Gestational Age–Dependent Abundance of Human Placental Transporters as Determined by Quantitative Targeted Proteomics

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ABSTRACT

Some women take medication during pregnancy to address a variety of clinical conditions. Because of ethical and logistical concerns, it is impossible to determine fetal drug exposure, and therefore fetal risk, during pregnancy. Hence, alternative approaches need to be developed to predict maternal-fetal drug exposure throughout pregnancy. To do so, we previously developed and verified a maternal-fetal physiologically based pharmacokinetic model, which can predict fetal exposure to drugs that passively cross the placenta. However, many drugs are actively transported by the placenta (e.g., human immunodeficiency virus protease inhibitors). To extend our maternal-fetal physiologically based pharmacokinetic model to these actively transported drugs, we determined the gestational age–dependent changes in the protein abundance of placental transporters. Total cellular membrane fractions from first trimester (T1; n = 15), second trimester (T2; n = 19), and term (n = 15) human placentae obtained from uncomplicated pregnancies were isolated by ultracentrifugation. Transporter protein abundance was determined by targeted quantitative proteomics using liquid chromatography tandem mass spectrometry. We observed that breast cancer resistance protein protein and P-glycoprotein abundance significantly decreased from T1 to term by 55% and 69%, respectively (per gram of tissue). Organic anion–transporting polypeptide (OATP) 2B1 abundance significantly decreased from T1 to T2 by 32%. In contrast, organic cation transporter (OCT) 3 and organic anion transporter 4 abundance significantly increased with gestational age (2-fold from T1 to term, 1.6-fold from T2 to term). Serotonin transporter and norepinephrine transporter did not change with gestational age. The abundance of bile salt export pump, multidrug resistance–associated protein 1–5, Na+/laurocholate cotransporting polypeptide, OATP1B1, OATP1B3, OCTN1–2, concentrative nucleoside transporter 1–3, equilibrative nucleoside transporter 2, and multidrug and toxin efflux pump 1 could not be quantified. These data can be incorporated into our maternal-fetal physiologically based pharmacokinetic model to predict fetal drug exposure to drugs that are actively transported across the placenta.

SIGNIFICANCE STATEMENT

We quantified the protein abundance of key placental uptake and efflux transporters [organic cation transporter (OCT) 3, P-glycoprotein (P-gp), breast cancer resistance protein (BCRP)] across gestational ages (first trimester, second trimester, and term) using quantitative targeted proteomics. We observed that the protein abundance of P-gp and BCRP decreased, whereas that of OCT3 increased with gestational age. Incorporating the protein abundance determined in this study into maternal-fetal physiologically based pharmacokinetic model can help us better predict fetal drug exposure to substrates of these transporters.

Introduction

To date, approximately 40%–80% of women take drugs during pregnancy and about 50% take at least one drug in the first trimester (Scaffidi et al., 2017). Pregnant women take drugs for a variety of reasons, including to treat preexisting disease (e.g., depression, epilepsy), for pregnancy-induced conditions (e.g., gestational diabetes and hypertension), to prevent vertical transmission of infectious agents (e.g., human immunodeficiency virus, malaria), or to treat fetal conditions, such as poorly developed lungs because of preterm birth (Sheffield et al., 2014).

Despite the striking prevalence of drug use during pregnancy, there is little information on the extent of maternal-fetal drug exposure throughout pregnancy. When a pregnant woman takes a drug, the fetus is de facto exposed to the drug even if s/he is not the target for drug therapy. Fetal drug exposure, and therefore fetal risks, are driven by maternal drug exposure, placental transport/metabolism, and fetal metabolism (Zhang et al., 2017). Physiologic and drug disposition changes throughout pregnancy result in time-dependent changes in maternal drug exposure (Anderson, 2005; Tasnif et al., 2016). In addition, the placenta is richly endowed with efflux transporters, such as P-glycoprotein (P-gp; ATP-binding cassette B1)/breast cancer resistance protein (BCRP; ATP-binding cassette G2) (Mathias et al., 2005; Han et al., 2018), as well as influx transporters, such as organic anion
transporter 4 (OAT4)/norepinephrine transporter (NET) (Fig. 1) (Vahakangas and Myllýnen, 2009). The abundance of these placent al transporters may change as pregnancy proceeds (Mathias et al., 2005). Consequently, pregnancy is a dynamic process whereby maternal-fetal drug exposure changes in a time-dependent manner. Thus, for optimum therapy of the pregnant woman and to minimize fetal risk, the challenge is to measure or predict maternal-fetal drug exposure throughout pregnancy.

