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Protein expression of various hepatic uridine 5′-diphosphate glucuronosyltransferase (UGT) enzymes and their inter-correlations: a meta-analysis

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ABSTRACT: Avoiding cytochrome P450 (CYP) related drug interactions in the development of new drug candidates means that glucuronidation by uridine 5′-diphosphate glucuronosyltransferase (UGT) enzymes is expected to become a more prominent pathway in the metabolism of new drug candidates designed by pharmaceutical companies. Therefore, determining the abundance and activity of these enzymes is of value in the process of scaling *in vitro* data to *in vivo* metabolic parameters. Many of the studies involving the measurement of UGTs were conducted with too few samples, which did not provide a good indication of population values and the level of variability. Meta-analysis is used in the current study to combine all reported values (eight studies that used LC-MS isotope-labelled standard targeted quantitative methods), detect inconsistencies between the various datasets and describe correlations of expression between the quantified UGT enzymes. Some heterogeneity was observed between studies, especially in the UGT1A4, 2B7 and 2B10 datasets. However, in the absence of information on the inter-laboratory consistency of assays, it is difficult to assign these differences to the heterogeneity of the samples. Large inter-individual variability was observed in the collated data across this family of enzymes. Positive correlations between the expression levels of certain UGT enzymes were found in the collated data. These included the pairs: UGT1A4/2B4 ($r_s = 0.71$, $p < 0.0001$, $n = 82$), UGT2B4/2B15 ($r_s = 0.63$, $p < 0.0001$, $n = 83$), UGT2B7/2B15 ($r_s = 0.81$, $p < 0.0001$, $n = 99$). These correlations can be explained by common regulatory mechanisms involved in the expression of these proteins. Copyright © 2014 John Wiley & Sons, Ltd.

Key words: uridine 5′-diphosphate glucuronosyltransferase; abundance; inter-individual variability; correlation of expression; *in vitro–in vivo* extrapolation

Introduction

Uridine 5′-diphosphate glucuronosyltransferase (UGT) enzymes are a family of transferases responsible for glucuronidation, a phase II conjugative reaction. Although these enzymes are mainly expressed in the liver, some UGT isoforms are expressed extra-hepatically in organs such as the intestine and the kidneys [1]. The survey by Wienkers and Heath [2] indicated the involvement of this family of enzymes in the direct metabolic clearance of more than 15% of the most prescribed therapeutic drugs in the USA in 2002. In addition, many therapeutic drugs are metabolized first by cytochrome P450 (CYP) enzymes and subsequently conjugated by UGTs. However, over the past decade, many pharmaceutical companies have started actively adopting strategies to avoid cytochrome P450 related metabolism for their candidate drugs,

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which has the consequential impact of an increased role of other metabolic pathways, especially those mediated by UGTs, in directly metabolizing new candidate drugs [3]. Hence, the abundance and activity data of these enzymes are of increased value as these data can be used for extrapolation from *in vitro* data to *in vivo* pharmacokinetic parameters (IVIVE) to describe the fate of new candidate drugs and clinically relevant drug–drug interactions [4,5]. These data can also be used for simulation experiments and in the process of producing virtual populations for the pharmacological and toxicological assessment of drugs [6]. Correlations of expression between drug metabolizing enzymes have been reported in the literature [7], and these correlations, if established, can assist in producing more reliable virtual populations.

Traditionally, quantitative methods, such as Western blotting and enzyme-linked immunosorbent assays (ELISA), were used to estimate relative quantities of UGT enzymes. These methods are extremely variable and can provide imprecise measurements of enzyme abundances mainly due to the extensive sequence homology between different UGT isoforms and the cross-reactivity of antibodies used for the quantification process. LC-MS based methods that use heavy-labelled peptide internal standards (AQUA/QconCAT) and highly sensitive and selective mass spectrometry platforms have made absolute quantification of UGT enzymes possible, providing abundance values expressed in units of picomoles per milligram microsomal protein. Recently, several studies that employed LC-MS to quantify UGT abundances have been published and the number of such studies is expected to increase.

