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Placental profiling of UGT1A enzyme expression and activity and interactions with preeclampsia at term

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Abstract

Placental UDP-glucuronosyltransferase (UGT) enzymes have critical roles in hormone, nutrient, chemical balance and fetal exposure during pregnancy. Placental UGT1A isoforms were profiled and differences between preeclamptic (PE) and non-PE placental UGT expression determined. In third trimester villous placenta, UGT1A1, 1A4, 1A6 and 1A9 were expressed and active in all specimens ($n = 10$), but UGT1A3, 1A5, 1A7, 1A8 and 1A10 were absent. The UGT1A activities were comparable to human liver microsomes per milligram, but placental microsome yields were only 2 % of liver (1 mg/g of tissue vs. 45 mg/g of tissue). For successful PCR, placental collection and processing within 60 min from delivery, including DNase and 300 ng of RNA in reverse transcription were essential and snap freezing in liquid nitrogen immediately was the best

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Conflict of interest The authors have nothing to declare.

preservation method. Although UGT1A6 mRNA was lower in PE ($P < 0.001$), there were no other significant effects on UGT mRNA, protein or activities. A more comprehensive tissue sample set is required for confirmation of PE interactions with UGT. Placental UGT1A enzyme expression patterns are similar to the liver and a detoxicative role for placental UGT1A is inferred.

Keywords

Developmental pharmacology; Glucuronidation; Obstetrics; Phase II metabolism

1 Introduction

The placenta is a unique organ of pregnancy that functions to provide and balance the nutritive, detoxicative and endocrine needs of the fetus, and therefore placental enzymes mediating these functions are critical for fetal development and pregnancy outcomes (Benirschke et al. 2006). The UDP-glucuronosyltransferases (UGTs, EC 2.4.1.17) are a superfamily of such enzymes with demonstrated roles in regulating maternal and fetal exposure to hormones, dietary compounds, drugs and environmental chemicals through biotransformation and elimination (Mackenzie et al. 1997).

In humans, the UGT superfamily is divided into five subfamilies, designated UGT1A, UGT2A and UGT2B, UGT3A and UGT8A. These subfamilies contain multiple isoforms, each with independent regulation and expression (Mackenzie et al. 2005; Meech and Mackenzie 2010). While the UGT2A subfamily is found only in the nasal mucosa and little is known about UGT3A or UGT8A, the UGT1A and UGT2B isoforms are widely distributed in most tissues (Radomska-Pandya et al. 1999). In the placenta, all UGT2B isoforms are transcribed and several are known to be active (Collier et al. 2002a, 2004; Ginsberg and Rice 2009). In contrast, the presence of placental UGT1A isoforms is more controversial. Encoded by a single-gene locus on human chromosome 2q37.1, UGT1A transcripts have identical exons 2-5a/5b, but alternative splicing of exon 1 is used to produce nine functional UGT1A proteins: 1A1, 1A3, 1A4, 1A5, 1A6, 1A7, 1A8, 1A9 and 1A10 (Radomska-Pandya et al. 1999). More recently, splice variants and epigenetic variants have been shown to increase UGT1A gene product diversity, particularly through differential splicing of exon 5 (Girard et al. 2007). Although one study reported no UGT1A mRNA expression in the placenta (Izukawa et al. 2009), others have reported evidence for non-specific UGT1A mRNA expression using an antibody raised to common exons 2–5 (Collier et al. 2002a, b) and the presence of UGT1A4 activity due to transplacental metabolism of the drug lamotrigine (Reimers et al. 2011). Thus, the UGT1A isoforms in human placenta have not been completely profiled.

Tight control of xenobiotics and hormones is of vital importance for the mother and fetus. In humans, placental UGTs are likely to have a vital role compensating for a lack of fetal hepatic glucuronidation, since although fetal liver expresses UGT, they are either inactive or have very low activity until after delivery (Burchell et al. 1989; Collier et al. 2012; Court et al. 2011; Kawade and Onishi 1981; Miyagi and Collier 2007, 2011, 2012). This is very interesting because with the exception of cats (*Felis sp.*) that have vastly reduced or absent glucuronidation throughout life (Court and Greenblatt 1997, 2000), UGT activities are

readily detected in the fetuses of most other mammalian species (Fyffe and Dutton 1975; Manzo et al. 1969; Radomska-Pandya et al. 1999). Therefore, placental UGT enzymes are of ongoing interest to reproductive and developmental pharmacologists and toxicologists.

