

# **A NOVEL METHOD FOR THE IMMUNOQUANTIFICATION OF UDP- GLUCURONOSYLTRANSFERASES IN HUMAN TISSUE**

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**Running Title:**

Quantification of UGT Expression in Human Tissue

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**Abbreviations used:**

UGT, UDP-glucuronosyltransferase; MAP, multiple antigenic peptide; VNTR, variable number tandem repeat.

## Abstract

Glucuronidation is a major pathway of drug and xenobiotic metabolism, catalysed by members of the UDP-glucuronosyltransferase (UGT) family. Predicting the contribution of individual UGTs to drug metabolism would be of considerable value in drug development, and would be greatly aided by the availability of detailed absolute expression levels of these proteins; this is hampered by the lack of purified protein standards due to the hydrophobic membrane-associated nature of UGTs and the consequential difficulties in expression and purification. Here we describe a novel solution to this problem by expressing UGTs in *E. coli* as fusion proteins with ribonuclease S-peptide, targeted to the periplasm with the pelB leader sequence. Following addition of ribonuclease S-protein to membrane extracts, a functional ribonuclease is reconstituted that provides a direct and absolute quantification of the amount of UGT fusion protein; this is subsequently used to generate standard curves for immunoquantification by immunoblotting. To illustrate the value of the method, we have quantified the expression of UGTs 1A1 and 1A6 in human liver and kidney microsomes using new isoform-specific antibodies developed against peptides from these proteins. Expression levels of both proteins in liver were highly variable (28-fold and 20-fold, respectively), and correlated strongly with UGT enzyme activity towards probe substrates bilirubin and 1-naphthol, respectively. The method is broadly applicable and provides a straightforward means of determining absolute, as opposed to relative, quantities of UGT proteins present in human tissues.

## Introduction

Glucuronidation is a key pathway for the metabolism, detoxification and elimination of numerous xenobiotics as well as endogenous compounds such as bilirubin and steroid hormones (Burchell and Coughtrie, 1989; Tukey and Strassburg, 2000; Wells et al., 2004). The glucuronidation reaction, which involves the transfer of the glucuronic acid moiety from co-substrate UDP-glucuronic acid, is catalysed by members of the UDP-glucuronosyltransferase (UGT) enzyme family. In humans, there are at least 20 members of the UGT enzyme family belonging to 4 different sub-families: 1A (where 9 enzymes are produced from a single gene), 2A, 2B and 3A. This diversity of UGTs facilitates glucuronidation of a vast array of chemical structures at several different functional groups, including phenols, alcohols, carboxylic acids and amines as well as rare examples of glucuronidation at carbon and sulfur atoms (Burchell and Coughtrie, 1989; Tukey and Strassburg, 2000).

Adverse drug reactions (ADR) are a major cause of morbidity and mortality. A recent survey (Pirmohamed et al., 2004) estimated that ADRs accounted for 6.5% of hospital admissions, with a financial burden to the UK health service of £0.5 billion per annum. It is increasingly clear that glucuronidation plays a significant role in individual response to drugs, and therefore in susceptibility to ADRs. For instance, treatment-limiting toxicity of the anticancer drug irinotecan is associated with mutations in *UGT1A1* (Nagar and Remmel, 2006; Hoskins et al., 2007) and a *UGT1A* haplotype involving mutations in *UGT1A1*, *UGT1A3* and *UGT1A7* predisposes to jaundice associated with atazanavir treatment (Lankisch et al., 2006). There is therefore considerable interindividual and inter-tissue variation in the expression of many UGTs, e.g. (Strassburg et al., 2000; Izukawa et al., 2009), which have significant functional sequelae (Wells et al., 2004). Most studies aimed at determining expression levels of UGTs have relied upon mRNA measurements (often as

relative rather than absolute values – e.g. (Aueviriyavit et al., 2007)) which have been shown to correlate poorly with protein expression levels (Izukawa et al., 2009). Therefore, there remains a need for quantitative data on functionally active UGT protein expression levels in human tissues. Such expression profiles have been generated for the cytochromes P450 in liver and intestine (Shimada et al., 1994; Paine et al., 2006) and also recently for the sulfotransferases in liver, kidney, intestine and lung (Riches et al., 2009b). Such detailed information contributes importantly to software tools (e.g. SimCyp; <http://www.simcyp.com/>) which aim to predict the *in vivo* fate of drugs. However, absolute quantification of UGT protein expression in human tissues using immunochemical approaches poses a significant technical problem since the technique requires both isoform-specific antibodies and authentic quantified standard protein. These standards would normally be in the form of the full length purified protein (e.g. (Shimada et al., 1994; Riches et al., 2009b)), but since UGTs are integral membrane proteins, their purification from recombinant expression systems is challenging. Here, we describe a novel approach to this problem using UGTs expressed as fusion proteins coupled to S-peptide as standards for immunoquantification, and validate this system using the important hepatic UGTs 1A1 and 1A6. The S-peptide (S•tag™) is a 15 amino acid N-terminal peptide derived from ribonuclease that allows direct quantification of the fusion protein without purification, since high affinity reconstitution with ribonuclease S-protein forms a functional ribonuclease enzyme whose readily quantified activity yields a direct measurement of the concentration of the UGT fusion protein (Kim and Raines, 1993).