Meta-analysis is a process of combining different studies in order to obtain an overall figure for a measured variable, detect the degree of heterogeneity between the individual studies, and to describe inconsistency and its possible sources [8]. With the growing number of studies that attempted to quantify pharmacokinetically relevant enzymes, especially UGT enzymes, there is a pressing need for a meta-analysis of these data to identify weighted mean enzyme abundances, the degree of variability in these abundance data and the degree of correlation between the expression levels of these enzymes at the protein level. This study represents an attempt to use the available literature on the abundances of UGT enzymes for a meta-analysis of the reported data, which were obtained experimentally based on LC-MS labelled standard targeted quantitative methods. The results of this study will help various groups working on physiologically based pharmacokinetic (PBPK) models to incorporate more representative values for the system parameters in relation to the abundance of UGT enzymes.

Methods

Collection of data

Two electronic databases, Medline (http://www.nlm.nih.gov/bsd/pmresources.html) and Web of Knowledge (http://wok.mimas.ac.uk/) (between the years 1980 and 2014), were searched for relevant literature on the abundance of enzymes using appropriate keywords (glucuronosyltransferase abundance; UGT abundance; correlation of expression). Other keywords were also used, e.g. quantity/ concentration/ content, quantification/ measurement, instead of abundance to widen the search scope. Resources cited by the collected papers were also inspected to locate further literature that could be used. Only human liver data from Caucasian adults were included and the sources of data were identified to ensure that none of the data used were duplicated in the analysis. Where data were not quoted and graphs contained data points, GetData Graph Digitizer version 2.25 was used to obtain abundance values.

Calculation of weighted means and coefficients of variation

For each enzyme, abundance values were tested for heterogeneity and normality of the distribution. The heterogeneity test [9–11] determines whether the eight studies are consistent with one another, whereas the normality test determines whether the combined data are normally distributed. The normality test was performed according to the method of Kolmogorov and Smirnov.

The data from the individual studies were combined. The weighted means and the weighted coefficients of variation of the abundances of the different enzymes from the collated data were calculated using Equations (1) and (2), respectively

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$$W\overline{X} = \sum_{j=1}^{J} n_j.\overline{X}_j \Big/ \sum_{j=1}^{J} n_j \quad (1)$$

$$WCV = \sum_{j=1}^{J} n_j.CV_j \Big/ \sum_{j=1}^{J} n_j \quad (2)$$

where $W\overline{X}$ and WCV represent the weighted mean and weighted coefficient of variation, respectively. Subscript 'j' indicates the study, n_j the number of samples in the study, and $\overline{X}_j$ the mean abundance of a particular enzyme reported in the study.

Assessment of heterogeneity between studies

To assess homogeneity between the means and coefficients of variation of individual studies and the overall mean and variability in the collated data, Equations (3–5) were used.

$$VarW\overline{X} = \sum_{j=1}^{J} w_j.\overline{X}_j \Big/ \sum_{j=1}^{J} w_j \quad (3)$$

$$w_j = 1 \Big/ (sd_j)^2 \quad (4)$$

$$Q = \sum_{j=1}^{J} \left(w_j.\left(\overline{X}_j - VarW\overline{X}\right)^2 \right) \quad (5)$$

where w_j is the weight of study j expressed as the variance and calculated using Equation (4) (the inverse of standard deviation, sd_j, squared), $VarW\overline{X}$ is the variance in the weighted mean of the data from all the studies. Q is the coefficient of heterogeneity (Eq. (5)) of the collated data (Cochran's Q test [9]) expressed as the collective weighted squared differences between the mean of each study and the variance in the weighted mean. A higher value of Q indicates greater heterogeneity.

The degree of heterogeneity can be assessed using the I^2 index, Equation (6), proposed by Higgins and Thompson [10,11]. This provides a percentage of overall heterogeneity that can be interpreted as proposed by Higgins *et al.* [11] as follows: around 0%, no heterogeneity; around 25%, low heterogeneity; around 50%, moderate heterogeneity; and around 75%, high heterogeneity.

$$I^2 = 100 \times \frac{Q - (k-1)}{Q} \quad (6)$$

where I^2 is the index of heterogeneity, Q is Cochran's heterogeneity coefficient, and $(k - 1)$ is the number of degrees of freedom defined as the number of studies, k, minus one. Where I^2 is negative, it is set to zero. The probability, p, of the test can be quoted by comparing the Q value to a chi-square distribution with the same number of degrees of freedom [11].

