0	62.4	20.0
#8	90.8	3.4	40.2	0.0	25.6	0.0	0.0	38.3	0.0	42.3	98.0	0.0	47.6	30.0
#9	55.4	7.0	44.6	0.0	11.9	0.0	0.0	52.9	0.0	47.4	125.6	13.7	57.4	0.3
#10	27.4	3.1	32.2	0.0	20.5	0.0	0.0	47.6	0.0	30.6	108.5	0.0	41.1	23.5
#11	28.1	5.3	44.3	0.0	23.6	0.0	0.0	59.3	0.0	17.8	80.3	0.0	40.4	0.3
#12	38.8	0.9	23.7	0.0	10.8	0.0	0.0	14.3	0.0	33.5	60.0	6.2	28.9	1.6
#13	114.8	3.0	49.0	0.0	12.1	0.0	0.0	14.1	0.0	68.3	47.9	36.1	31.7	0.0
#14	22.5	3.4	27.5	0.0	10.1	0.0	0.0	27.4	0.0	34.2	100.3	0.0	39.4	29.1
#15	86.7	19.7	81.4	0.0	23.3	0.0	0.0	33.8	0.0	92.9	106.3	23.7	31.0	0.2
#16	36.2	13.2	42.9	0.0	16.8	0.0	0.0	18.3	0.0	31.8	58.5	8.3	34.8	0.3
#17	10.7	2.9	27.4	0.0	8.5	0.0	0.0	20.5	0.0	29.6	72.5	0.0	32.4	20.1
#18	33.7	6.6	50.2	0.0	13.8	0.0	0.0	19.9	0.0	36.3	83.0	13.0	22.6	5.9
#19	12.7	1.7	21.3	0.0	4.7	0.0	0.0	19.1	0.0	29.2	80.6	29.5	33.3	0.5
#20	47.1	4.6	35.3	0.0	9.6	0.0	0.0	19.3	0.0	38.3	83.8	2.3	32.7	13.5
#21	11.5	2.5	13.5	0.0	3.3	0.0	0.0	6.8	0.0	14.0	45.2	10.1	17.9	0.3
#22	32.5	3.8	31.6	0.0	10.3	0.0	0.0	19.5	0.0	32.7	66.3	11.0	34.9	4.5
#23	6.0	6.1	21.1	0.0	7.2	0.0	0.0	17.8	0.0	18.7	65.8	14.7	28.4	34.4
#24	26.2	1.3	20.0	0.0	7.4	0.0	0.0	16.1	0.0	27.2	68.9	14.0	31.1	0.3
#25	23.2	0.7	10.5	0.1	3.9	0.0	0.0	6.3	0.0	20.1	28.0	0.0	18.0	0.3
#26	7.9	2.8	25.0	0.0	10.5	0.0	3.7	10.4	0.0	25.2	42.1	6.8	16.5	0.3
#27	17.0	11.3	22.1	0.0	16.5	0.0	0.0	28.7	0.0	31.9	54.6	7.6	35.5	0.3
#28	13.2	14.7	55.4	0.0	12.7	0.0	0.0	19.6	0.0	39.3	124.1	26.6	45.4	0.4
#29	13.1	6.7	56.3	0.0	21.8	0.0	0.0	76.7	0.0	32.9	57.3	0.0	29.8	0.1
#30	57.6	6.5	39.5	0.0	15.3	0.0	0.0	43.8	0.0	56.8	117.9	0.0	62.1	0.5
#31	22.1	14.6	43.0	0.1	16.3	0.0	0.0	27.9	0.0	41.2	100.6	12.2	39.4	0.3
#32	28.2	0.9	16.0	0.0	8.6	0.0	0.0	19.2	0.0	18.8	41.0	8.1	24.0	0.1
#33	17.8	2.4	25.4	0.0	7.4	0.0	0.0	28.5	0.0	30.7	101.1	8.1	48.4	0.3
#34	13.0	9.9	27.8	0.0	8.8	0.0	0.0	18.9	0.0	29.1	54.5	9.8	16.7	0.0
#35	39.3	2.7	17.0	0.0	9.3	0.0	0.0	17.6	0.0	26.2	24.4	0.0	15.0	1.7
#36	20.8	4.7	33.7	0.0	7.8	0.0	0.0	7.1	0.0	25.8	38.7	4.5	24.9	4.1
#37	37.3	25.7	60.0	0.0	20.9	0.0	0.0	35.4	0.0	62.4	94.1	0.0	37.9	0.2
#38	100.0	8.6	37.8	0.1	18.7	0.0	0.0	21.8	0.0	65.0	119.0	0.0	48.9	0.4
#39	17.0	1.1	14.8	0.0	7.1	0.0	0.0	11.4	0.0	30.4	53.9	0.0	32.9	0.3
#40	58.1	5.1	46.1	0.0	7.1	0.0	0.0	25.7	0.0	28.0	37.2	4.2	13.4	0.0
#41	9.2	9.1	19.0	0.0	6.8	0.0	0.0	10.2	0.0	18.3	33.8	0.0	8.7	10.5
#42	10.4	2.7	25.2	0.0	4.1	0.0	0.0	18.2	0.0	28.4	61.6	5.3	24.9	13.5
#43	37.0	3.9	26.9	0.0	7.4	0.0	0.0	15.1	0.0	27.8	34.6	5.2	12.0	4.0
#44	9.8	4.2	19.7	0.0	6.0	0.0	0.0	13.7	0.0	15.8	44.9	4.5	11.6	0.0
#45	44.8	12.8	46.4	0.0	6.6	0.0	0.0	24.6	0.0	32.3	28.9	3.8	6.6	0.0
#46	16.7	5.2	20.3	0.0	3.7	0.0	0.0	11.6	0.0	16.6	32.8	5.8	11.4	12.2
#47	33.9	4.7	27.6	0.0	9.4	0.0	0.0	25.4	0.0	21.0	45.1	4.7	17.9	0.5
#48	31.2	3.0	19.2	0.0	5.4	0.0	0.0	12.5	0.0	27.4	35.0	5.1	18.1	0.1

**Figure S1:** The UGT1A1 genotype for the promoter variation (TA<sub>6>7</sub>; rs8175347) in relation to UGT1A1 protein expression and enzyme activity.

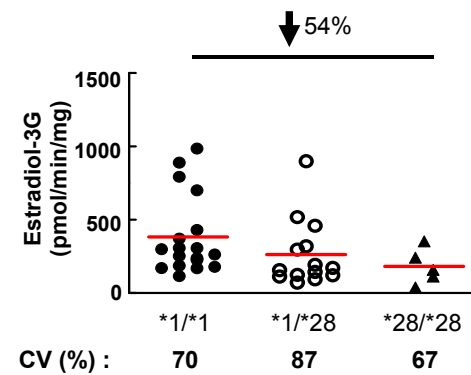
### A. UGT1A1



### B. Bilirubin



### C. Estradiol



Legend: The genotype of donors of liver microsomal fractions (n=39) is given as a function of: A. absolute concentration of UGT1A1; B. glucuronidation of bilirubin; and C. glucuronidation of estradiol. Coefficient of variation (CV%) are given below each graph.

\* $p < 0.05$ , \*\* $p < 0.01$