Although fetal drug exposure can be determined by sampling cord blood (umbilical vein) only at the time of delivery, such sampling is not possible earlier in gestation (Scaffidi et al., 2017). Also, sampling umbilical vein blood at term provides only a snapshot of fetal blood drug concentration at a given time and does not provide information on fetal drug exposure (i.e., fetal plasma/blood area under the curve) (Zhang et al., 2017). Moreover, even though pharmacokinetic studies of a drug can theoretically be conducted in pregnant women throughout gestation, such studies pose considerable logistical and ethical challenges. Thus, alternative methods need to be developed to predict (rather than determine) maternal-fetal drug exposure throughout pregnancy. To do so, we have developed and verified a maternal-fetal physiologically based pharmacokinetic model that can predict time-dependent changes in maternal-fetal exposure to drugs metabolized by CYP enzyme and cross the placenta by passive diffusion (Zhang and Unadkat, 2017). The model was validated using data from human placental transporters (Enders and Blankenship, 1999; Myllýnen et al., 2005; Joshi et al., 2016). To extend our maternal-fetal physiologically based pharmacokinetic model to predict maternal-fetal exposure to drugs that are transported into or out of the placenta, we determined the gestational age–dependent changes in the abundance of placental transporters by targeted quantitative proteomics using LC-MS/MS.

**Materials and Methods**

**Chemicals and Reagents.** Homogenization buffer reagents and the protease inhibitor cocktail, Pefabloc SC, were purchased from Sigma-Aldrich (St. Louis, MO) and Roche, Basel, Switzerland. Omni Bead Ruptor Homogenizer, 7-ml soft tissue tubes, and metal beads for homogenization were purchased from Omni International (Kennesaw, GA). Bicinchoninic acid assay kit, dithiothreitol, iodoacetamide, and sequencing grade trypsin were obtained from Pierce Biotechnology (Rockford, IL). Isotope-labeled heavy internal standard peptides were obtained from Thermo Fischer Scientific (Rockford, IL), and corresponding unlabeled surrogate peptides were purchased from New England Peptide (Gardner, MA) (Supplemental Table 1). High-performance liquid chromatography–grade acetonitrile, methanol, chloroform, formic acid, and ammonium bicarbonate were obtained from Thermo Fischer Scientific (Fair Lawn, NJ). Sodium deoxycholate (98% purity) was purchased from MP Biomedicals (Santa Ana, CA).

**Procurement of Human Placental Tissue Samples.** Collection of placental tissue from uncomplicated pregnancies was approved and classified as nonhuman subject research by the Institutional Review Board of the University of Washington. Placentae were classified into three gestational age groups (mean ± S.D.): first trimester (T1: 63.1 ± 10.8 days, n = 15), second trimester (T2: 117 ± 19.6 days, n = 19), and term (n = 15) (Supplemental Table 3; Table 1). To account for variability in digestion efficiency, protein abundance was normalized to the maximum observed BSA digestion efficiency. In addition, to account for intertissue variability in membrane isolation, the enrichment of the apical membrane marker [alkaline phosphatase (ALP)] and basal membrane marker [Na+/K+-ATPase] was assessed. ALP and Na+/K+-ATPase were selected because these proteins are highly abundant in the apical membrane and basal membrane, respectively. Membrane marker enrichment was defined as the ratio of marker abundance in 1 mg of total membrane protein (MP) to marker abundance in 1 mg of homogenate protein (HP) (eq. 1). Transporters preferentially expressed in apical or basal membranes were corrected for the corresponding membrane marker enrichment (dividing by the marker enrichment value), which then was scaled to abundance per gram tissue as shown in eq. 2. Additionally, the resulting value per gram tissue was scaled to picomole analyte per predicted weight of the whole placenta. The weight of each placenta was estimated as a function of gestational age using a method described earlier (eq. 3) (Abudjallil et al., 2012). Predicted placental weight values were used because of the lack of information on the exact weight of each placenta.

**Marker Enrichment (fold)**

\[
\text{Marker Enrichment (fold)} = \frac{\text{pmol Membrane marker} \text{ per mg MP}}{\text{pmol Membrane marker} \text{ per mg HP}}
\]

**Protein abundance (pmol protein/g tissue)**

\[
\text{Protein abundance (pmol protein/g tissue)} = \frac{\text{pmol protein} \times \text{Marker Enrichment (fold)}}{\text{mg HP}}
\]

**Placental weight (g)**

\[
\text{Placental weight (g)} = \frac{0.0122 \times \text{GA}^2 + 0.9149 \times \text{GA} - 0.716 \times \text{GA}}{1.048}
\]
Abundance values for organic anion–transporting polypeptide (OATP) 2B1 in several T2 samples were below the lower limit of quantification (LLOQ) as defined by LC-MS/MS signal less than five times background noise. Such values were conservatively assigned the value of LLOQ. Data were analyzed by nonparametric Kruskal-Wallis and Dunn’s multiple comparison test in GraphPad Prism 7 with statistical significance of P < 0.05. Site-dependent and interday variability data were analyzed by Kruskal-Wallis test with Dunn’s multiple comparisons (Supplemental Fig. 2). Continuous data were analyzed by Pearson correlation, and significance cut-off was defined as R² ≥ 0.5 (Supplemental Fig. 5; Table 2).