## Materials and Methods

**Materials.** pET-15b, pET-20b, pET-32b and BL21(λDE3) and all S•tag™ reagents were obtained from Merck Chemicals, Nottingham, UK, and *Pfu* polymerase and pGEM-T Easy were from Promega (Southampton, UK). Uridine diphosphate glucuronic acid

DMD #41699

[glucuronosyl-<sup>14</sup>C] (>6.7GBq/mmol) was purchased from PerkinElmer, Cambridge, UK. Multiple antigenic peptides were synthesised by Alta Bioscience, Birmingham, UK. Anti-goat IgG conjugated to horseradish peroxidase was from Sigma-Aldrich, Gillingham, UK. All other reagents were from commonly used local suppliers.

***Tissue Samples and Preparation of Microsomal Fractions.*** Human liver samples used in this study have been described previously (Thomas and Coughtrie, 2003; Riches et al., 2007; Riches et al., 2009b). Human kidney samples were obtained from the UK Human Tissue Bank, Leicester, UK. Ethical approval for local use of samples was obtained from the Tayside Research Ethics Committee. Frozen tissue was weighed and kept on ice, and 5ml of SHM buffer (250mM sucrose, 10mM HEPES, 3mM 2-mercaptoethanol, pH7.4) were added for every gram of tissue. Homogenates were prepared in a Teflon-glass homogeniser, and subjected to differential centrifugation at 10,000 x g for 15 minutes with the resulting supernatant centrifuged at 100,000 x g for 1 hour. The pellet, representing the microsomal fraction, was resuspended in 1ml per gram of original tissue used and homogenised by hand then aliquoted, snap frozen in liquid nitrogen and stored at -80°C until use (usually within 6 months).

***Production of Antibodies against UGT1A1 and UGT1A6.*** For UGT1A1, we selected a unique 19 amino acid sequence (ENDSFLQRVIKTYKKIKKD) from the variable N-terminal region for synthesis as a multiple antigenic peptide (MAP). For UGT1A6, a 100 amino acid peptide (from Pro61 to Ala160) was selected from within the N-terminal variable region on the basis of predicted antigenicity and sequence divergence from its nearest relatives.

Antigenicity was assessed using a web based application

(<http://www.bioinformatics.org/JaMBW/3/1/7/>), and amino acid sequence similarity was

determined by alignment of all the UGT1A family members using ClustalW

(<http://www.ebi.ac.uk/clustalw/>) and BLAST searches

(<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). The sequence coding for this peptide was cloned into pET-15b with a C-terminal 6-histidine tag to facilitate purification and expressed in *E. coli* BL21 ( $\lambda$ DE3) cells following induction with IPTG. Briefly, the selected region for UGT1A6 was amplified using the following primers: 1A6VF PCR - 5' CATATGCCATGGCCTGAAGTTAATTTGCTTTTGAAA and 1A6VR PCR - 5' CTCGAGTCAAGCCAGGATCACCCCACAGGGTAA. The PCR was performed using the pET-20b/pelB-UGT1A6 vector (see below) as template and *Pfu* polymerase (Promega). The PCR product was cloned into pGEM-T easy (Promega) and sequenced, then excised with *Nde*I and *Xho*I and ligated with similarly digested pET-15b to produce an N-terminal His6 tagged peptide of 1A6 corresponding to the region Pro61 to Ala160. The expressed peptide was found in the pellet after centrifugation at 12,000 x g, suggesting it was localised in inclusion bodies. Cells were harvested and the pellet resuspended in denaturing extraction buffer (50mM sodium phosphate, pH7, 300mM NaCl and 6M guanidine-HCl) and incubated on ice for 4 hours with gentle shaking. The extract was centrifuged at 12,000 x g for 20 minutes at 4°C to pellet insoluble material. 1ml of the supernatant was applied to a TALON® cobalt affinity spin column (Clontech, Saint-Germain-en-Laye, France) as described by the manufacturer. A total of 3ml of extract were passed through spin columns, and the eluant pooled and dialysed against 500 volumes of 45mM sodium phosphate pH7, 150mM NaCl, 20% (v/v) glycerol. This procedure produced a total of 9mg of purified fusion peptide.

The immunisation procedure was carried out by Alba Bioscience (Edinburgh, UK), and 0.5mg of peptide or MAP was used per injection in sheep. 4 immunisations were administered 4 weeks apart with blood collected for analysis one week after the 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> immunisations.