We hypothesized that placental UGT activity may be particularly relevant in preeclampsia (PE). Considered one of the most serious syndromes in pregnancy (Landau and Irion 2005), PE is characterized by proteinuria and hypertension after 20 weeks of gestation, and is commonly comorbid with gestational diabetes (Duckitt and Harrington 2005). It has been associated with structural and functional placental changes that can compromise fetal nutrition, increase placental/fetal oxidative stress and lead to low birth weight and premature birth (Landau and Irion 2005). Previous studies have demonstrated that in a mouse model, dysregulated UGT activities are concurrent with placental inflammation and oxidative stress (Collier et al. 2012; Raunig et al. 2011). Since both occur in PE and have been independently associated with negative fetal and pregnancy outcomes (Ashida et al. 2008; Lampe 2007), UGT enzymes were a logical potential target for PE deregulation.

The present study was designed to profile, for the first time, the complete expression and activities of UGT1A isoforms in the human placenta. There are few reports of UGT1A expression and these are not comprehensive for all UGT1A isoform mRNA and protein expression (Reimers et al. 2011; Izuwaka et al. 2009; Collier et al. 2002a, b, 2004). Moreover, under the hypothesis that UGT enzymes differ in PE, differences in UGT1A expression and activities with pre-eclampsia were also studied. It is noted that if differences occur, it would not be possible to tell if these were causes or consequences of PE. Finally, within the study we have nested a tissue collection and mRNA quality study to assist ourselves and others to optimize expression studies for UGTs in placenta. We conclude that the placental profile for UGT1A isoforms is more similar to the liver than either the intestine or kidney (the other major organs where UGTs are expressed) and are not substantially different at the protein level for PE, although significantly lower UGT1A6 mRNA levels are observed. Evidence points to a primarily detoxicative and endocrine role for placental UGT enzymes.

2 Materials and methods

2.1 Tissue collection and processing

Term non-PE and PE placentas were collected with IRB approval from women undergoing cesarean section at Kapiolani Women and Children's Medical Center, Honolulu, Hawaii. Villous placental tissues were collected by blunt dissection within 30 min of delivery and either immersed in RNeasyTM (Qiagen, Valencia, CA, USA) or snap frozen in liquid nitrogen and then stored at -80 °C until use. All tissue collection and use were reviewed by the University Committee on Human Experimentation and the Hospital Institutional Review Board and deemed 'exempt from regulations'. Therefore, the patient data collected was minimal and conformed to Health Insurance Portability and Accountability Act 1996 (HIPAA) regulations.

Tissues in this study were as follows: non-PE third trimester placentas ($n = 5$): 28 weeks (premature membrane rupture), 34 weeks (premature membrane rupture), 37, 38 and 40

weeks (no complications). The PE placenta characteristics ($n = 5$) were: 28 weeks (PE with severe features), 32 weeks (PE with severe features), 35 weeks (mild PE), 36 weeks (PE with severe features) and 37 weeks (PE without severe features). All placentas were collected by cesarean section. There was no significant difference in the gestational ages between each group (t test $P = 0.51$). No other clinical information on mothers or placentas was collected: a limitation of the study.

For studies of RNA quality and quantity, two pieces of placenta were collected from a centrally situated non-PE placental cotyledon, immediately after delivery and placed on ice or left at room temperature (21 °C). Thereafter, a sample of each tissue was frozen in RNA later (Qiagen, Valencia, CA) after every 30 min up to 6 h post-delivery and stored at -80 °C. Upon completion of the time course, all samples were thawed and RNA extracted for analysis. RNA quality was determined by the absorbance ratio at 260 and 280 nm and only absorbance ratios of 1.8 or higher were acceptable. Moreover, RNA quantity was determined using a Nanodrop 2000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA).

Control tissues used for RNA and protein studies of UGT isoforms 1A1, 1A3, 1A4, 1A6 and 1A9 were from liver samples collected by the Hawaii Human Biorepository Core from postmortem donors within 1 h of cross-clamp. All tissues were snap frozen in liquid nitrogen within 6 h of death and stored at -80 °C until use, whereupon pieces of tissue were excised (frozen) and RNA or protein extracted. Positive control RNA for UGT 1A5, 1A7 and 1A8 (single donor, jejunum RNA), as well as UGT1A10 (single donor, stomach RNA) were purchased from Biochain (Hayward, CA, USA).

