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RESEARCH ARTICLE

Quantitative Proteomics Reveals Changes in Transporter Protein Abundance in Liver, Kidney and Brain of Mice by Pregnancy

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ARTICLE HISTORY

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Abstract: ***Background*:** Few studies have systematically investigated pregnancy-induced changes in protein abundance of drug transporters in organs important for drug/xenobiotic disposition.

***Objective*:** The goal of this study was to compare protein abundance of important drug/xenobiotic transporters including Abcb1a, Abcg2, Abcc2, and Slco1b2 in the liver, kidney and brain of pregnant mice on gestation day 15 to that of non-pregnant mice.

***Methods*:** The mass spectrometry-based proteomics was used to quantify changes in protein abundance of transporters in tissues from pregnant and non-pregnant mice.

***Results*:** The protein levels of hepatic Abcc2, Abcc3, and Slco1a4 per µg of total membrane proteins were significantly decreased by pregnancy by 24%, 72%, and 70%, respectively. The protein levels of Abcg2, Abcc2, and Slco2b1 per µg of total membrane proteins in the kidney were significantly decreased by pregnancy by 43%, 50%, and 46%, respectively. After scaling to the whole liver with consideration of increase in liver weight in pregnant mice, the protein abundance of Abcb1a, Abcg2, Abcc2, Abcb11, Abcc4, Slco1a1, and Slco1b2 in the liver was ~50-100% higher in pregnant mice, while those of Abcc3 and Slco1a4 were ~40% lower. After scaling to the whole kidney, none of the transporters examined were significantly changed by pregnancy. Only Abcg2 and Abcb1a were quantifiable in the brain and their abundance in the brain was not influenced by pregnancy.

***Conclusion*:** Protein abundance of drug transporters can be significantly changed particularly in the liver by pregnancy. These results will be helpful to understand pregnancy-induced changes in drug/xenobiotic disposition in the mouse model.

Keywords: Pregnancy, pregnant mice, transporter, liver, kidney, brain, quantitative proteomics, mass spectrometry.

1. INTRODUCTION

Pregnant women undergo extensive physiological changes such as increase in production of pregnancy-related hormones which are essential for maintenance of normal pregnancy and proper fetal growth and development [1, 2]. Such changes can affect drug disposition during pregnancy by altering expression of drug disposition genes [3]. While changes in expression or activity of drug-metabolizing enzymes by pregnancy have been extensively studied [4-8], few studies have systematically investigated pregnancy-induced changes in protein abundance or activity of drug transporters in organs important for drug disposition, such as the liver, kidney and brain. Several studies have evaluated changes in mRNA expression for some transporters during pregnancy [9, 10]; however, mRNA expression is not always correlated with protein expression or activity. To the best of our knowledge, few previous studies have offered a comprehensive evaluation of the effects of pregnancy on protein abundance of a variety of drug transporters important for drug disposition simultaneously in multiple organs. In the present study, we chose pregnant mice as the animal model to conduct such analyses as we have previously shown that the Pharmacokinetic (PK) changes of several drugs in pregnant women can be replicated in the pregnant mouse model [5, 7, 11]. Knowledge about the changes in drug transporter abundance by pregnancy is important for understanding of pregnancy-induced alternations in drug/xenobiotic PK, efficacy and safety.

Thus, the goal of this study was to quantitatively examine the effects of pregnancy on protein abundance of various drug transporters important for drug/xenobiotic disposition, including Abcb1a, Abcg2, Abcc2, Slco1a1 and other transporters, in the liver, kidney and brain of mice. Using the highly sensitive liquid chromatography tandem-mass spectrometry (LC-MS/MS)-based quantitative proteomics with Multiple Reaction Monitoring (MRM) [12, 13], we ob-

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served tissue-specific effects of pregnancy on protein abundance of drug transporters in mice.

2. MATERIALS AND METHODS

2.1. Materials

Optima grade or high-performance liquid chromatography grade methanol, acetonitrile and water were from Thermo Fisher Scientific (Waltham, MA) or Acros Organics (Pittsburgh, PA). Isoflurane was from Piramal Healthcare (Mumbai, India) through the University of Washington Medical Center Pharmacy. Synthetic heavy signature peptides were obtained from Pierce Biotechnology (Rockford, IL). The corresponding stable-isotope-labeled (SIL) peptides were from Thermo Fisher Scientific. ProteoExtract native membrane protein extraction kit was from Calbiochem (Temecula, CA). Ammonium bicarbonate and sodium deoxycholate were from Thermo Fisher Scientific and MP Biomedicals (Santa Ana, CA), respectively. BCA protein assay and in-solution trypsin digestion kits, iodoacetamide and dithiothreitol were obtained from Pierce Biotechnology (Rockford, IL).