**Results**

**Interday and Site-Dependent Variability in Transporter Abundance.** Transporter protein abundance in three T2 placentae was independent of the sampling site and day of preparation (Supplemental Fig. 2, B and C), and hence, site 1 was chosen as the sampling site for further analyses. Overall, placenta H27108 showed greater variability than other two placentae (unpublished data), perhaps because of its earlier gestational age.

**Total Membrane Yield, Marker Enrichment, and Scaling Approach.** As expected, membrane protein yield was about 3% of that in the homogenate. There was modest but significant difference in protein yields between the trimesters (Fig. 2; Table 1). Na+/K⁺-ATPase enrichment did not change significantly between three gestational age groups. ALP enrichment was significantly different at term compared to the above proteins, the remaining transporters targeted for quantification were below the LLOQ (5-fold signal-to-noise ratio) (Table 1).

**Abundance of Apical and Basal Membrane Transporters in Human Placentae of Various Gestational Ages.** Of the four apical membrane transporters, BCRP and P-gp showed gestational age–dependent decrease in protein abundance (pmol of analyte per gram of tissue) between T1 and term (55% and 69%, respectively) and between T2 and term (42% and 52%, respectively (Fig. 3; Supplemental Table 4). OCT3 and OAT4 showed, respectively, 2-fold (between T1 and term) and 1.6-fold (between T2 and term) increase in protein abundance. OATP2B1 showed significant (32%) decrease in protein abundance between T1 and T2 (Fig. 3; Supplemental Table 4). Neither SERT nor NET showed significant change in protein abundance with gestation. Of the three basal membrane transporters, BCRP and P-gp showed gestational age-dependent decrease in protein abundance (55% and 69%, respectively) and between T2 and term (42% and 52%, respectively). Hence, we incorporated membrane marker enrichment values into our scaling approach (eqs. 1–3) using ALP for transporters expressed on the apical membrane and Na⁺/K⁺-ATPase for transporters expressed on the basal membrane of the syncytiotrophoblast.

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**Table 1**

Gestational age grouping, protein yield, and membrane marker enrichment of placentae

|                | Day 1–98  | Day 99–196 | Term Day 273–287 |
|----------------|-----------|------------|-----------------|
| Gestational age (days) | 63.1 ± 10.8 | 117 ± 19.6 | N/A             |
| Number of samples     | 15        | 19         | 15              |
| Homogenate total protein yield (mg HP/g tissue) | 26.6 ± 8.5† | 20.1 ± 3.0† | 28.5 ± 7.4†     |
| Membrane total protein yield (mg MP/g tissue)  | 0.8 ± 0.4§  | 0.7 ± 0.3   | 0.5 ± 0.2‡      |
| Membrane marker enrichment (fold) | 3.0 ± 1.7*   | 2.7 ± 1.2*  | 4.2 ± 1.5*      |
| Alkaline phosphatase  | 3.1 ± 2.3  | 2.7 ± 1.0  | 2.2 ± 1.1       |
| Na⁺/K⁺-ATPase         | 3.0 ± 1.7*  | 2.7 ± 1.2*  | 4.2 ± 1.5*      |

Data shown as mean ± S.D. Identical symbols next to the values (†, ‡, §, or †) denote significant differences between the respective values (Kruskal-Wallis test with Dunn’s multiple comparisons, P < 0.05).
Transporter Abundance at Three Gestational Ages. When abundances of all proteins across the three gestational age groups were compared (Fig. 4), term placentae looked notably distinct from T1 and T2. When relative transporter protein abundance was compared (Fig. 4, pie charts), the decrease in apical membrane transporters (black bars: from 55% in T1 to 33% at term) and increase in basal membrane transporters (gray bars: from 45% in T1 to 67% at term) were observed.

Protein-Protein Correlations of Placental Transporters. Multiple pairs of transporters showed significant protein-protein correlation (Pearson correlation with \( P < 0.05 \)) (Supplemental Fig. 5; Table 2). Strong correlations (defined as \( R^2 > 0.5 \)) were observed between BCRP and P-gp \((R^2 = 0.78)\), BCRP and SERT \((R^2 = 0.62)\), P-gp and SERT \((R^2 = 0.63)\), and OAT4 and OATP2B1 \((R^2 = 0.53)\).