2.2 Protein extraction, RNA extraction and cDNA synthesis

Tissue lysates and microsomes were prepared from placentas and livers as previously described (Collier et al. 2000). Lysates were normalized to 2 mg/mL of protein for Western blotting and microsomes normalized to 0.5 mg/mL for enzyme activity assays using the bicinchoninic acid method with bovine serum albumin as the protein standard (Smith et al. 1985).

Total RNA was extracted from 30 mg of thawed placenta or liver. The tissue was homogenized with a rotor-stator homogenizer in RLT buffer (Qiagen, Valencia, CA, USA) followed by RNA isolation using RNeasy Mini kit with on-column DNase digestion according to the manufacturer's instruction (Qiagen). The RNA was stored at -80 °C until use. On use, 1 µg of total RNA was reverse-transcribed with qSCRIPT™ cDNA synthesis kit (Quanta BioSciences, Gaithersburg, MD, USA) using a blend of oligo (dT) and random primers as follows: 5 min, 22 °C; 30 min, 42 °C; and 5 min at 85 °C.

2.3 Real-time PCR, primer selection and relative quantitation

Primer sequences of the different UGT1A isoforms used for real-time PCR were either from published studies or designed with Primer Premier 6.0 software (Premier Biosoft International) and are described in Table 1, with references. The primers for UGT1A6 were

designed in the specific exon 1 of the UGT1A gene and checked for specificity using BLAST.

Real-time PCR was performed using 6 μ L of cDNA with 10 pmol of forward and reverse primers (Table 1) on a BioRad iCycler IQTM using SYBR Green detection in a total volume of 20 μ L with 10 μ L of PerfeCTa SYBR green SuperMix for IQ (Quanta BioSciences, Gaithersburg, MD). Cycling conditions were 1 cycle for 5 min at 95 °C, followed by 40 cycles of 15 s at 95 °C and 1 min at 62 °C. The threshold value detection (C_t) was set in the exponential phase of amplification. Amplicon sizes of the PCR products were verified on 2 % agarose gel (UltraPure; Invitrogen) by electrophoresis, stained with 1 μ g/mL ethidium bromide (Bio-Rad, Hercules, CA) and then visualized with UV light. For quantification, values were normalized to the value of 18S ribosomal RNA measured for each sample using primers as previously described (Snodgrass et al. 2010). The C_T values were calculated in the exponential part of the curve and target genes (UGT1A isoforms) were analyzed with respect to the housekeeping gene 18S by comparing the C_T for each (DC_T). These DC_T levels of expression were converted to fold differences between the non-PE (“normal”) placentas and the PE (“disease state”) tissues using the $2^{(-\Delta C_T)}$ method (here called DDC_T) for relative quantitation without efficiency correction (Pfaffl 2001).

2.4 Western blotting

Western blotting was performed for expression of UGT1A1, 1A4, 1A6 and 1A9 as previously described by us (Collier et al. 2002a). Briefly, lysates (20 μ g) of five non-PE and five PE placentas were resolved on 10 % SDS-PAGE gels under reducing conditions (MiniProtean III, Biorad, Hercules, CA, USA). Gels were transferred to PVDF membrane using a semi-dry transfer apparatus (Biorad) and blocked in TBS-T with 5 % non-fat milk powder overnight. Even protein loading was determined with Coomassie blue gel staining. The next day, membranes were washed for 6 $\times$ 5 min in TBS-T and incubated with primary antibody (1 : 2,500, UGT1A1, 1A4, 1A6 or 1A9 as provided by Professor Michael Coughtrie, University of Dundee, UK) for 2 h at room temperature. Membranes were washed again and then incubated with secondary antibody (donkey anti-goat biotin, 1 : 8,000 Jackson Immunolabs, Westgrove, PA, USA) for 1 h at room temperature. Finally, membranes were incubated with Streptavidin–HRP–biotin (1 : 10,000, GE Healthcare, Piscataway, NJ, USA) for 1 h at room temperature. Proteins were detected by Amersham ECL plusTM Western Blotting Detection System (GE Healthcare). The protein bands were sized by a comparison to a Rainbow MarkerTM (GE Healthcare). Additionally, each membrane contained a human liver positive control (5 μ g) sourced from a set of pooled livers ($n = 8$) from the Hawaii Human Biorepository. This internal positive control band was used for ratiometric area: density analysis to assign relative levels of enzyme proteins using the program ImageJ (NIH, Bethesda MD). Briefly, a single box was generated then moved horizontally along the gel, controlling for selection bias. Background was subtracted for each blot and normalization of protein to the positive control band occurred.