**Expression of UGT-S•tag Fusion Protein.** A PCR product representing part of the conserved C terminal domain of the UGT1A family plus the S•tag sequence was generated using *Pfu* polymerase and cloned into pGEM-T easy. After sequence confirmation, the PCR product was subcloned into existing *E. coli* expression constructs, pET-20b/pelB-UGT1A1 and pET-20b/pelB-UGT1A6, which contain the pelB leader sequence (which targets the protein to the bacterial periplasm (Better et al., 1988; Skerra and Pluckthun, 1988)) substituted for the natural N-terminal UGT signal sequence (Ouzzine et al., 1994). Briefly, three primers were designed to bind in the constant region of the UGT1A (UGT1A(S)F); to link the UGT1A sequence to the S-tag sequence (UGT1A(S) Linker) and as an S-tag reverse (S-tag R). The sequences of these primers were: UGT1A(S)F: 5' ACCTGGTACCAGTACCATTCTTG 3' – this binds across a unique internal restriction endonuclease site (*KpnI*) noted in italics. UGT1A(S) Linker: 5' AAATCCAAGACCCATAAAGAAACCGCTGCTGCT 3' – this comprises the last 15 nucleotides of the UGT1A constant region before the stop codon (noted in italics) and the first 18 nucleotides of the S-tag sequence. S-tagR: 5'CTCGAGGCGGCCGCCTAGCTGTCCATGTGCTG 3' – this comprises the final 15 nucleotides of the S-tag sequence, a stop codon and restriction endonuclease sites, *NotI* and *XhoI*. The cloning strategy comprised a two stage PCR. In the first stage a small PCR product of 74bp is produced using the UGT1A(S) Linker and S-tag R primers and pET-32b as template. In the second stage the purified product from the first PCR is used alongside pET-20b/pelB-UGT1A6 as the template, using the primers UGT1A(S) F and S-tag R. In the second PCR the product was expected to be 217bp. The PCR product was purified and sequenced. It was digested with *KpnI* and *XhoI* and ligated with similarly digested pET-20B/pelB-UGT1A1 or 1A6 to produce an S-tag version of these UGT isoforms. The modified expression constructs were transformed into the *E. coli* expression strain BL21



DMD #41699

( $\lambda$ DE3). For expression of UGT1A1(S), a 500ml culture of modified terrific broth was inoculated from a small starter culture. The cells were incubated for 16 hours at 37°C with no induction. Subcellular fractionation was performed and the membrane pellet was resuspended in TSE buffer (50mM tris-acetate pH7.6, 250mM sucrose, 0.25mM EDTA). Expression of UGT1A1(S) was confirmed using the S•tag rapid assay kit as described by the manufacturer (Merck), and by immunoblotting with peroxidase-conjugated S•protein and anti-UGT1A1 antibody. For expression of UGT1A6(S) a starter culture was used to inoculate 500ml LB with ampicillin selection. The culture was incubated until the optical density was 0.57. After adding IPTG (1mM) to induce expression the culture was incubated at 27°C for 2 hours. The membrane pellet was prepared, and expression confirmed, as for UGT1A1(S).

***Quantitative Immunoblot Analysis.*** Crude IgG fractions of antisera were prepared by 50% ammonium sulfate precipitation followed by extensive dialysis against PBS. For anti-UGT1A1, the best immunoblotting results were obtained by using affinity purified IgG which was prepared using the same peptide as for immunization, as described by the manufacturer (Alta Bioscience). IgG prepared from both antisera was used at a concentration of 10 $\mu$ g/ml for immunoblotting.

Proteins were resolved on denaturing SDS-PAGE gels (10% acrylamide monomer) and transferred electrophoretically for 16 hours at 27V to PVDF membranes (Immobilon-P, Millipore, Watford, UK) as described by Towbin et al. (Towbin et al., 1979). Following transfer, the membranes were incubated in blocking buffer (5% (w/v) dried milk powder in 20ml TBS-T (10mM Tris-HCl, pH9.0; 150mM NaCl; 0.1% (v/v) Tween 20)) for 1 hour then with the IgG, diluted to 10 $\mu$ g/ml in 20ml blocking buffer, for 3 hours. Gels were stained with Coomassie blue to ensure complete transfer of proteins; gels were routinely seen to be clear, suggesting efficient transfer of standards and test samples. Membranes were washed in

DMD #41699

TBS-T and then exposed to the anti-goat IgG-peroxidase conjugate, diluted at 1:20,000 in blocking buffer, for 2 hours. After extensive washing in TBS-T, blots were developed using Western Lightning (PerkinElmer) and ECL Hyperfilm (GE Healthcare, Little Chalfont, UK). Standard curves for quantification were generated using samples of UGT(S) loaded in a range between 0.25ng and 5 ng UGT(S).

Scans of the developed x-ray films were made using a desktop scanner attached to a personal computer, and analysed using Quantiscan 3.1 (Biosoft, Cambridge, UK) as described previously (Riches et al., 2009a). Following pilot experiments, the quantity of microsomal protein loaded onto the gels was adjusted such that the densities of resulting bands were within the linear region of the standard curves on each blot. Each sample was analyzed on at least three separate blots with each antibody, and thus individual data points quoted are the means of these determinations.