Assessing correlations between the abundances of drug-metabolizing enzymes

The normality of the data was assessed using the Kolmogorov Smirnov method. The non-parametric Spearman rank test [12] was used to assess correlations of the expression levels of the enzymes. Before correlation calculations, collated data were normalized using mean values of a reference study (Eq. (7)), here weighted mean data obtained in this report were used (Table 1). The calculations of the correlation coefficients, r_s, and probabilities, p, were carried out according to Equation (8) and the distribution of the term described in Equation (9).

$$x_{j,\ \text{normalized}} = \frac{x}{\overline{x}_j} \cdot \overline{x}_{\text{reference}} \quad (7)$$

where $x_{j,\text{normalized}}$ is the normalized abundance value of the abundance measurement x in study j, $\overline{x}_j$ is the mean abundance value of study j, and $\overline{x}_{\text{reference}}$ is the mean abundance of the same enzyme in the reference study used for the normalization process.

$$r_s = 1 - \frac{6\sum d^2}{n^3 - n} \quad (8)$$

$$t = r_s \sqrt{\frac{n-2}{1 - r_s^2}} \quad (9)$$

where d is difference between ranks of the two sets of abundance data for which the correlation is being tested, and n is the number of pairs of measurements used in the correlation test. The

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Table 1. The weighted means, coefficients of variation (CV), ranges and heterogeneity analysis of the analysed hepatic UGT enzyme abundance data

Enzyme	Weighted mean (pmol/mg)	CV (%)	Range[a] (pmol/mg)	Number of livers	Q	I^2 (%)	Heterogeneity	Studies
UGT1A1	41	60	4–138	154	1.73	0	None	[13–20]
UGT1A3	31.4	75	2–488	124	2.01	0	None	[13,15,18–20]
UGT1A4	55.4	37	4–106	107	18.13	83	High	[15,18–20]
UGT1A6	40	51	1–285	134	8.64	42	Medium	[13–15,18–20]
UGT1A9	32	39	9–123	125	3.03	0	None	[13,15,18–20]
UGT2B4	57	37	18–136	99	2.27	12	Low	[18–20]
UGT2B7	87	36	5–178	132	24.43	84	High	[13,17–20]
UGT2B10	20	44	1–15	76	10.17	90	High	[18,20]
UGT2B15	52	39	13–130	116	3.75	20	Low	[13,18–20]
UGT2B17	18	126	0–50	76	1.39	28	Low	[18,20]

Q, Cochran's heterogeneity coefficient; I^2, Higgins and Thompson's heterogeneity index.
[a]Ranges are included where available.

probability is calculated using the t-value on Student's t-distribution and the appropriate number of degrees of freedom ($n - 2$). The ranks of the abundance data are used for the correlation test instead of their actual values [12]. A Bonferroni correction was used to define the p-value for statistical significance for the number of tests applied on each enzyme (nine tests), which provides a corrected probability value of $p = 0.0055$.

Results

Studies used for the meta-analysis

The number of studies used in this meta-analysis is eight out of 24 studies collected using the search strategy [13–20]. There were several criteria used to choose the studies to be the basis of the meta-analysis. Studies were excluded where the samples were not hepatic microsomes (six studies, which were based on cell cultures or recombinantly expressed enzymes), and where enzymatic activity or mRNA levels of expression were the only quantification data (six studies). Additionally, studies quoting abundance data in arbitrary, relative or non-standard units were also excluded (five studies). Finally, one study was excluded because a label-free LC-MSMS method was used for the quantification process. Furthermore, two studies were excluded for more than one of the above reasons. Quantification in the eight remaining studies used for the meta-analysis was carried out using LC-MS labelled-isotope standard targeted quantitative methods.