2.5 Enzyme activity assays

The activity of UGT1A1 was determined with the substrate bilirubin (125 μ M) as described (Heirwegh et al. 1972). The substrate was synthesized in-house and used as described by us

previously (Miyagi and Collier 2011). Bilirubin glucuronide concentrations were calculated using $\epsilon = 44.4 \times 10^3 \text{ L mol}^{-1} \text{ cm}^{-1}$. Pooled adult human liver microsomes ($n = 200$, Xenotech, Lenexa KS) were the positive control with a coefficient of variation (CV) of 25 %.

The activity of UGT1A4 was measured using the substrate trifluoperazine (100 μM) and assay conditions as previously described (Uchaipichat et al. 2006). Detection of glucuronidation was by monitoring the fall in fluorescence in solution at wavelengths described by Rele et al. (2004), a modification previously validated by us (Miyagi and Collier 2007). Quantitation was by comparison to a standard curve with $r^2 = 0.988 \pm 0.001$. Pooled adult human liver microsomes ($n = 200$) were the positive control with CV = 7.5 %.

The activity of UGT1A6 was measured with serotonin (100 μM) as previously described (Krishnaswamy et al. 2003) and used by us (Miyagi and Collier 2011). Pooled adult human liver microsomes ($n = 200$) were the positive control with CV = 2.8 %.

The activity of UGT1A9 was determined using the specific inhibitor 2.5 μM niflumic acid as described (Miners et al. 2011). The general UGT substrate 100 μM 4-methylumbelliferone sodium (Sigma, St Louis, MO) was used as described (Collier et al. 2000) except that alamethicin (0.5 $\mu\text{g}/\text{mg}$ protein from *trichoderma viridae*) was used as the activator and 2 % BSA was included in reactions. Standard curve was $r^2 = 0.996 \pm 0.001$. Pooled adult human liver microsomes ($n = 200$) were the positive control with CV = 2.6 %.

2.6 Statistical analyses

Statistical analyses were performed using Prism 5.0 with statistical significance set at $\alpha = 0.05$. (GraphPad Prism, San Diego CA). Parametric statistics were performed since all data approximated Gaussian distributions (as assessed with the D'Agostino–Pearson test). Since all data were divided into discrete categories, two-tailed Student's t tests were used to assess differences between groups with an F test to compare variances.

3 Results

3.1 mRNA expression of UGT1A isoforms in the term placenta

Among the UGT1A isoforms tested, UGT1A1, 1A4, 1A6 and 1A9 were expressed in all ten placentas tested. The isoforms UGT1A3, 1A5, 1A7, 1A8 and 1A10 were not detected in any placentas, but were detected in positive control tissues (intestine: UGT1A3, 1A5, 1A7 and 1A8; stomach: UGT1A10). Table 2 shows the Ct values of expressed genes in placenta and positive control tissues. In the placenta, the expression of UGT1A isoforms was lower than that in positive control tissues (i.e., liver).

While there were no differences in UGT mRNA between the five non-PE and five PE placentas, inter-patient variability in mRNA expression was observed in both groups of samples. For UGT1A1 the variability in mRNA expression ranged 1.7-fold (UGT1A1), 0.9-fold (UGT1A4), 15.4-fold (UGT1A6) and 1.8-fold (UGT1A9). In PE placentas, mRNA expression of UGT1A isoforms was consistently lower than in non-PE placentas, with only UGT1A6 being significantly lower (15-fold, $P < 0.001$, Table 2).