Discussion

Although placental transporter abundance has been previously quantified by us (Mathias et al., 2005) and others (Gil et al., 2005; Meyer zu Schwabedissen et al., 2006; Sun et al., 2006), these studies have used either Western blotting/ELISA to quantify the transporters or qPCR to quantify transporter mRNA expression (Nishimura and Naito, 2005). Western blot is inherently semiquantitative and cannot be used to compare the abundance of multiple transporters without the availability of protein standards. mRNA abundance does not always correspond to protein abundance and cannot be used for PBPK modeling and simulation. To address the shortcomings of previous studies, we incorporated several unique features in the study presented here. First, we used the state-of-the-art targeted quantitative proteomics method to quantify the abundance of multiple transporters, a method that does not depend on the availability of protein standards. These transporters were chosen based on previously published gene expression data (mRNA or protein) indicating that they are present in the human placenta (Gil et al., 2005; Mathias et al., 2005; Nishimura and Naito, 2005; Meyer zu Schwabedissen et al., 2006; Sun et al., 2006). Second, we quantified the abundance of transporters across multiple gestational ages from placentae obtained from uncomplicated pregnancies. Third, we extended the transporters quantified to those not previously studied (e.g., SERT, NET). Fourth, we used a greater number of placentae in each gestational age \((n = 15)\) than prior studies. Fifth, we determined whether the abundance of the transporters in the placenta was sample site–dependent. Sixth, we corrected the intertissue variability in membrane isolation by utilizing enrichment of validated membrane markers, ALP, and Na+/K+-ATPase.

We did not observe any significant differences when preparations were made on different days or sampled from different sites (Supplemental Fig. 2). Lack of interday variability indicated our technical consistency in preparation methodology, and lack of site–dependent variability implied homogeneous distribution of transporters throughout placenta and was also observed before in term placentae (Memon et al., 2014). Because of this lack of variability, we chose to prepare membrane fractions using tissue obtained from site 1 (Supplemental Fig. 2) for all the placenta samples.

We observed a 3–4-fold enrichment of membrane markers in all the preparations. Membrane marker enrichment value was incorporated into the scaling strategy to control for the variability in membrane loss between preparations. ALP and Na+/K+-ATPase were chosen as highly abundant markers detectable in both homogenate and membrane fractions. Two separate markers were chosen because of the possible differences in enrichment of apical or basal membranes. Overall, the fold-enrichment values for both markers were comparable for each gestational age except for higher ALP enrichment values at term (Table 1). Both enrichment values were relatively low compared with those reported in the literature (separate isolation of apical and basal membranes) (Kelley et al., 1983; Illsley et al., 1990; Jimenez et al., 2004). The reason for this difference is unknown but could be due to greater contamination from other membranes during our preparation. Nonetheless, the low enrichment values did not detract from our ability to quantify the most abundant and important xenobiotic transporters (i.e., P-gp, BCRP, and OCT3) while the abundance of other transporters was below limits of detection (i.e., multidrug resistance-associated protein 1–5, OATP1B1/3, multidrug and toxin extrusion 1, OCTN1/2, concentrative nucleoside transporter 2/3, bile salt export pump, and Na+/taurocholate cotransporting polypeptide) (Fig. 1; Supplemental Table 1).

We found the most pronounced differences with gestational age in abundance of BCRP, P-gp, and OCT3, whereas that of OAT4 and OATP2B1 proteins was less affected (Fig. 3). This differential gestational age–dependent transporter abundance indicates that the observed changes are not an artifact of our method. The mechanistic basis of this differential effect remains to be elucidated. Teleologically,
scaled to the whole organ, a consistent gestational age–dependent increase in abundance of all seven proteins was observed (Supplemental Fig. 3B). This finding is due to the dramatic increase in placental weight (up to 500-fold) with gestation (eq. 3) in comparison with modest changes (2–3-fold) in protein abundance per gram of tissue. Hence, placental weight becomes the major determinant for the differences observed in the whole organ.

Our quantification results captured some elements of placentogenesis, which is a very dynamic and multifaceted process (Abduljalil et al., 2012; Burton and Fowden, 2015). The pattern of placental transporter protein abundance was similar between T1 and T2 but less similar between T1/T2 and term (Fig. 4). Such observations can be explained by the T1 and T2 placenta being closer in gestational age than term placentae (Table 1) and, hence, more similar in developmental processes. Additionally, induction of parturition-responsive genes can alter placental gene expression as the organ reaches term (Peng et al., 2011). Hence, at earlier gestational ages (T1 and T2), fetal drug exposure to transporter substrates may be more similar than at term.

The observed strong correlation between pairs of protein abundances (Supplemental Fig. 5; Table 2) may indicate the involvement of common regulatory mechanisms (e.g., by the same nuclear receptor) or possibly protein-protein interactions as reported between OATP1B3 and OCT1 in human hepatocytes (Shoop et al., 2015).