Weighted mean UGT abundance levels and overall variability

Results of heterogeneity testing are shown in Table 1. Most of the data from the individual studies were homogeneous except for those for UGT1A4, 2B7 and 2B10, where the number of studies is small, mostly indicating little heterogeneity between these studies. Weighted means, coefficients of variation and ranges of the collected data for the different UGT enzymes are also presented in Table 1. Figure 1 shows the results of the meta-analysis.

Correlation of expression between UGT enzymes

Where two or more enzymes were quantified in the same samples, the collated data were used for correlation analysis using the non-parametric Spearman rank test as the data did not exhibit normal distribution. An additional study [21] was used for the correlation of UGT1A1 and 1A6 although the authors did not quote the abundances using the conventional units: pmol per mg microsomal protein. In this study immunoquantification was the method used for quantification. Table 2 shows the results of correlation testing. Figure 2 shows examples of statistically significant weak and strong correlations of the collated data.

Discussion

Glucuronidation constitutes the main reaction in phase II metabolism, an integral part of the bioconversion process of drugs and xenobiotics,

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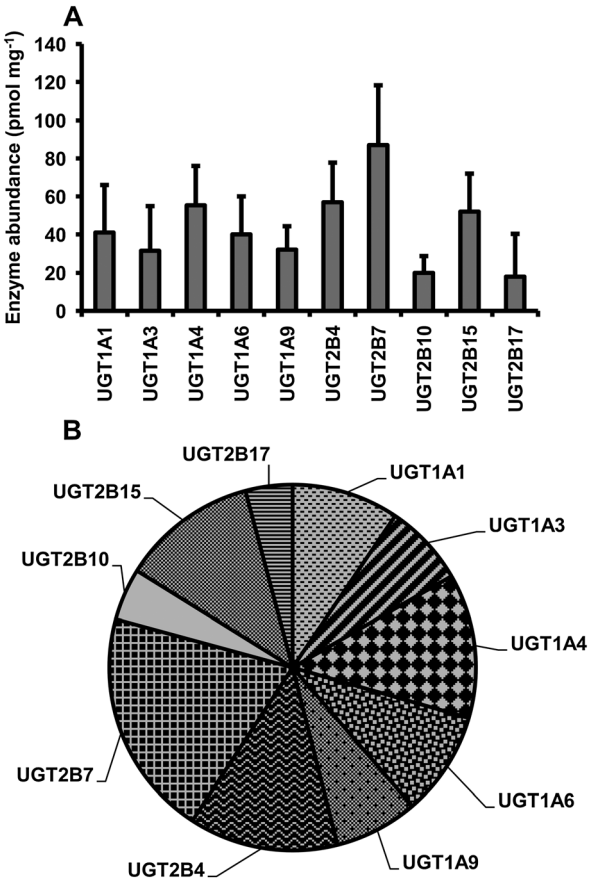

Figure 1. Bar graph (A) and pie chart (B) of weighted means of hepatic UGT abundances. Error bars represent standard deviation values. Abundances are expressed in units of picomoles per milligram microsomal protein

facilitating renal excretion of therapeutic and toxic compounds. This reaction is mediated by UDP-glucuronosyltransferase (UGT) enzymes which are ubiquitously expressed in different organs, especially in the liver, the main organ involved in metabolic clearance of xenobiotics [1,22]. In addition to the liver, some UGT enzymes, such as UGT1A7, 1A8 and 1A10, are expressed exclusively in the gastrointestinal system [1], indicating the relevance of these organs in drug metabolism involving UGT mediated pathways. UGT enzymes are likely to become more important in the metabolic clearance of new drug candidates as pharmaceutical companies seem to be actively avoiding new therapeutic agents that can be metabolized by cytochrome P450 enzymes in order to reduce the impact of drug–drug interactions [3].