2.2. Animal Studies and Quantitative Proteomics Analysis

Animal studies were approved by the Institutional Animal Care and Use Committee of the University of Washington. Animals were cared for in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Research Council. Wild-type FVB mice, 7-10 weeks old, were purchased from Taconic Farms (Germantown, NY). Mice were maintained under 12-h light/dark cycles, and food and water were provided *ad libitum*. Pregnant mice on gestation day (gd) 15 were obtained and maternal tissues (liver, kidney and brain) were collected as previously described [7, 14]. Gd 15 was selected as we previously showed that mRNA levels of most drug-metabolizing enzymes and transporters in the liver and kidney reached the maximal changes on gd 15 and remained relatively unchanged after gd 15 [10].

Protein quantification of transporters in the liver, kidney and brain of pregnant and non-pregnant mice by quantitative LC-MS/MS proteomics analysis. Quantification of changes in protein levels of Abcb1a (a P-gp isoform), Abcb1b (a P-gp isoform), Abcg2 (Bcrp), Abcb11 (Bsep), Abcc2 (Mrp2), Abcc3 (Mrp3), Abcc4 (Mrp4), Slco1a1 (Oatp1a1), Slco1a4 (Oatp1a4), Slco2b1 (Oatp2b1) and Slc22a3 (Oct3) in the liver, kidney and brain (Table **1**) of pregnant and non-pregnant mice was carried out using a surrogate peptide-based LC-MS/MS method as previously described [7, 14, 15]. Transporter protein was quantified by the surrogate peptide LC-MS/MS method (https://ascpt.onlinelibrary.wiley.com/doi/abs/10.1002/cpt.819). Briefly, the total membrane fraction was first isolated from the tissues and the transporter proteins were digested using trypsin. A unique surrogate peptide for each protein was identified using peptide databases, verified in the sample, and then quantified by LC-MS/MS proteomics. Corresponding stable labeled peptides were used as internal standard to address post-digestion sample-to-sample variability including matrix effect or ionization suppression in the LC-MS/MS. The relative quantification data were generated by normalizing the ratio of native peptide to internal standard signals by the total protein amount used for the digestion. The precision of the method relies on a reasonable assumption that trypsin digestion generates a consistent amount of surrogate peptides each time. One or two signature peptides unique for the transporters used to quantify each transporter were shown in supplemental Table **S1**. The corresponding heavy peptides containing labeled [$^{13}C_6$ $^{15}N_2$]-lysine or [$^{13}C_6$ $^{15}N_4$]-arginine were used as internal standards (Table **S1**). Total membrane proteins were isolated from mouse tissues, reduced, denatured, alkylated, desalted and digested as previously described [7, 13, 14]. Bovine serum albumin (BSA) and heavy peptide internal standard cocktail were added to each sample before trypsin digestion [16].

The chromatographic conditions and instruments used were the same as previously described [7, 14]. Relative protein levels of individual transporters were presented as the peak area ratios of analyte peptides to the corresponding IS peptides, and then the ratios were normalized to BSA and the ratios of respective transporters in pooled tissue homogenates. Pooled tissue homogenates were prepared by pooling individual liver, kidney and brain samples from the same experiments for the purpose of normalization. Normalization to BSA and internal standard was necessary to reduce variations and correct for ion suppression between sample preparations [16]. Normalization to pooled tissue homogenates was used as batch-to-batch quality controls. All experiments were performed with the same amount of total membrane proteins for trypsin digestion. Relative protein levels of individual transporters in total membrane proteins were expressed as relative protein levels of individual transporters per μg of total membrane proteins used for trypsin digestion. Relative protein levels of individual transporters in total membrane proteins were then scaled to the whole organ using the following equation.

Relative protein abundance of a transporter in a whole mouse organ (liver, kidney or brain) = relative protein levels of the transporter per μg of total membrane proteins × yield of total membrane protein isolation (μg per g of tissue) × the weight of the whole mouse organ (g) isolated from respective mouse.

Data obtained from the above analyses can only be used to compare relative protein abundance of the same transporter in the same type of tissue from pregnant and non-pregnant mice, but not across different transporters in the tissues.

2.3. Statistical Analysis

All data were presented as means of SD (n = 9) of three independent mouse tissues from three different mice, with each tissue analyzed in three technical replicates. The yields of total membrane protein isolation and tissue weights were also expressed as means ± SD (n = 3) of three independent mouse tissues from three different mice. Statistical analysis by unpaired Student's *t*-test was performed using the GraphPad Prism version 5 software (La Jolla, CA). Differences between pregnant and non-pregnant groups with p values of < 0.05 were considered statistically significant.

Table 1. Summary of the patterns of pregnancy-induced changes in protein levels or abundance of drug transporters analyzed in this study.