Our approach to transporter quantification has several limitations. We assumed that all measured transporter proteins are active and localized to the membrane indicated in Fig. 1 rather than internalized or present in cells other than the syncytiotrophoblast layer (Vahakangas and Myllynen, 2009; Joshi et al., 2016). Since the enrichment values for both ALP and Na+/K+-ATPase were similar, misclassification as to which membrane (apical vs. basal) the transporter was localized will not have a large impact on the quantification of the transporters and eventual use of these values in in vitro to in vivo extrapolation. Although methods are available to separate the apical from the basal membrane, such methods do not yield complete purification of each membrane (Jimenez et al., 2004). Therefore, we believe that our approach of using a membrane marker is superior to experimentally attempting to separate the apical from the basal membrane. The use of biotinylation assay can potentially address localization in in vitro systems (Kumar et al., 2017), although such estimation in ex vivo tissue has not been evaluated. Furthermore, in addition to syncytiotrophoblast, some transporters (e.g., OCT3, BCRP) have been reported to be present on other placental cell types, such as the endothelial cells (Joshi et al., 2016; Lee et al., 2018). Thus, our approach may lead to an over-estimation of total transporter proteins at the plasma membrane of syncytiotrophoblast.

Collectively, these data can be used to populate a maternal-fetal PBPK model to predict fetal exposure to xenobiotic transporter substrates at various gestational ages. Failing to account for placental transporter abundance changes (e.g., P-gp) may lead to biased estimates of fetal exposure to transporter substrates and therefore fetal drug toxicity and efficacy (for drugs in which fetus is a therapeutic target; e.g., antenatal corticosteroids or human immunodeficiency virus drugs). To predict fetal drug exposure, these data should be married with drug transport kinetics (i.e., Michaelis-Menten constant and V_{max}) determined in vitro, wherein the in vitro V_{max} can be extrapolated to that in vivo using the proteomics data presented here. Additionally, this transporter abundance can also help estimate the fraction transported in vivo by a given transporter. Estimate of fraction transported will also aid in prediction of placental drug-drug interactions that can modulate fetal drug exposure. This is especially important because when monitored from maternal plasma, perpetrator-driven inhibition of drug efflux (e.g., P-gp) can go undetected in maternal plasma while considerably modulating fetal drug exposure.
drug exposure and therefore fetal toxicity or efficacy (Patilea-Vrana and Unadkat, 2016). Collectively, gestational age–dependent abundance of transporters in the placenta is valuable in predicting fetal drug exposure and therefore fetal efficacy and toxicity associated with drug administration during pregnancy (Zhang and Unadkat, 2017).

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Authorship Contributions

Participated in research design: Anoshchenko, Prasad, Unadkat.

Conducted experiments: Anoshchenko.

Contributed new reagents or analytic tools: Wang, Mao.

Performed data analysis: Anoshchenko.

Wrote or contributed to the writing of the manuscript: Anoshchenko, Prasad, Neradugomma, Wang, Mao, Unadkat.

References

Abduljalil K, Furness P, Johnson TN, Rostami-Hodjegan A, and Soltani H (2012) Anatomical, physiological and metabolic changes with gestational age during normal pregnancy: a database for parameters required in physiologically based pharmacokinetic modelling. Clin Pharmacokinet 51:365–396 DOI: 10.2165/11597440-000000000-00000.

Anderson (2005) Pregnancy-induced changes in pharmacokinetics: a mechanistic-based approach. Clin Pharmacokinet, doi: 10.2165/00003088-200544100-00001.

Atkinson DE, Sibley CP, Fairbairn LJ, and Greenwood SL (2006) MDR1 P-gp expression and activity in intact human placental tissue; upregulation by retroviral transduction. Placenta 27:707–714 DOI: 10.1016/j.placenta.2005.06.008.

Balkovetz DF, Timpanath C, Leibach FH, Mahesh VB, and Ganapathy V (1989) Evidence for an imipramine-sensitive serotonin transporter in human placental brush-border membranes. J Biol Chem 264:2195–2198.

Blanco-Castañeda R, Galaviz-Hernández C, Souto PCS, Lima VV, Giachini FR, Escudero C, Damiano AE, Barragán-Záiga L, Martínez-Aguilar G, and Sousa-Macias M (2020) The role of xenobiotic-metabolizing enzymes in the placenta: a growing research field. Expert Rev Clin Pharmacol 13:247–263 DOI: 10.1080/17512433.2020.1733412.

Bottalico B, Larsson I, Brodský J, Hernandez-Andrade E, Caslín B, Marsál K, and Hansson SR (2004) Noradrenaline transporter (NET), serotonin transporter (SERT), vesicular monoamine transporter (VMAT2) and organic cation transporters (OCT1, 2 and EMT) in human placenta from pre-eclamptic and normotensive pregnancies. Placenta 25:518–529 DOI: 10.1016/j.placenta.2003.10.017.