3.2 Placental tissue handling, mRNA levels and UGT expression

The quality and quantity of extracted mRNA (260/280 ratio) when tissue was kept at room temperature and at +4 °C demonstrated that after 3 h at room temperature, the quantity of mRNA per mg of tissue was approximately 50 % less than placental tissue snap frozen in liquid nitrogen within 30 min of delivery (positive control, gold standard). Moreover, RNA quality was routinely below the accepted ratio of 1.8 and significantly lower than time zero at 3, 5 and 6 h (Table 3). Tissue kept on ice up to 6 h did not show any significant decay in the amount of RNA or quality according to the average 260/280 ratio. Additionally, when 18S rRNA expression was quantified by qRT-PCR to determine the stability of the housekeeping gene, significant differences were observed at different time points, with tissue at room temperature having less stable 18S rRNA than tissue kept on ice (Table 3). It is important to collect all samples within the same time point if 18S rRNA is to be used for relative quantitation. Moreover, PCR studies indicated that to successfully detect UGT isoforms, high levels (above 300 ng) of mRNA, purified by an additional DNase step, were necessary for successful amplification of UGT transcripts.

3.3 Protein expression of UGT1A isoforms

Since only UGT1A1, 1A4, 1A6 and 1A9 mRNA were detected, the protein expression of these isoforms only was confirmed by Western blot (Fig. 1). In general, women who had a high protein expression for one isoform tended to have similarly high levels for the other isoforms.

Protein levels were quantitated relative to the protein expression in a pool of adult human livers ($n = 200$) that were included on all blots (Fig. 1). Even though the level of protein expression of the UGTs studied was higher in non-PE placentas, there was no significant difference in the level of expression using density:area measurements.

3.4 Enzyme activity of UGT and correlation of activities to protein levels

Microsomal yields from placenta were approximately 1 mg/g tissue (average 1.3 ± 0.56), which is roughly 2 % of the amount of microsomes routinely extracted from human liver [45 mg microsomes per gram of tissue (Houston 1994)]. Despite this, the activities of UGT isoforms were comparable to that of human liver on a per milligram of microsomal protein basis (data not shown). However, if future studies using placenta are to be scaled for UGT metabolism, a scaling factor of 1 mg/g of tissue is recommended for placenta as opposed to 45 mg/g used in liver.

Enzyme activity for placental UGT1A1, 1A4, 1A6 and 1A9 was also detected and activities did not differ significantly between non-PE and PE third trimester placentas (Fig. 2). Moreover, protein levels did not correlate with enzyme activities (data not shown).

4 Discussion

Here, we present for the first time the complete expression and activity profile of UGT1A isoforms in villous human placenta. The mRNA, protein and activities of UGT1A1, 1A4, 1A6 and 1A9 can be detected in both non-PE and PE third trimester human placenta, but

UGT1A3, 1A5, 1A7, 1A8 and 1A10 cannot be detected. The expression of UGT1A mRNA did not correlate with protein expression, and protein levels did not correlate with enzyme activities. We have also demonstrated that the same isoforms as detected in non-PE placentas were detected in PE and that, while mRNA levels of UGT1A6 were significantly lower in PE and protein levels for all isoforms trended lower, PE did not significantly alter UGT1A activities.

The large inter-individual variability of protein expression observed here is consistent with the known existence of genetic polymorphisms that occur with significant frequencies in the population, especially in UGT1A1 (Bock 2010), which has the most variability. In addition, intra-placental variability within the same individuals has also been documented for several other metabolizing enzymes, such as glutathione peroxidases (Mistry et al. 2010). To minimize the potential for confounding from placental variability, all of our samples were excised from the central part of the villous placenta, close to the umbilical cord insertion point and hence should reflect a similar regional placental profile. However, we cannot discount that some variability in the data reported are not genetic variations, but effects of the local placental environment. Also, in these studies protein levels did not correlate with enzyme activities in either non-PE or PE placentas. Although a small sample set was used, lack of protein:activity correlation was most likely due to post-transcriptional and post-translational modifications that regulate UGT enzyme catalysis. Glycosylation is critical for modulating UGT2B activities (Barbier et al. 2000) and phosphorylation is also vital for both UGT1A and 2B activities (Basu et al. 2008). Further, chemicals such as xenobiotic soy compounds can alter human UGT activities in an allosteric manner (Nishimura et al. 2007). Allosteric signaling and/or protein modifications would allow UGT protein to remain steady, but enzyme activity to vary. Such mechanisms may be relevant for unraveling the disconnect observed here and elsewhere (Miyagi and Collier 2007; Divakaran et al. 2014) between UGT protein expression and enzyme activity. This being said, because significant changes in protein or activity were not noted, the authors cannot rule out that lower levels of mRNA for UGT1A6 were due to degradation rather than a true difference between PE and non-PE placentas.