**Assay of UGT Activity.** UGT1A1 activity was assayed with bilirubin as substrate using a method described previously (Heirwegh et al., 1972), and UGT1A6 activity was assessed using 500 $\mu$ M 1-naphthol by the universal HPLC assay method described by Ethell et al. (Ethell et al., 1998; Taskinen et al., 2003).

**Total protein concentration analysis.** Quantification of total protein levels in microsomal samples and recombinant protein preparations was carried out using the method originally described by Lowry *et al.* (Lowry et al., 1951), using BSA as standard.

## Results

We sought to develop a method that would overcome the problems posed by the lack of availability of purified UGT standards and facilitate the absolute immunoquantification of UGT expression in human tissue microsomes. The S-peptide/S-protein system provides a

convenient mechanism for producing quantified protein standards, and is readily applicable to membrane proteins such as UGTs since it does not rely upon purifying the protein from the expression system. The functional ribonuclease activity was reconstituted when incubating the recombinant *E. coli* membrane fraction containing the UGT/S•tag™ fusion protein with purified S-protein (the binding is of very high affinity –  $K_d = 10^{-9}$  M), and the enzymatic breakdown of the substrate polycytidylic acid (poly(C)) was readily followed by measuring  $A_{280}$ . By comparison with results obtained with a known S-peptide standard, the molar concentration of UGT in the extract was determined. We used expression constructs generated with the pelB leader sequence to target the UGT expression to the periplasmic membrane, since full-length UGTs are normally expressed in inclusion bodies in *E. coli* and require harsh conditions to solubilise them.

***Antibodies against UGT1A1 and UGT1A6.*** We designed two peptides which were used to generate isoform-specific antibodies against human UGT1A1 and UGT1A6 in sheep. For UGT1A1, we chose a short region in the N-terminal variable region which was synthesized as a MAP, and for UGT1A6 a 100-amino acid peptide was expressed in *E. coli* as a fusion protein with a 6xHis tag and purified by affinity chromatography. We tested the specificity of the antibodies produced using immunoblot analysis against recombinant human UGTs as shown in Figure 1.

To validate the immunoquantification method, we chose to quantify the expression of UGTs 1A1 and 1A6 in microsomes prepared from 29 individual human liver samples. For each liver sample and antibody, conditions were optimized in order that the bands were within the linear range of the standard curves generated using the S•tag™-labelled UGTs. A representative immunoblot for each antibody is shown in Figure 2.

***UGT1A1 and UGT1A6 Expression in Human Tissues.*** There was considerable variation in the expression of UGTs 1A1 and 1A6 in the various liver samples (Table 2 and Figure 3),

DMD #41699

although expression could be detected in each sample. The quantity of UGT1A1 found in these liver samples varied from 0.04  $\mu\text{g/g}$  tissue to 1.14  $\mu\text{g/g}$  tissue, a 28-fold variation, with a mean of 0.34  $\mu\text{g/g}$  tissue. There is a common variable number tandem repeat (VNTR) polymorphism in the TATA box of the *UGT1A1* gene (*UGT1A1*\*28) that is associated with the reduced expression of UGT1A1, reduced glucuronidation of bilirubin and mild unconjugated hyperbilirubinemia seen in Gilbert's syndrome (Bosma et al., 1995; Monaghan et al., 1996), which presumably accounts for a significant proportion of the variability seen here. UGT1A1 expression was not detected in microsomes prepared from the human kidney samples.

With the expression of UGT1A6 we again observed extensive variation in levels within liver microsomal samples, ranging from 0.07 to 1.42  $\mu\text{g/g}$  tissue (one sample had no detectable expression) with a mean of 1.18 – a 20 fold variation in the amount of UGT1A6. Expression of UGT1A6 was observed in microsomal samples prepared from human kidney, although this was approximately 3-fold lower than in the liver.

To confirm that expression levels measured with this assay were representative of enzyme function, we carried out assays for UGT activity using the hepatic microsomal samples. We used bilirubin as a probe substrate for UGT1A1 and 1-naphthol (at a concentration of 500 $\mu\text{M}$ ) as a probe substrate for UGT1A6, and performed correlation analysis between the protein expression and enzyme activity datasets. The results are presented in Table 1 and Figure 4. There was a strong correlation between UGT1A1 expression and bilirubin UGT activity in the human liver microsomes (Pearson's  $r = 0.82$ ,  $p < 0.0001$ ) and a somewhat lower, but still significant, degree of correlation between UGT1A6 protein expression and the glucuronidation of 500 $\mu\text{M}$  1-naphthol (Pearson's  $r = 0.61$ ,  $p < 0.005$ ).