Changes in Transporter Protein Levels Per µg of Total Membrane Proteins (First Column Under Tissue) and in Transporter Protein Abundance Scaled to Whole Organ (second column under tissue)						
	Liver		**Kidney**		**Brain**	
Abcb1a (a P-gp isoform)	↔	↑	↔	↔	↔	↔
Abcb1b (a P-gp isoform)	NQ	NQ	NQ	NQ	NQ	NQ
Abcb11 (Bsep)	↔	↑	ND	ND	ND	ND
Abcg2 (Bcrp)	↔	↑	↓	↔	↔	↔
Abcc2 (Mrp2)	↓	↑	↓	↔	ND	ND
Abcc3 (Mrp3)	↓	↓	↔	↔	ND	ND
Abcc4 (Mrp4)	↔	↑	NQ	NQ	NQ	NQ
Slc22a3 (Oct3)	NQ	NQ	NQ	NQ	NQ	NQ
Slco1a1 (Oatp1a1)	↔	↑	ND	ND	NQ	NQ
Slco1a4 (Oatp1a4)	↓	↓	↔	↔	NQ	NQ
Slco1b2 (Oatp1b2)	↔	↑	ND	ND	ND	ND
Slco2b1 (Oatp2b1)	NQ	NQ	↓	↔	NQ	NQ

Statistically significant increase or decrease in protein levels or abundance of transporters in the liver, kidney or brain of pregnant mice compared to non-pregnant controls is designated as ↑ or ↓, respectively. No significant changes are denoted as ↔. NQ indicates transporters at levels detectable, but below the quantification limit. ND indicates transporters at levels below the detection limit.

Abcb1a, ATP-binding cassette, subfamily B member 1a; Abcb1b, ATP-binding cassette, subfamily B member 1b; Abcb11, ATP-binding cassette, subfamily B member 11; Abcg2, ATP-binding cassette, subfamily G member 2; Abcc2, ATP-binding cassette, subfamily C member 2; Abcc3, ATP-binding cassette, subfamily C member 3; Abcc4, ATP-binding cassette, subfamily C member 4; Slc22a3, Solute carrier family 22 member 3; Slco1a1, solute carrier organic anion transporter family member 1a1; Slco1a4, solute carrier organic anion transporter family member 1a4; Slco1b2, solute carrier organic anion transporter family member 1b2; P-gp, P-glycoprotein; Bsep, bile salt export pump; Bcrp, breast cancer resistance protein; Mrp, multidrug resistance-associated protein; Oatp, organic anion-transporting polypeptide; Oct, organic cation transporter.

3. RESULTS

3.1. Overall Protein Expression Patterns of Drug Transporters

We first quantified relative protein levels of 12 drug transporters known to be important for drug disposition in total membrane proteins isolated from the liver, kidney and brain of pregnant and non-pregnant FVB mice. Several previous studies have determined relative protein expression of some of these transporters in pregnant mice with less sensitive and non-specific immunoblotting [17-19]. In the present study, we used highly sensitive and selective LC-MS/MS proteomics to simultaneously analyze the impact of pregnancy on protein expression of these transporters in mice. Of the 12 drug transporters, all transporters were detectable and 9 were quantifiable in the liver (Table **1**). However, only 6 transporters were quantifiable, 3 detectable and 3 undetectable in the kidney; 2 quantifiable, 6 detectable and 4 undetectable in the brain. Therefore, it appears that drug transporters generally have higher levels of protein expression in the liver than in the kidney and brain, with most of the transporters quantifiable in the liver, but only detectable or undetectable in the kidney or brain (Table **1**). Notably, Abcb1a, but not Abcb1b, could be quantified in the liver, suggesting that Abcb1a is the major murine P-gp isoform in the liver. Slo22a3 (Oct3) could be detected, but not quantifiable in all three organs.

3.2. Relative Protein Abundance of Drug Transporters in the Liver

When the protein levels of quantifiable transporters were expressed as relative protein levels per µg of total membrane proteins in the liver, we found that pregnancy significantly decreased the protein levels of Abcc2, Abcc3, and Slco1a4 by ~24%, ~72%, ~70%, respectively (Fig. **1**). The relative protein levels of Abcg2, Abcb11, Abcb1a, Abcc4, Slco1a1 and Slco1b2 per µg of total membrane proteins in the liver did not significantly differ between pregnant and non-pregnant mice (Fig. **1**).