The abundances and activities of enzymes involved in metabolism are used in drug development as scaling factors in the process of *in vitro–in vivo* extrapolation (IVIVE), and in simulations of drug trials [6]. In order to obtain meaningful scaling factors and realistic simulations, abundances of enzymes have to be obtained for a large population of patients, which is not always possible, and correlations of expression have to be taken into account [19]; both of these can be achieved by collating all the abundance data published for these enzymes and deriving a weighted mean for each of them using meta-analysis. The only meta-analysis for the expression data of different hepatic UGT enzymes, to the best of our knowledge, was based on mRNA data from only three studies [22], and this may be of limited usefulness since mRNA data do not always correlate with enzyme abundance or activity levels [18,23,24]. Hence, there was clearly a need for a meta-analysis of the published data on the abundance of UGT proteins, especially in the liver. Furthermore, there was a requirement for an understanding of correlations between the expression patterns of the different UGT enzymes, preferably in a large number of samples. The present work successfully addresses both these gaps in knowledge.

The results presented in this study provide weighted mean abundances, coefficients of variation and abundance ranges for the main hepatic UGT enzymes relevant to drug metabolism. The number of samples analysed for these enzymes (76–154 livers per enzyme) provides confidence in these abundances (Table 1 and Figure 1). The method used to obtain the data in the analysed studies was based on LC-MS measurement of UGT enzyme abundances in the microsomal fraction extracted from human liver tissue samples. LC-MS based quantification offers an improvement over immunoquantification both in terms of accuracy and reproducibility, which provides more confidence in the reliability of the meta-analysis. The enzyme abundance distribution pie presented in this study is the first to be published for hepatic UGT enzymes. We note that, of the eight studies considered here, two were published during the first three months of 2014, suggesting that there is current interest in the field and that the hepatic UGT pie may be refined quite frequently in the near future.

Correlations analysis can be carried out when simultaneous quantification of different enzymes

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Table 2. Correlation matrix of the expression of hepatic UGT enzymes in Caucasian subjects. Statistically non-significant (-), statistically significant and strong ([a], $0.5 \le r_s < 0.6$), and statistically significant and very strong ([b], $r_s \ge 0.6$) correlations are shown. r_s, the Spearman correlation coefficient; p, probability value; n, number of subjects

	UGT1A1	UGT1A3	UGT1A4	UGT1A6	UGT1A9	UGT2B4	UGT2B7	UGT2B10	UGT2B15	UGT2B17
UGT1A1	1	-	-	$r_s = 0.40$ $p < 0.0001$ $n = 137$	$r_s = 0.30$ $p = 0.003$ $n = 99$	$r_s = 0.42$ $p < 0.0001$ $n = 83$	-	-	$r_s = 0.36$ $p = 0.0003$ $n = 99$	-
UGT1A3		1	$r_s = 0.41$ $p = 0.0001$ $n = 82$	-	-	-	-	-	-	-
UGT1A4			1	$r_s = 0.45$ $p < 0.0001$ $n = 82$	$r_s = 0.53$[a] $p < 0.0001$[a] $n = 82$[a]	$r_s = 0.71$[b] $p < 0.0001$[b] $n = 82$[b]	$r_s = 0.49$[a] $p < 0.0001$[a] $n = 82$[a]	-	$r_s = 0.41$ $p = 0.0001$ $n = 82$	-
UGT1A6				1	$r_s = 0.51$[a] $p < 0.0001$[a] $n = 99$[a]	$r_s = 0.46$ $p < 0.0001$ $n = 82$	-	-	$r_s = 0.37$ $p = 0.0002$ $n = 98$	-
UGT1A9					1	$r_s = 0.52$[a] $p < 0.0001$[a] $n = 83$[a]	$r_s = 0.47$ $p < 0.0001$ $n = 99$	-	$r_s = 0.48$ $p < 0.0001$ $n = 99$	-
UGT2B4						1	$r_s = 0.57$[a] $p < 0.0001$[a] $n = 83$[a]	-	$r_s = 0.63$[b] $p < 0.0001$[b] $n = 83$[b]	-
UGT2B7							1	$r_s = 0.42$ $p = 0.0008$ $n = 60$	$r_s = 0.81$[b] $p < 0.0001$[b] $n = 99$[b]	-
UGT2B10								1	-	-
UGT2B15									1	-
UGT2B17										1

and transporters is carried out in the same tissue samples [13,19,25]. Correlation of UGT expression was assessed and some relationships were found, with the strongest correlation between the pairs: UGT1A4/UGT2B4, UGT2B4/UGT2B15, UGT2B7/UGT2B15 with correlation coefficients (r_s) exceeding 0.6 ($p < 0.0001$) (Table 2). These correlations highlight the common transcriptional regulation previously proposed between different drug metabolizing enzymes [19,26].