## Discussion

The ability to reliably predict the contribution of individual drug metabolising enzymes to the *in vivo* fate of xenobiotics would be of considerable value and benefit to chemical and pharmaceutical industries, as well as the regulatory bodies that oversee them. For many conjugating enzyme families, including the UGTs, we still do not have a good idea of the relative contributions of the major isoforms in this regard, not least because of the lack of reliable methods for quantifying protein expression. This is mainly due to the fact that UGTs are integral membrane proteins that are difficult to express and purify from e.g. *E. coli*, which means it is extremely challenging to produce accurately and absolutely quantified protein for the generation of standard curves for immunoquantification approaches. Another drawback is the lack of high quality, isoform specific antibodies for immunoquantification. Here we have described a novel approach to the immunoquantification of UGT protein expression in human tissues. The method, which uses expressed and readily quantified UGT/S•tag<sup>TM</sup> fusion proteins to generate standard curves, was validated using two important human UGTs, UGT1A1 and UGT1A6, against which we also produced new isoform-specific antibodies.

The UGT/S•tag<sup>TM</sup> fusion proteins with the pelB leader sequence were expressed in *E. coli* and were found in the membrane fraction upon ultracentrifugation, indicating that they had been correctly targeted to the periplasm. When combined with an excess of S-protein, they formed an active ribonuclease activity that was readily quantified spectrophotometrically using the S•tag rapid assay. These proteins were then successfully used to develop standard curves for quantification of UGT protein levels by immunoblotting.

Because of its role in the detoxification and elimination of bilirubin, UGT1A1 is considered one of the most important (if not the most important) UGTs, and is expressed primarily in the liver and to a lesser extent in the gastrointestinal tract (Ohno and Nakajin,

2009). A proportion of the variability of UGT1A1 expression in the population is due to the common VNTR polymorphism in the *UGT1A1* gene promoter associated with Gilbert's syndrome (Bosma et al., 1995; Monaghan et al., 1996) (or other mutations in different populations, e.g. (Koiwai et al., 1995)), however measurements of mRNA levels show very wide interindividual variation for all UGTs (e.g. (Izukawa et al., 2009)) suggesting that other environmental, genetic and/or epigenetic factors impact significantly on expression levels. UGT1A6 is responsible for the glucuronidation of many xenobiotics, and a number of drugs are known to be substrates for this enzyme, including paracetamol (Court et al., 2001), valproate (Ethell et al., 2003) and deferiprone (Benoit-Biancamano et al., 2009). UGT1A6 also metabolises a number of important endogenous substrates such as serotonin (Krishnaswamy et al., 2004). As with UGT1A1, previously reported mRNA-based quantification and other studies have demonstrated very widespread variability in expression of UGT1A6 in liver (Krishnaswamy et al., 2005; Izukawa et al., 2009). One study failed to find an association between a variety of polymorphisms in *UGT1A6* and expression levels of the protein, suggesting instead that environmental factors such as alcohol consumption may play an important role in interindividual variability (Krishnaswamy et al., 2005), however other studies have suggested an impact of the variant UGT1A6\*2 on salicylic acid pharmacokinetics (Chen et al., 2007). We noticed that there is a reasonable similarity in the expression profiles of UGT1A1 and UGT1A6 in this set of liver samples. We know from Figure 1 that there is no evidence for any significant cross-reactivity of these two new antibodies, so the possibility remains that there is a degree of co-ordinate regulation of these two UGTs.

The method reported here for producing recombinant protein standards as S•tag™ fusion proteins is readily applicable to potentially any recombinant protein expression system, particularly where proteins such as membrane proteins are difficult to purify, and we

DMD #41699

have recently successfully applied this method to the quantification of the ABC efflux transporters BCRP, MRP2 and P-glycoprotein in human tissues (Tucker TGHA *et al.*, manuscript submitted). The main drawbacks associated with this method include the requirement for the generation and characterisation of high quality, isoform-specific antibodies (which are often not available commercially) and the somewhat labour intensive processes of gel electrophoresis and immunoblotting. However, unlike quantitative proteomic approaches such as AQUA, it does not rely on highly specialised and expensive mass spectrometry equipment or expensive labelled peptides (Gerber *et al.*, 2003; Kettenbach *et al.*, 2011); such a method has been described for the quantification of UGT1A1 and UGT1A6 (Fallon *et al.*, 2008). Another problem with proteomic approaches is the potential for incomplete digestion of target proteins, particularly in complex samples such as microsomal membrane preparations where UGTs are located. A very recent publication (Sakamoto *et al.*, 2011) details a mass spectrometry method for the quantification of a number of transporters and drug metabolising enzymes (including UGT1A1 and UGT2b7), however only data on the reliability and reproducibility of the method are presented, not the absolute expression values for the proteins concerned.

In summary, we have devised a novel approach to the immunoquantification of UGT expression in human tissue samples. The method is straightforward to establish and is broadly applicable, and provides the opportunity to determine the relative contributions of different UGT isoforms to the metabolism of drugs and other xenobiotics.