We noticed that the yields of total membrane protein isolation from the mouse livers were 0.08 ± 0.01 µg and 0.10 ± 0.02 µg per mg of liver for non-pregnant and pregnant mice, respectively. The mean liver weights were 1.3 ± 0.1 g and 2.1 ± 0.1 g per liver per mouse for non-pregnant and pregnant mice, respectively. Hence, this increase in liver weight of pregnant mice versus non-pregnant mice suggests that transporter protein abundance in the whole liver can be increased even if there is no difference in relative protein levels of transporters per µg of total membrane proteins. Indeed, after scaling to the whole liver, relative protein abundance of Abcg2, Abcb11, Abcb1a, Abcc2, Abcc4, Slco1a1 and Slco1b2 in the whole liver were ~50-100% higher in pregnant mice compared to non-pregnant mice (Fig. **2**). The relative protein abundance of only Abcc3 and Slco1a4 in the

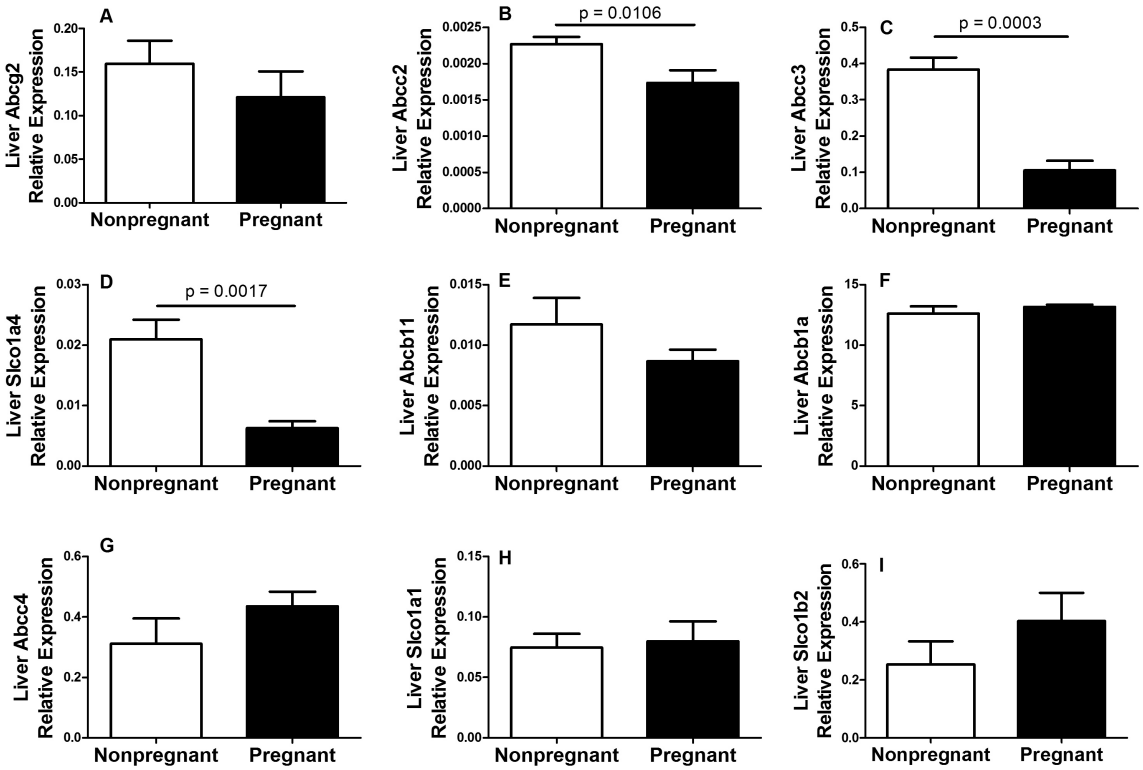

Fig. (1). Relative protein expression of transporters in total membrane proteins isolated from the livers of pregnant and non-pregnant mice. Relative protein levels of Abcg2 (**A**), Abcc2 (**B**), Abcc3 (**C**), Slco1a4 (**D**), Abcb11 (**E**), Abcb1a (**F**), Abcc4 (**G**), Slco1a1 (**H**) and Slco1b2 (**I**) in total membrane proteins isolated from the livers of pregnant (filled bars) and non-pregnant (open bars) mice.