The findings in this study are in line with correlation analysis of UGT mRNA levels in 25 human livers reported by Izukawa *et al.* [23], which indicated very strong correlations between different UGT expression levels including the pairs UGT1A4/UGT1A9 ($r_s = 0.79$), UGT1A6/UGT1A9 ($r_s = 0.84$) and UGT2B4/UGT2B15 ($r_s = 0.74$), as well as correlations between UGT2B4 and UGT1A4 ($r_s = 0.80$) and between UGT2B4 and UGT1A9 ($r_s = 0.80$). The correlations between protein levels reported in the present study may be relatively weaker due to the fact that the data used for correlation analysis were collated from different studies and that the correlations tested are influenced by transcription and translation, which are independently regulated processes.

Correlations of expression of different UGT isoforms have been attributed to liver-enriched transcriptional factors such as hepatocyte nuclear factor (HNF)1α and 4α, which bind and activate UGT gene promotors leading to co-induction, and therefore correlation of expression of UGT1 and UGT2 enzymes [23,26]. Examples of such an effect include the reported correlation at the mRNA level between UGT1A9 and 2B7 due to the involvement of HNF1α [27] and that between the mRNA levels of UGT1A6 and 1A9 attributed to HNF1α and HNF4α [28]. Other modulating factors, such as hormones, bile acids and xenobiotics can contribute to these correlations by interacting with receptors, which include the pregnane X receptor (PXR), the constitutive androstane receptor (CAR), the peroxisome proliferator-activated receptor (PPAR)α and the aryl hydrocarbon receptor (AhR) [29,30]. In particular, it has been reported that the PPARα receptor, which is activated by ligands such as eicosanoids and unsaturated fatty acids [26], has various target genes in the UGT family including both UGT1A and 2B enzymes [31–33]. Examples of modulated enzymes include UGT1A4 [31] and UGT2B4 [33] shown to correlate in the present study.

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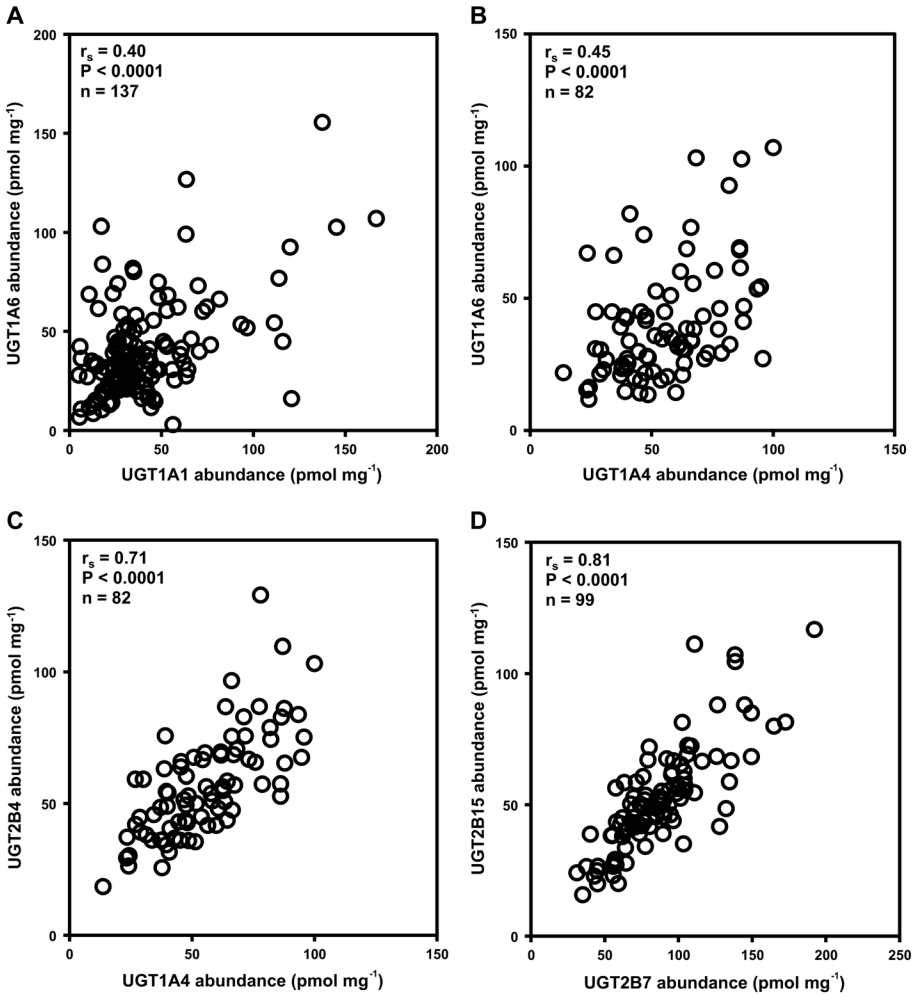