DMD #41699

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## Authorship Contributions

*Participated in research design:* Milne, Burchell, Coughtrie

*Conducted experiments:* Milne

*Contributed new reagents or analytical tools:* Milne

*Performed data analysis:* Milne, Coughtrie

*Wrote or contributed to the writing of the manuscript:* Milne, Burchell, Coughtrie



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DMD #41699

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DMD #41699

## **Footnote**

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## Figure Legends

**Figure 1** *Specificities of anti-UGT antibodies.* Recombinant UGT isoforms were expressed in V79 cells or in FT293 cells as indicated and cell lysates were prepared from frozen cell pellets resuspended in 200 $\mu$ l SHM buffer (see Materials & Methods) and sonicated 3 x 5 seconds. Lysates along with samples of human liver and kidney microsomes (30 $\mu$ g of each), were resolved on SDS-PAGE gels and transferred to PVDF membranes which were probed with each antibody as described in Materials and Methods. HLM, human liver microsomes; HKM, human kidney microsomes.

**Figure 2.** *Representative quantitative immunoblots with anti-UGT antibodies.* Microsomes prepared from a number of liver samples were resolved on SDS-PAGE gels, along with the appropriate recombinant UGT/S•tag™ fusion protein (values in ng). Following transfer to PVDF membranes, blots were immunostained with either ant-UGT1A1 (A) or anti-UGT1A6 (B).

**Figure 3** *Expression of UGTs 1A1 and 1A6 in human liver microsomes.* Microsomes prepared from 29 human liver samples were subjected to immunoquantification for the expression of UGT1A1 (A) and UGT1A6 (B). Data are shown as mean  $\pm$  SD for between 3 and 7 separate determinations.

**Figure 4** *Correlation between UGT protein expression and enzyme activity.* Microsomal fractions were subjected to immunoquantification as described and enzyme activity measurements with probe substrates bilirubin (A – for UGT1A1) and 1-naphthol (B – for

DMD #41699

UGT1A6). Correlation (linear regression) analysis was carried out using Prism 4 (GraphPad Software, La Jolla, CA, USA).

**Table 1**

*Quantification of the expression and enzyme activity of UGTs 1A1 and 1A6 in human liver and kidney microsomes*

<b>Tissue</b>	<b>UGT1A1 Expression (<math>\mu\text{g/g tissue}</math>)</b>	<b>UGT1A1 Activity (<math>\text{nmol/min/g tissue}</math>)</b>	<b>UGT1A6 Expression (<math>\mu\text{g/g tissue}</math>)</b>	<b>UGT1A6 Activity (<math>\text{nmol/min/g tissue}</math>)</b>
Liver ( $n = 29$ )	$0.36 \pm 0.26$	$4.07 \pm 3.06$	$1.18 \pm 1.07$	$4.89 \pm 4.32$
<i>Range</i>	<i>0.04 – 1.14</i>	<i>0.59 – 14.1</i>	<i>0.087 – 4.59</i>	<i>BLQ – 13.34</i>
Kidney ( $n = 4$ )	BLQ	BLQ	$0.44 \pm 0.07$	$3.28 \pm 0.31$
<i>Range</i>			<i>0.35 – 0.51</i>	<i>2.82 – 3.45</i>

Data are means  $\pm$  SD for quantification in  $n$  tissue samples. Measurements were carried out on between 3 and 7 replicates and the range of values obtained is given in italics.

BLQ, below limit of quantification

Figure





Figure 2

A.



B.