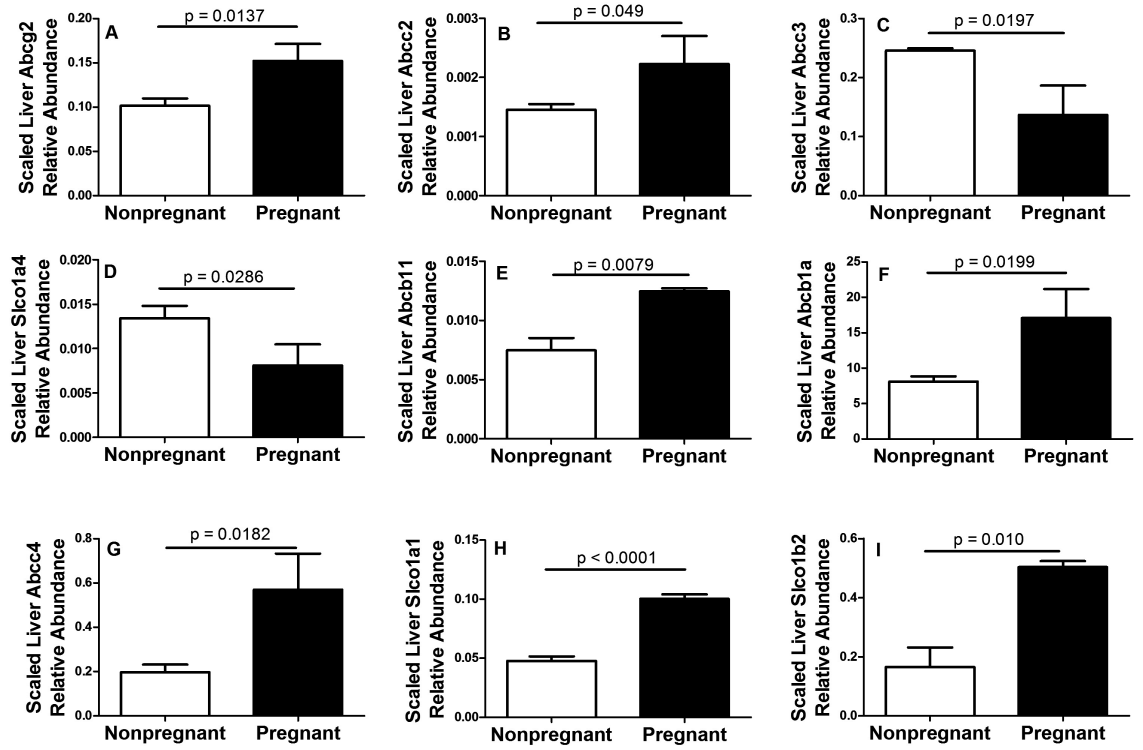

Fig. (2). Relative protein abundance of transporters in the liver of pregnant and non-pregnant mice after scaling to the whole liver. Relative protein abundance of Abcg2 (**A**), Abcc2 (**B**), Abcc3 (**C**), Slco1a4 (**D**), Abcb11 (**E**), Abcb1a (**F**), Abcc4 (**G**), Slco1a1 (**H**) and Slco1b2 (**I**) after scaling to the whole liver of pregnant (filled bars) and non-pregnant (open bars) mice.

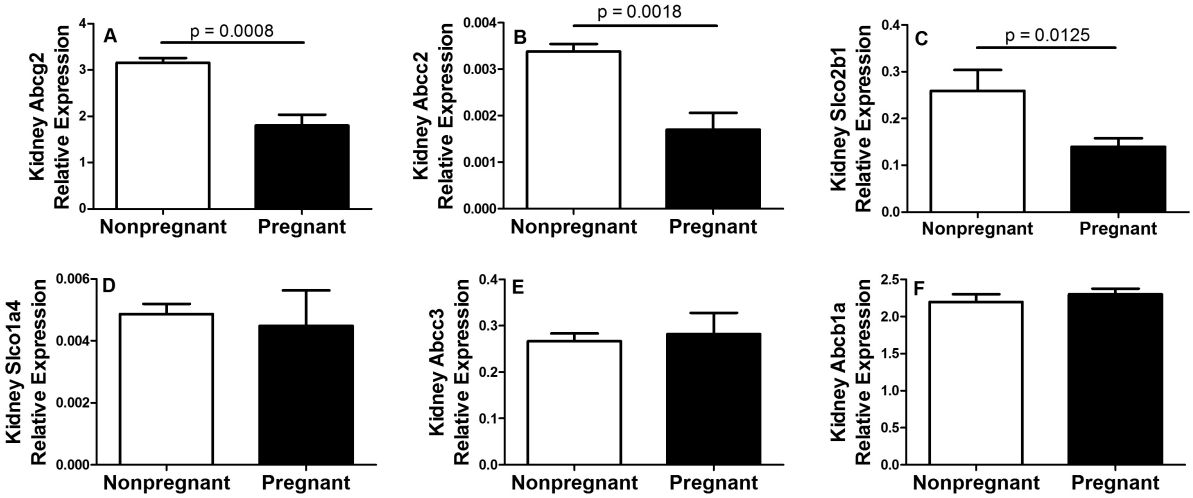

Fig. (3). Relative protein expression of transporters in total membrane proteins isolated the kidneys of pregnant and non-pregnant mice. Relative protein levels of Abcg2 (**A**), Abcc2 (**B**), Slco2b1 (**C**), Slco1a4 (**D**), Abcc3 (**E**) and Abcb1a (**F**) in total membrane proteins isolated from the kidneys of pregnant (filled bars) and non-pregnant (open bars) mice.