Barton GJ and Fowden AL (2015) The placenta: a multifaceted, transient organ. Philos Trans R Soc Lond B Biol Sci 370:20140066 DOI: 10.1098/rstb.2014.0066.
Supplementary Information

Gestational Age-Dependent Abundance of Human Placental Transporters as Determined by Quantitative Targeted Proteomics

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Figure S1. Workflow for isolation of total membranes from the placental tissues (A) followed by quantitative targeted proteomics (B).
Table S1. Surrogate peptides and LC-MS/MS parameters for quantification of placental drug transporters and membrane markers.

| Protein Name  | Surrogate Peptide | Peptide Type | Parent Ion | Fragment Ions                      | Declustering Potential | Collision energy |
|---------------|-------------------|--------------|------------|------------------------------------|------------------------|------------------|
| **Apical Membrane Transporters** |                  |              |            |                                    |                        |                  |
| BCRP          | SLLLVDLAAR        | Light        | 522.8      | 644.3, 757.4, 270.1               | 69                     | 27, 25, 27       |
| BCRP          | SLLLVDLAAR        | Heavy        | 527.8      | 654.3, 767.5, 270.1               | 69                     | 28, 28, 27       |
| P-gp          | NTTGALTTR         | Light        | 467.8      | 719.4, 618.4                     | 70                     | 23, 26           |
| P-gp          | NTTGALTTR         | Heavy        | 472.8      | 729.4, 628.4                     | 70                     | 23, 26           |
| SERT          | LIITPGTFK         | Light        | 495.3      | 227.18, 763.43, 549.3             | 80                     | 27               |
| SERT          | LIITPGTFK         | Heavy        | 499.3      | 227.2, 771.4, 557.3               | 80                     | 27               |
| NET           | FTPAAEFYER        | Light        | 615.8      | 814.4, 982.5, 885.4               | 80                     | 30               |
| NET           | FTPAAEFYER        | Heavy        | 620.8      | 824.4, 992.5, 895.4               | 80                     | 30               |
| **Alkaline**  |                  |              |            |                                    |                        |                  |
| Phosphatase   | EAAEALGAAK        | Light        | 465.8      | 201.1, 346.2, 530.3               | 80                     | 24               |
| Phosphatase   | EAAEALGAAK        | Heavy        | 469.8      | 201.1, 354.2, 538.3               | 80                     | 25               |
| MATE1         | GGPEATLEVR        | Light        | 514.8      | 274.2, 457.8, 688.4               | 101                    | 37, 25, 31       |
| MATE1         | GGPEATLEVR        | Heavy        | 519.8      | 274.2, 457.8, 698.4               |                        |                  |
| MRP2          | LTIIQPDLFSGLSR    | Light        | 885.8      | 1329.6, 989.6, 310.2              | 146                    | 37, 57, 43       |
| MRP2          | LTIIQPDLFSGLSR    | Heavy        | 890.5      | 1339.6, 999.6, 310.3              |                        |                  |
| MRP3          | ADGALTQEEK        | Light        | 531.4      | 634.3, 747.4, 875.4               | 91                     | 25               |
| MRP3          | ADGALTQEEK        | Heavy        | 535.4      | 642.3, 755.4, 883.5               |                        |                  |
| MRP4          | AEAAALTETAK       | Light        | 538.4      | 875.4, 733.4, 201.0               | 76                     | 25               |
| MRP4          | AEAAALTETAK       | Heavy        | 542.4      | 883.4, 741.4, 201.0               |                        |                  |
| OCTN1         | AFILDLFR          | Light        | 497.8      | 776.5, 663.4, 550.3               | 67                     | 27               |
| OCTN1         | AFILDLFR          | Heavy        | 502.8      | 786.5, 673.4, 560.3               |                        |                  |
| OCTN2         | TWNIR             | Light        | 345.2      | 588.3, 402.2                      | 56                     | 21               |
| Transporter | Sequence | Localization | Mass | Description |
|-------------|----------|--------------|------|-------------|
| OCTN2       | TWNI R   | Heavy        | 350.2 | 598.3, 412.2 |
| OAT4        | ATTALLLSFLGR | Light | 636.9 | 579.3, 692.4, 805.5 |
| OAT4        | ATTALLLSFLGR | Heavy | 636.9 | 589.3, 702.4, 815.5 |
| OATP2B1     | VLAVTDSRP | Light | 514.9 | 816.4, 646.3, 745.4 |
| OATP2B1     | VLAVTDSR  | Heavy | 519.8 | 826.4, 656.3, 755.4 |
| OCT3        | GIALPETVDDVEK | Light | 693.4 | 242.2, 1031.5, 516.3 |
| OCT3        | GIALPETVDDVEK | Heavy | 697.4 | 242.2, 1039.5, 520.3 |
| Na⁺/K⁺ ATP-ase | AAVPDAGVK | Light | 414.2 | 685.4, 586.3 |
| Na⁺/K⁺ ATP-ase | AAVPDAGK  | Heavy | 418.2 | 693.4, 594.3 |
| MRP1        | TPSGNLVNR | Light | 479.3 | 759.4, 672.4, 428.8 |
| MRP1        | TPSGNLVR  | Heavy | 484.3 | 769.4, 682.4, 428.8 |
| MRP5        | SLSEASVAVDR | Light | 567.3 | 717.4, 943.5 |
| MRP5        | SLSEASVADV | Heavy | 572.3 | 727.4, 943.5 |
| OATP1B1     | NVTGFFQSFK | Light | 587.8 | 961.5, 860.4 |
| OATP1B1     | NVTGFFQSFK | Heavy | 591.8 | 969.5, 868.4 |
| OATP1B3     | NVTGFFQSLK | Light | 570.8 | 927.49, 826.5, 622.3 |
| OATP1B3     | NVTGFFQSLK | Heavy | 574.8 | 935.51, 834.5, 630.3 |