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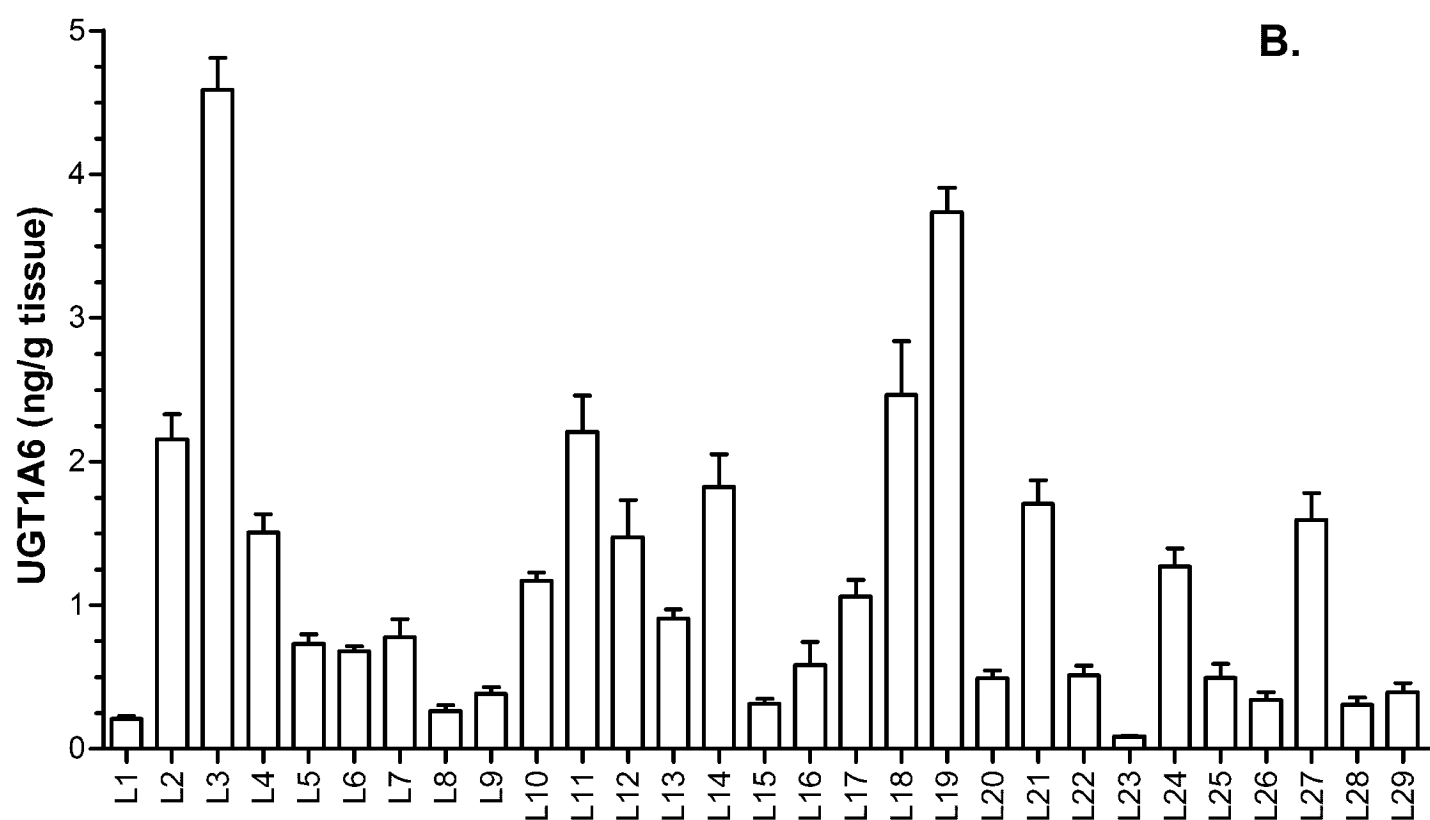
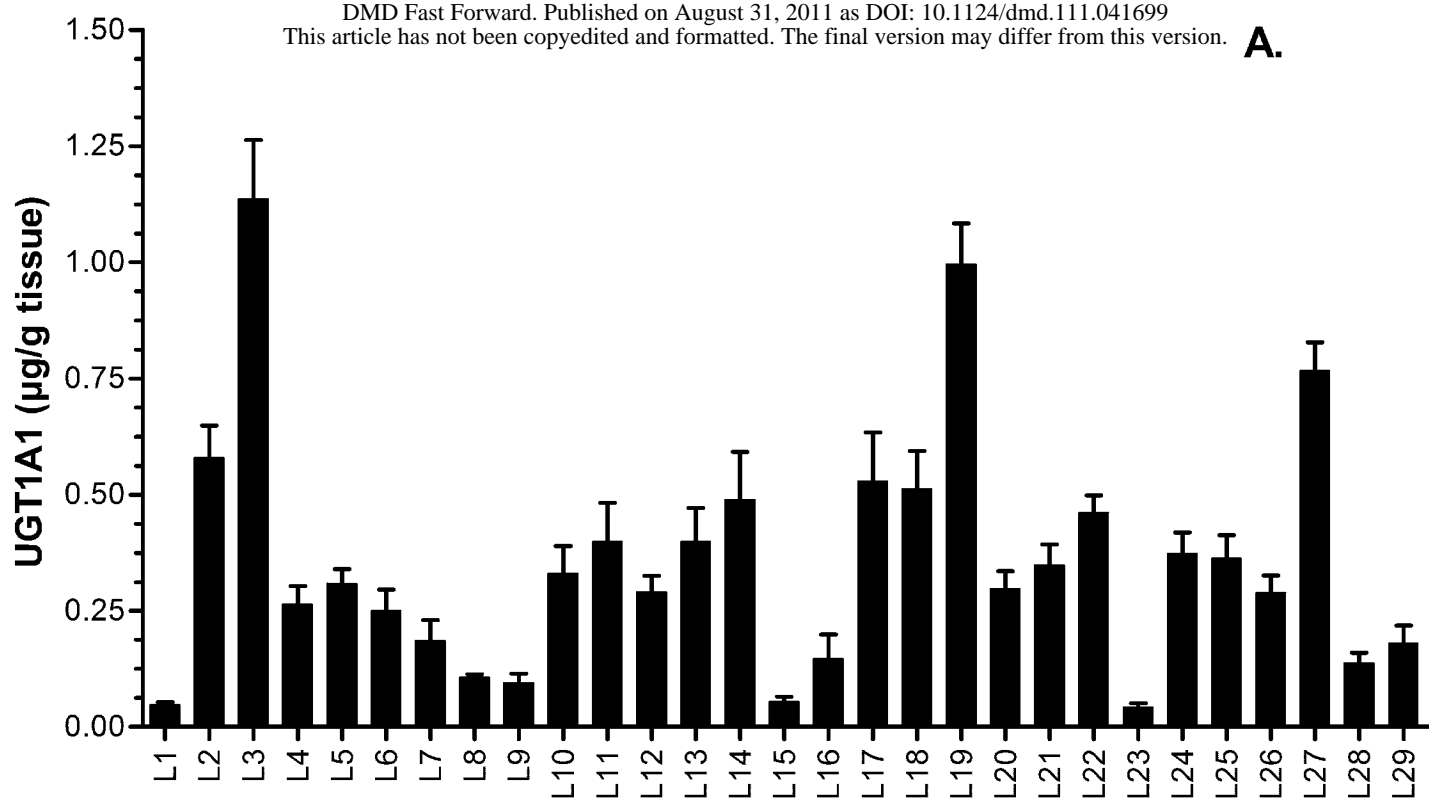