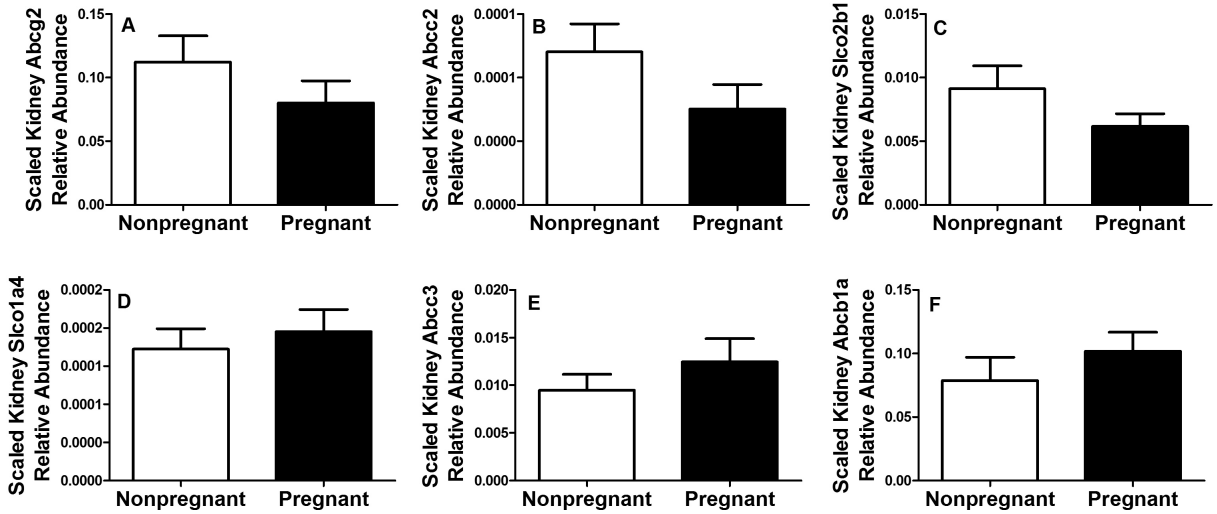

Fig. (4). Relative protein abundance of transporters in the kidney of pregnant and non-pregnant mice after scaling to the whole kidney. Relative protein abundance of Abcg2 (**A**), Abcc2 (**B**), Slco2b1 (**C**), Slco1a4 (**D**), Abcc3 (**E**) and Abcb1a (**F**) after scaling to the whole kidney of pregnant (filled bars) and non-pregnant (open bars) mice.

whole liver were decreased by ~40% in pregnant mice (Fig. **2**), which is because the increase in liver weight does not compensate the drastic decrease in relative protein levels of Abcc3 and Slco1a4 per μg of total membrane proteins.

3.3. Relative Protein Abundance of Drug Transporters in the Kidney

Among the transporters quantifiable in the kidney, we found that pregnancy significantly decreased the relative protein levels of Abcg2, Abcc2 and Slco2b1 per μg total membrane proteins by approximately 40-50% (Fig. **3**). The relative protein levels of Slco1a4, Abcc3 and Abcb1a per μg of total membrane proteins in the kidney were not significantly changed by pregnancy (Fig. **3**).

The kidney weights were 94 ± 2 mg and 108 ± 2 mg per kidney per mouse for non-pregnant and pregnant mice, respectively. The kidney weights of pregnant mice were ~16% larger than those of non-pregnant mice, which is similar to a previous report [20]. The yields of total membrane protein isolation from the mouse kidneys were 0.06 ± 0.01 μg and 0.07 ± 0.01 μg per mg of kidney for non-pregnant and pregnant mice, respectively, which did not significantly differ between pregnant and non-pregnant mice. After scaling to the whole kidney, the relative protein abundance of all the transporters quantifiable in the whole kidney was not significantly altered by pregnancy (Fig. **4**).

3.4. Relative Protein Abundance of Drug Transporters in the Brain

Among 12 transporters analyzed, only two, namely Abcg2 and Abcb1a, were quantifiable in the brain (Fig. **5**), indicating that mouse Abcg2 and Abcb1a are the major transporters in the brain. Indeed, Abcg2 and Abcb1a have been shown to be the two major efflux transporters in the

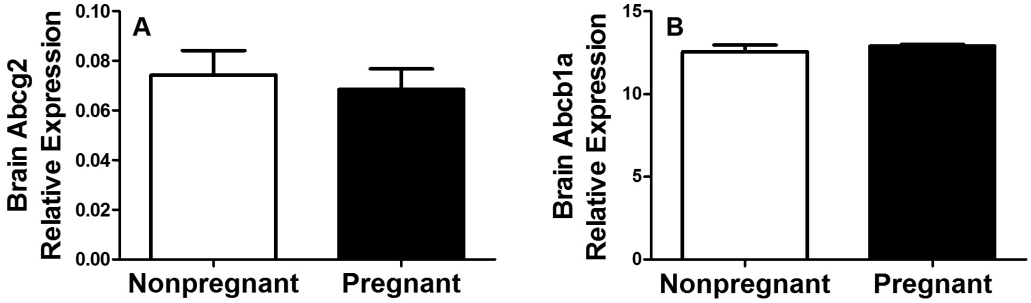

Fig. (5). Relative protein expression of transporters in total membrane proteins isolated from the brains of pregnant and non-pregnant mice. Relative protein levels of Abcg2 (**A**) and Abcb1a (**B**) in total membrane proteins isolated from the brains of pregnant (filled bars) and non-pregnant (open bars) mice.