### Basal Membrane Transporters

| Transporter | Sequence | Localization | Mass | Description |
|-------------|----------|--------------|------|-------------|
| BSEP        | STALQLIQR | Light | 515.3 | 657.4, 529.3, 770.5 |
| BSEP        | STALQLIQR | Heavy | 520.3 | 667.4, 539.4, 780.5 |
| CNT2        | LAYPEVEESK | Light | 582.8 | 817.4, 980.5, 720.3 |
| CNT2        | LAYPEVEESK | Heavy | 585.8 | 823.4, 986.5, 726.3 |
| CNT3        | DHFFAFK  | Light | 456.2 | 659.4, 365.2, 512.3 |
| CNT3        | DHFFAFK  | Heavy | 461.2 | 669.4, 375.2, 522.3 |
| NTCP        | GIYDGDLK | Light | 440.7 | 710.3, 547.3 |
| NTCP        | GIYDGDLK | Heavy | 444.7 | 718.4, 555.3 |

### Transporters with Unknown Localization

| Transporter | Sequence | Localization | Mass | Description |
|-------------|----------|--------------|------|-------------|
| CNT3        | DHFFAFK  | Heavy | 461.2 | 669.4, 375.2, 522.3 |
| NTCP        | GIYDGDLK | Heavy | 444.7 | 718.4, 555.3 |
Note: LLOQ for all quantified transporters except for OATP2B1 was 3.1 fmol on-column (per 5 μL injection volume). LLOQ for OATP2B1 was 2.4 fmol on-column. R and K in bold in heavy peptides represent stable-labeled ($^{13}$C and $^{15}$N) residues.
Table S2. LC conditions for surrogate peptide quantification

| Column | UPLC column (ACQUITY UPLC® HSS T3 column, 1.8 µm, 2.1 mm x 100 mm, Waters) |
| Guard Column | Security Guard column (C18, 4 mm x 2.0 mm, Phenomenex) |
| Run Time | 26 min |
| Injection Volume | 5 µL |
| Column Oven Temperature | 25°C |
| Autosampler Temperature | 8°C |

| Gradient Table | Flow Rate (ml/min) | %A | %B | Curve |
|----------------|-------------------|----|----|-------|
| Time (min)     |                   |    |    |       |
| Initial        | 0.3               | 97 | 3  | Initial |
| 4              | 0.3               | 97 | 3  | 6     |
| 8              | 0.3               | 87 | 13 | 6     |
| 18             | 0.3               | 75 | 25 | 6     |
| 21             | 0.3               | 66.7 | 33.3 | 6     |
| 22             | 0.3               | 50 | 50 | 6     |
| 23             | 0.3               | 20 | 80 | 6     |
| 24             | 0.3               | 20 | 80 | 6     |
| 24.5           | 0.3               | 97 | 3  | 6     |
| 26             | 0.3               | 97 | 3  | 6     |

A = 0.1% formic acid in water; B = 0.1% formic acid in acetonitrile
Table S3. Placentae donor demographics