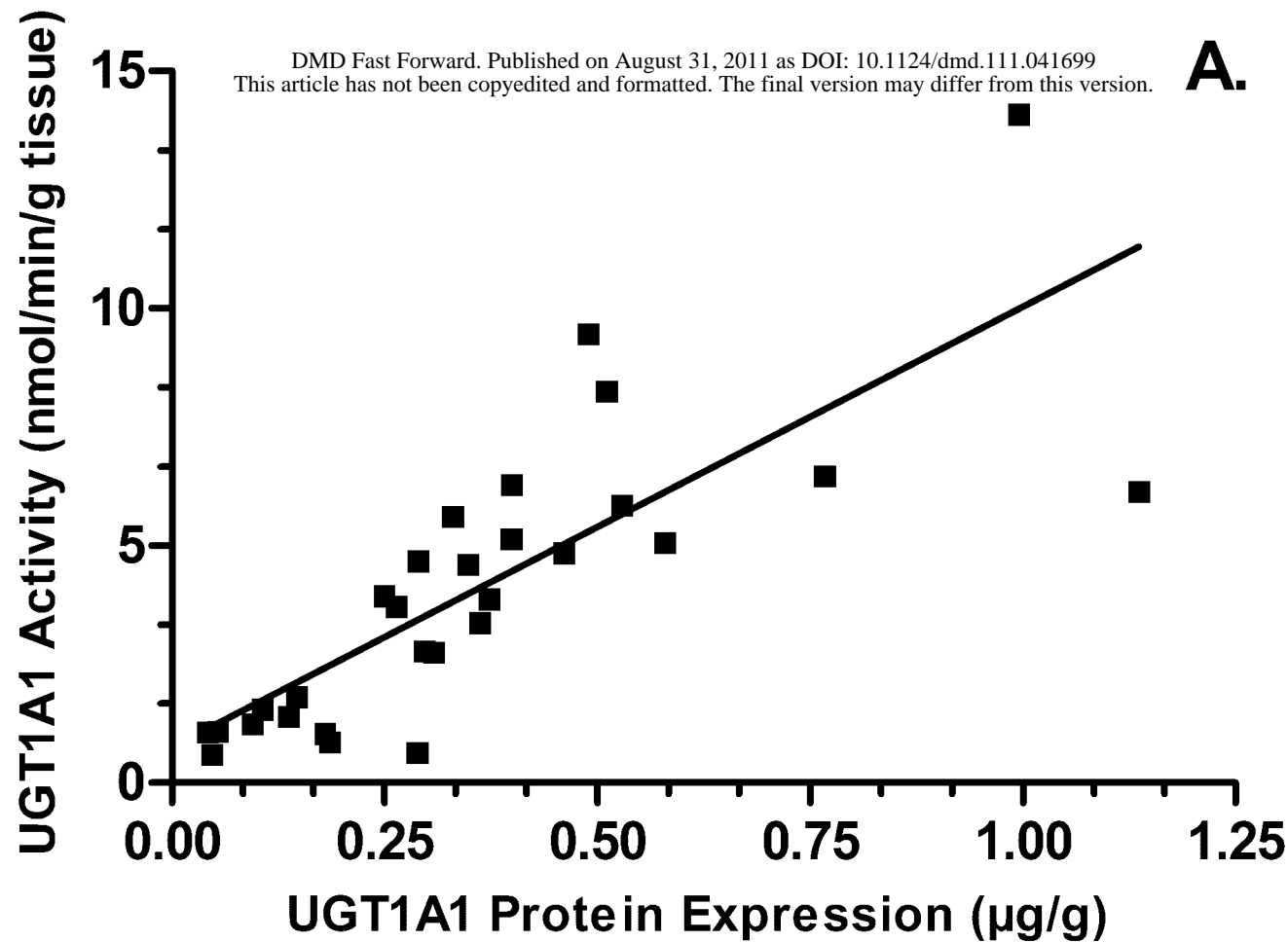


Figure 4

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**A.**



**B.**