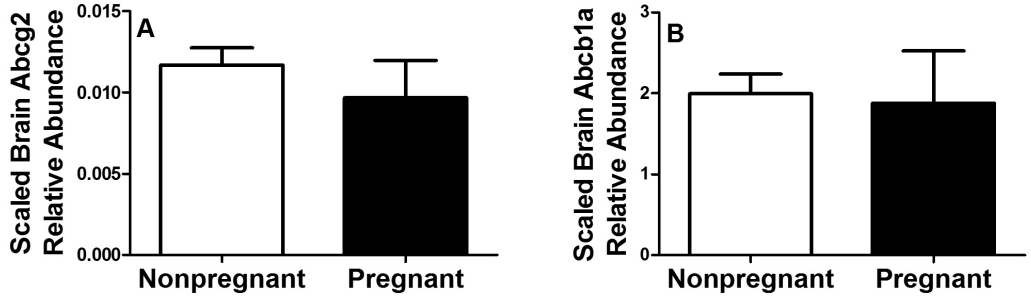

Fig. (6). Relative protein abundance of transporters in the brain of pregnant and non-pregnant mice after scaling to the whole brain. Relative protein abundance of Abcg2 (**A**) and Abcb1a (**B**) after scaling to the whole brain of pregnant (filled bars) and non-pregnant (open bars) mice.

blood-brain barrier [21]. Since pregnancy did not significantly change brain weight (504 ± 12 mg per brain per pregnant or non-pregnant mouse) and the yields of total membrane protein isolation from the mouse brains (0.05 ± 0.01 μg per mg of brain for pregnant or non-pregnant mouse), we found that pregnancy had no significant effects on relative protein abundance of Abcg2 and Abcb1a in the brain, no matter whether based on normalization to total membrane proteins (Fig. **5**) or after scaling to the whole brain (Fig. **6**).

4. DISCUSSION

The present study systematically investigated the effects of pregnancy on protein expression and abundance of a variety of drug transporters in the liver, kidney, and brain in the mouse model. Pregnancy caused significant changes in transporter protein expression or abundance in a tissue-dependent manner, with most changes occurring in the liver (Table **1**). Relative protein levels of Abcc2, Abcc3, and Slco1a4 per μg of total membrane proteins in the liver were downregulated by pregnancy (Fig. **1**), which were similar to prior reports of these transporters for protein (Abcc2 and Abcc3) or mRNA (*Abcc3* and *Slco1a4*) expression [10, 19, 22, 23]. Relative protein levels of hepatic Abcg2, Abcb11, Abcb1a, Abcc4, Slco1a1 and Slco1b2 in total membrane proteins did not significantly differ between pregnant and non-pregnant mice (Fig. **1**), which are consistent with previous studies with immunoblotting [19, 22, 24].

After scaling to the whole liver by incorporating changes in liver weight and yield of total membrane protein isolation, relative protein abundance of Abcg2, Abcb11, Abcb1a, Abcc2, Abcc4, Slco1a1 and Slco1b2 in the whole liver of pregnant mice was significantly increased compared to non-pregnant mice with the exception of Abcc3 and Slco1a4 (Fig. **2**). A previous study reported no effects of pregnancy on relative protein levels of mouse P-gp in hepatic S-9 fractions detected by immunoblotting [24], similar to what we showed in Fig. (**1F**). However, the relative protein abundance of Abcb1a in the whole liver of pregnant mice was increased by ~50% compared to non-pregnant mice (Fig. **2F**). Therefore, caution should be taken when we use data of relative protein levels of transporters that are normalized to total membrane proteins to explain changes in drug disposition *in vivo*. Based on our findings, protein abundance data of transporters scaled to the whole liver is recommended and would be more reliable to explain changes in *in vivo* PK. We recently showed that the systemic exposure of norbuprenorphine-β-D-glucuronide, a possible Abcg2 substrate, was significantly decreased in pregnant mice compared to non-pregnant controls, even if hepatic metabolic formation of norbuprenorphine-β-D-glucuronide was significantly increased [7]. One possible explanation of this observation may be the increase in hepatic Acbg2 protein abundance in the whole liver as we showed in this study (Fig. **2**) which could increase biliary excretion of norbuprenorphine-β-D-glucuronide to the bile, thus facilitating norbuprenorphine-β-D-glucuronide elimination in pregnant mice. The increase in protein abundance of Abcc2 and Abcb11 in the whole liver may contribute to alterations of bile acid disposition pathway during pregnancy [19] and may be a compensatory effect for pregnancy-induced down-regulation of hepatic Abcc3 [25]. Consistent with protein expression changes observed in this study, Abcc3 showed the most significant reduction in mRNA levels among all transporters analyzed in the liver [10].