| Demographic Characteristic       | n  |
|----------------------------------|----|
| **Sex of the Fetus**             |    |
| Male                             | 15 |
| Female                           | 10 |
| Not Available                    | 24 |
| **Ethnicity/Race**               |    |
| White                            | 8  |
| White/Native American            | 2  |
| Hispanic                         | 7  |
| Asian                            | 1  |
| Black/African-American           | 3  |
| Biracial                         | 3  |
| Not Available                    | 25 |
| **Smoking history**              |    |
| Yes                              | 7  |
| No                               | 5  |
| Not Available                    | 37 |
| **Marijuana use**                |    |
| Yes                              | 9  |
| No                               | 0  |
| Not Available                    | 40 |
| **Other medication use**         |    |
| Prenatal Vitamins                | 3  |
| Adderal, Albuterol, calcium carbonate, Divalaproex, ferrous sulfate, Omeprazole, Propylthiouracil, Sumatriptan, Varex, Xanax, Zafran, Zyrtec | 1 woman per medication |
| Not Available                    | 34 |
Figure S2. Placental sites (A) used in the analyses of site-dependent (B) and inter-day variability (C) of transporter abundance in three T2 placentae. Protein abundance was independent of the site of sampling or of the day of analyses when sampled from site 1 (C). Each bar represents mean±SD of data from 3 placentae (each trypsin-digested twice). Placental ID: H26938 (152 gestational days), H27003 (137 gestational days) and H27108 (115 gestational days). Kruskal-Wallis test with Dunn’s multiple comparisons was used for statistical analysis.
Figure S3. Protein abundance of apical and basal membrane transporters in human placentae of three gestational ages. Panel (A) represents values expressed per mg of MP. Abundance of P-gp was 56% lower at term than in T1 and 41% lower at term than in T2. Abundance of NET was 1.9-fold higher in T2 than in T1 and 2.8-fold higher at term than in T1. Abundance of OATP2B1 was 37% lower at T2 than in T1 and 40% lower at term than in T1. Five measurements for OATP2B1 in T2 group were below LLOQ and were assigned the conservative value of LLOQ (2.3 fmol on column). Gestational age did not affect BCRP, SERT, OAT4 and OCT3 protein abundance. Panel (B) represents values expressed per placenta. All the transporters showed significant increase with gestational age. Dots are observed values, lines are mean and standard deviations (T1 n=15; T2 n=19; Term n=15); Only significant differences are shown; Kruskal-Wallis Test with Dunn’s multiple comparisons, p>0.05.
Table S4. Protein abundance of apical and basal membrane transporters in human placentae of three gestational ages. Values are given as Mean ± SD and expressed in pmol/g tissue (N=49). T1 – 1st trimester, T2 – 2nd trimester.

| Transporter | Protein Abundance | %CV | Protein Abundance | %CV | Protein Abundance | %CV |
|-------------|-------------------|-----|-------------------|-----|-------------------|-----|
| Apical      |                   |     |                   |     |                   |     |
| BCRP        | 16.3±9.79         | 60.1| 12.8±5.97         | 46.6| 7.41±2.28         | 30.8|
| P-gp        | 14.7±8            | 54.4| 9.57±5.3          | 55.4| 4.57±2.67         | 58.4|
| SERT        | 8.78±3.93         | 44.8| 8.78±5.36         | 61.0| 5.95±1.86         | 31.3|
| NET         | 5.52±2.99         | 54.2| 8.55±4.61         | 53.9| 9.12±4.72         | 51.8|
| Basal       |                   |     |                   |     |                   |     |
| OAT4        | 17.1±7.11         | 41.6| 12.8±7.15         | 55.9| 20.7±11.6         | 56.0|
| OATP2B1     | 7.82±3.34         | 42.7| 5.31±5.21         | 98.1| 8.83±7.25         | 82.1|
| OCT3        | 12.4±4.47         | 36.0| 16±7.28           | 45.5| 25.1±11           | 43.8|
Figure S4. Comparison of transporter protein abundance in human placenta, kidney and liver as measured by quantitative targeted proteomics. Placental protein abundance data are from the current study and represent pooled data across the three gestational age groups; liver data (1, 2); kidney data (3). Data comparing abundance of SERT, NET and OCT3 are not shown as the corresponding data in the liver and kidney cortex are not available. N/A - data not available; # - below LLOQ.

Figure S8 References

1. Prasad B, Evers R, Gupta A, Hop CE, Salphati L, Shukla S, Ambudkar SV, Unadkat JD. Interindividual variability in hepatic organic anion-transporting polypeptides and P-glycoprotein (ABCB1) protein expression: quantification by liquid chromatography tandem mass spectroscopy and influence of genotype, age, and sex. Drug metabolism and disposition: the biological fate of chemicals. 2014;42(1):78-88.
2. Wang L, Collins C, Kelly EJ, Chu X, Ray AS, Salphati L, Xiao G, Lee C, Lai Y, Liao M, Mathias A, Evers R, Humphreys W, Hop CE, Kumer SC, Unadkat JD. Transporter Expression in Liver Tissue from Subjects with Alcoholic or Hepatitis C Cirrhosis Quantified by Targeted Quantitative Proteomics. Drug metabolism and disposition: the biological fate of chemicals. 2016;44(11):1752-8.
3. Prasad B, Johnson K, Billington S, Lee C, Chung GW, Brown CD, Kelly EJ, Himmelfarb J, Unadkat JD. Abundance of Drug Transporters in the Human Kidney Cortex as Quantified by Quantitative Targeted Proteomics. Drug metabolism and disposition: the biological fate of chemicals. 2016;44(12):1920-4.
Figure S5. Protein-protein correlation of placental transporter abundance (N=49). Correlations with Pearson correlation of $R^2 > 0.5$. 