Figure 2. Plots of example correlations of the expression of hepatic UGT enzymes. Correlations between UGT1A6 and UGT1A1 (A) and UGT1A6 and UGT1A4 (B) are weak ($r_s < 0.5$), whereas correlations between UGT1A4 and UGT2B4 (C) and UGT2B7 and UGT2B15 (D) are very strong ($r_s \geq 0.6$). Plots show data normalized based on the weighted means as indicated in the Methods

It is important, however, to note that the unit used for abundance values (picomoles per gram microsomal protein) is in fact a *relative* unit, comparing the quantity of enzyme to total protein in the sub-cellular fraction. Some background correlation can therefore often be observed. While a statistical correction, a Bonferroni correction, for example, can be used to tackle this statistical artefact (as is the case in this study), the problem still persists if an unrelated enzyme or protein of high abundance is down-regulated to a sufficient extent, which has the effect of increasing the abundance of different UGT enzymes relative to the total microsomal protein. This can lead to an apparent up-regulation of all UGT enzymes and, as a result, the detection of some level of correlation based on the statistical test. We suggest, therefore, that this inaccuracy may be resolved by using a more appropriate abundance/concentration unit, such as 'copy number per cell' or 'micrograms/picomoles per gram tissue'. Indeed, the latter unit was used in a recent publication [21].

After application of the Bonferroni correction to the data considered here, it can be seen that genuine correlations are stronger within the 2B subfamily than within the 1A subfamily. The 2B proteins are expressed from independent genes, whereas the 1A proteins are produced from a single gene locus

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by alternate splicing of the mRNA product [22]. Thus, any induction of 2B enzymes is likely to lead to positive correlations, whereas the 1A case is more complicated. An increase in mRNA encoding 1A UGTs would be expected to yield a positive correlation; however, an mRNA transcript is spliced to yield a single protein, and any change in the regulation of splicing will deliver a negative correlation between the abundances of the final gene products. The correlation that we are testing is a combination of these two regulatory factors; it seems that neither one of these is dominant.

In conclusion, the quantification of inter-individual variability and correlations of expression of drug-metabolizing enzymes including cytochrome P450 [34] and UGT enzymes is necessary to improve IVIVE based drug development and computational physiologically based pharmacokinetic (PBPK) models designed for *in silico* ADME (absorption, distribution, metabolism and excretion) prediction. The accuracy of extrapolation and modelling has implications on the prediction of the safety and efficacy of new drug candidates in patient populations. As an extension of this work, we look forward to quantifying the abundance and activity of different polymorphs of UGT enzymes where polymorphism has clinical implications in terms of disease (e.g. Gilbert's syndrome) and drug metabolism [35]. In addition, extending the correlation analysis to other families and between different families of enzymes as well as transporters can provide useful insights into the reported cross-talk between different proteins involved in drug metabolism and disposition [19].

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Conflict of Interest

The authors have declared that there is no conflict of interest.

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