The roles of UGT1A isoforms throughout the body are many and varied. In particular for reproduction, UGT1A1 is known to metabolize estrogenic hormones, thereby terminating estrogen actions and facilitating elimination of the steroid. Our own laboratory has demonstrated assisted reproduction technologies in the mouse model alter steroid glucuronidation at the level of the placenta, in part through transcriptional induction of *Ugt1a* genes (Collier et al. 2009, 2012; Raunig et al. 2011). Moreover, we and others have demonstrated that UGT isoform expression is tissue specific and transcription, as well as activities, can be related to levels of sex steroids (Buckley and Klaassen 2007, 2009; Collier et al. 2012). Therefore, increased scrutiny of both UGT1A and 2B isoforms and their roles in hormonal homeostasis, pregnancy and reproductive toxicology is warranted.

In addition to hormonal homeostasis, the placenta is a significant detoxification organ that prevents xenobiotics from reaching the fetus through transplacental metabolism and also aids waste excretion through metabolism and transport activities. The UGT1A isoform activities observed in this study occur at equal or higher rates (on a per milligram of protein

level) than those reported in pooled adult liver. This being said, in our experience, levels of microsomal protein extracted from placentas are around 2 % of that extracted from livers, per milligram of tissue. Despite this, the physical proximity of the placenta to the fetus and at least one recent report of high placental extraction of the environmental chemical bisphenol A and the drug AZT by glucuronidation (Ginsberg and Rice 2009; Collier et al. 2004) argue strongly for a vital role of placental UGT enzymes in the protection of the fetus from xenobiotics.

This is also relevant to our decision to study PE and the findings presented. We did not see significant changes in UGT activities or protein in any isoforms expressed, although UGT1A6 mRNA levels were decreased significantly by PE ($P < 0.001$). While these results do not provide strong evidence for UGTs as a primary mechanism or consequence of PE, it does suggest that these enzymes may be ancillary targets at a transcriptional level. Given the known involvement of post-transcriptional modifications in UGT activity (Barbier et al. 2000; Basu et al. 2008; Nishimura et al. 2007), the question arises as to how severe transcriptional interference needs to become and/or how long PE occurs during pregnancy before activity differences are observed. This was a small sample set and UGT enzymes vary in the population. If greater numbers of placentas had been assessed, the results may have been more definitive. Additionally, since clinical data were not collected, we could not exclude confounding by other factors such as parity, pregnancy syndromes (e.g., gestational diabetes), environmental exposures including smoking and prescription or illicit drug use, or the onset of labor before cesarean section. Moreover, all of these placentas were third trimester, so the current study has limited applicability to developmental changes across gestation.

The vital role of the placenta in fetal development is underscored by a lack of fetal hepatic UGT activity in general. Of the isoforms studied to date, hepatic UGT1A4, 1A9, 2B7 and 2B15 are absent or have sparingly low activity at birth (Divakaran et al. 2014; Miyagi and Collier 2007, 2011; Miyagi et al. 2012; Zaya et al. 2006), while UGT1A1 activity increases from 0.1 to 1.0 % of adult activities between 30 and 40 weeks of gestation (Kawade and Onishi 1981) and UGT1A6 may be up to 50 % of adult activities at birth (Burchell et al. 1989). Hence glucuronidation primarily develops in the postnatal period, independent of gestational age at birth (Burchell et al. 1989; Coughtrie et al. 1988; Kawade and Onishi 1981; Strassburg et al. 2002). Thus, during pregnancy UGT1A isoforms seem to be important for balancing maternal–placental–fetal homeostasis, fetal development and regulating chemicals, nutrients and waste exposure (Collier et al. 2004, 2009; Ginsberg and Rice 2009; Raunig et al. 2011; Reimers et al. 2011).

During these studies, we confirmed that placental tissue handling and processing are critical to the success of gene expression studies in this organ. Therefore, we recommend that placental tissues are collected within an hour of delivery and processed immediately for optimal extraction, although collection on ice for up to 6 h or freezing at -80°C is also acceptable. Additionally, high levels of mRNA were necessary for PCR amplification, above 300 ng. Our experience with gestational tissue research allowed us to identify collection and processing issues that may escape researchers who primarily study other organ systems and/or was not controlled for when purchasing samples from commercial

vendors. These observations have also been reported by others and may be particularly important for studying placentas and pregnancy in the context of reproductive/developmental pharmacology and toxicology (Fajardy et al. 2009; Lanoix et al. 2012).

In summary, the human placenta expresses UGT1A1, 1A4, 1A6 and 1A9 and their activity can be detected. This expression profile is more similar to the liver than other major sites of glucuronidation such as the kidney, intestine or esophagus (Radomska-Pandya et al. 1999). Since PE did not strongly affect UGT1A expression in villous placentas, dysregulation of these enzymes may not be the primary mechanism of deleterious effects from PE. However, several recent studies have highlighted the importance of human placental glucuronidation in fetal exposure to drugs and environmental chemicals (Ginsberg and Rice 2009; Reimers et al. 2011). Therefore, understanding placental glucuronidation can contribute to our understanding of fetal development and pregnancy outcomes, particularly with regard to hormonal balance and chemical detoxification.

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Fig. 1.

There were no significant differences in expression of UGT1A proteins in the third trimester non-preeclamptic and preeclamptic placentas, although a trend toward lower protein expression for all isoforms was observed. **a** Representative Western blots of each protein. **b** Semi-quantitative analysis of levels of UGT1A proteins relative to an internal positive control (human liver). *Box and whisker plots* are presented where the *midline* is the median, *upper* and *lower boxes* are the 75th and 25th quartiles, respectively, and *top* and *bottom whiskers* are maximum and minimum values, respectively

Fig. 2. The activities of UGT1A1 (a), 1A4 (b), 1A6 (c) and 1A9 (d) are detected in non-preeclamptic and preeclamptic placentas, but there are no significant differences between the groups. Bars are means $\pm$ SEM of $n = 5$ individuals

Table 1

Nucleotide sequences of primers used for PCR analysis and their associated references

Gene	Forward primer	Reverse primer	Amplicon size
UGT1A1 ^a	5' TTT TGT CTG GCT GTT CCC ACT 3'	5' GAA GGT CAT GTG ATC TGA ATG AGA 3'	251
UGT1A3 ^b	5' ATG TGC TGG GCC ACA CTC AAC T 3'	5' TCA TTA TGC AGT AGC TCC ACA CAA 3'	130
UGT1A4 ^b	5' CCT GCT GTG TTT TTT TGG AGG T 3'	5' ATT GAT CCC AAA GAG AAA ACC AC 3'	430
UGT1A5 ^c	5' TGG CAA TTA TGA ACA ATA TGT CT 3'	5' GAT GCA TGG CTG ACA AGA T 3'	417
UGT1A6 ^b	5' CAA CTG TAA GAA GAG GAA AGA C 3'	5' ATT GAT CCC AAA GAG AAA ACC AC 3'	101
UGT1A7 ^b	5' CCC CTA TTT TTT CAA AAA TGT CTT 3'	5' ATT GAT CCC AAA GAG AAA ACC AC 3'	260
UGT1A8 ^b	5' GGT CTT CGC CAG GGG AAT AG 3'	5' ATT GAT CCC AAA GAG AAA ACC AC 3'	422
UGT1A9 ^d	5' GAA CAT TTA TTA TGC CAC CG 3'	5' ATT GAT CCC AAA GAG AAA ACC AC 3'	274
UGT1A10 ^b	5' CTC TTT CCT ATG TCC CCA ATG A 3'	5' ATT GAT CCC AAA GAG AAA ACC AC 3'	363
18S ^e	5' CAC GGC CGG TAC AGT GAA A	5' AGA GGA GCG AGC GAC CAA	71

^a Congiu et al. (2002)^b Iizukawa et al. (2009)^c Finel et al. (2005)^d Designed with Primer Premier 6.0^e Snodgrass et al. (2010)

Table 2

Using q-RT-PCR, the isoforms UGT1A1, 1A4, 1A6 and 1A9 were detected in non-preeclamptic and preeclamptic placenta, while UGT1A3, 1A5, 1A7, 1A8 and 1A10 were not

Gene of interest	Control Tissue Average Ct	Non-preeclamptic		Preeclamptic		DDCt DCt Non-preeclamptic - DCt Preeclamptic	Fold difference in UGT preeclamptic relative to non-preeclamptic
		Average Ct	DCt UGT-18S	Average Ct	DCt UGT-18S		
1A1	21.81 ± 0.32	27.25 ± 0.45	17.27 ± 0.72	28.09 ± 0.84	18.05 ± 1.09	-0.78 ± 1.09	1.7 (0.8-3.6)
1A3	23.2 ± 0.19	ND	ND	ND	ND	ND	ND
1A4	24.2 ± 0.08	27.21 ± 0.09	17.23 ± 0.10	27.18 ± 0.24	17.14 ± 0.34	0.09 ± 0.34	0.9 (0.8-1.3)
1A5	28.77 ± 0.39	ND	ND	ND	ND	ND	ND
1A6	22.94 ± 0.10	25.06 ± 0.16	15.08 ± 0.23	29.06 ± 0.05	19.02 ± 0.19	-3.94 ± 0.19	15.4 (12.6-16.3)
1A7	29.85 ± 0.07	ND	ND	ND	ND	ND	ND
1A8	22.08 ± 0.11	ND	ND	ND	ND	ND	ND
1A9	25.35 ± 0.09	27.72 ± 0.07	17.74 ± 0.09	28.59 ± 0.17	18.55 ± 0.20	-0.81 ± 0.20	1.8 (1.5-2.0)
1A10	24.02 ± 0.09	ND	ND	ND	ND	ND	ND
18S	10.56 ± 0.52	9.98 ± 0.09	ND	10.04 ± 0.18	ND	ND	ND

Preeclampsia decreased UGT1A6 expression significantly by 15-fold

ND not-determined

Table 3

The effects of time from delivery to mRNA extraction on mRNA quantity, integrity and 18S amplification in human placenta

Time (h)	Room temperature			On ice		
	Total RNA (ng/L)	Average 260/280 ratio ($\pm$ SD)	Average 18S Ct ($\pm$ SD)	Total RNA (ng/L)	Average 260/280 ratio ($\pm$ SD)	Average 18S Ct ($\pm$ SD)
0.0	965.4	1.92 $\pm$ 0.04	10.4 $\pm$ 0.05	974.8	1.89 $\pm$ 0.03	10.8 $\pm$ 0.09
0.5	984.3	1.91 $\pm$ 0.05	9.6 $\pm$ 0.09	1002.6	1.94 $\pm$ 0.05	10.0 $\pm$ 0.07
1.0	945.3	1.93 $\pm$ 0.02	10.6 $\pm$ 0.02	982.3	1.86 $\pm$ 0.07	11.1 $\pm$ 0.10
1.5	873.1*	1.86 $\pm$ 0.04	11.7 $\pm$ 0.10	965.3	1.91 $\pm$ 0.02	10.9 $\pm$ 0.07
2.0	738.2*	1.79 $\pm$ 0.08	12.0 $\pm$ 0.08	912.9	1.87 $\pm$ 0.09	9.2 $\pm$ 0.07
2.5	631.1*	1.78 $\pm$ 0.10	10.9 $\pm$ 0.09	934.4	1.91 $\pm$ 0.06	10.5 $\pm$ 0.12
3.0	495.2*	1.72 $\pm$ 0.06*	13.8 $\pm$ 0.18	898.1	1.86 $\pm$ 0.06	12.1 $\pm$ 0.09
4.0	382.2*	1.75 $\pm$ 0.07	12.0 $\pm$ 0.12	911.2	1.90 $\pm$ 0.05	9.9 $\pm$ 0.08
5.0	318.9*	1.70 $\pm$ 0.03*	13.1 $\pm$ 0.20	877.7	1.87 $\pm$ 0.04	10.1 $\pm$ 0.09
6.0	274.1*	1.62 $\pm$ 0.15*	15.0 $\pm$ 0.14	876.5	1.82 $\pm$ 0.12	11.2 $\pm$ 0.13

* $P < 0.05$ versus freshly collected tissue (time zero)