The data regarding changes in relative protein expression of transporters in the kidney by pregnancy are generally consistent with previous studies. For example, Abcc2 protein levels in membrane protein preparations of mouse kidney

were also shown to be significantly decreased; and Abcc3 protein expression in mouse kidney was not changed, by pregnancy [17]. Likewise, previous studies also have shown that the protein levels of mouse P-gp in total membrane proteins of the kidney were not significantly altered by pregnancy [17, 24]. The mRNA levels of *Slco2b1* in mouse kidney were not significantly affected by pregnancy [17], which is not consistent with the protein data of this study for Slco2b1 based on total membrane proteins (Fig. **3C**). Similarly, we previously showed that *Abcb1a* mRNA in mouse kidney was significantly decreased by pregnancy [10], but in this study, we found no significant changes in Abcb1a protein levels in the mouse kidney based on total membrane proteins (Fig. **3F**). This is likely due to the fact that mRNA expression is not always correlated with protein expression. Of noted, the protein levels of Abcg2 in the kidney based on total membrane proteins were previously shown to be not significantly affected by pregnancy [17]; however, we found a 43% reduction (Fig. **3A**). Nevertheless, after scaling to the whole kidney with consideration of the increase in kidney weight by pregnancy, all differences in protein abundance of these transporters quantifiable in the kidney disappeared (Fig. **4**). Thus, caution should be taken when we use data of relative protein levels of transporters in the kidney that are normalized to total membrane proteins to explain changes in renal clearance. Again, protein abundance data of transporters scaled to the whole kidney is recommended to explain changes in renal drug clearance *in vivo*. This study also have shown that protein expression or abundance of two major drug transporters Abcb1a and Abcg2 in the blood-brain barrier are not affected by pregnancy (Fig. **5** and Fig. **6**), suggesting that pregnancy possibly does not influence transfer of drugs that are substrates of the two transporters across the blood-brain barrier.

We also observed a 30% decrease in Na^+/K^+-ATPase per μg of total membrane proteins in the liver, but not in the kidney and brain (data not shown). Since Na^+/K^+-ATPase is marker protein in the plasma membrane, pregnancy-induced changes in transporter protein expression particularly in the liver appears to be a general phenomenon. Mechanisms of the diverse changes in transporter protein expression observed in this study remain elusive. It is possible that pregnancy-related hormones that are increasingly produced over the course of pregnancy may contribute to the changes (induction or down-regulation) in transporter protein expression during pregnancy. For example, it was shown that ethinylestradiol, the semisynthetic estrogen, administered to rats decreased Mrp2 protein levels in the liver [26]. Thus, the increased estrogen levels during pregnancy could possibly down-regulate Mrp2 protein expression in the liver. Post-transcriptional regulation that alters protein synthesis and degradation [27] may also contribute to the decrease in Abcc2 protein expression in the liver and kidney during pregnancy. Epigenetic regulation through miRNAs may repress Abcc3 expression in the liver [28].

We realize that this study is not without limitations. This study did not evaluate pregnancy-induced changes in protein expression of transporters in small intestine. Small intestine is critical for drug absorption, and expression of drug transporters in the small intestine could also be altered by pregnancy. Such analyses will be an important topic of investigation in future studies. Due to technical limitations and small amounts of tissues available, we were not able to separate different membranes (*e.g.*, plasma and intracellular membranes) or specific cells in tissues and therefore this study could not evaluate changes in protein expression or abundance of drug transporters in specific membranes or cells within the tissues. However, the relative changes in protein expression or abundance of the transporters reported in this study remain the same as long as pregnancy does not affect membrane or cellular localization of these transporters. Future studies would be important to confirm whether pregnancy induces similar changes in activity of these transporters *in vivo* as the changes in protein abundance observed in this study.

CONCLUSION

In summary, quantitative proteomics analysis of this study revealed significant pregnancy-induced changes in protein abundance of major drug/xenobiotic transporters in mice particularly in the liver. Besides changes in protein levels of the transporters based on total membrane proteins isolated from mouse organs, the increases in organ weight by pregnancy also contributed to changes in protein abundance of transporters in the whole organ (*e.g.* the liver) even if the protein levels of transporters per μg of total membrane proteins were not changed (*e.g.*, Abcg2 in the liver) or even decreased (*e.g.*, Abcc2 in the liver). The data obtained in this study will be helpful to understand pregnancy-induced changes in drug/xenobiotic disposition by altering protein abundance of transporters in the liver in the mouse model.

AUTHOR'S CONTRIBUTIONS

Participated in research design: Liao, Prasad, Mao

Conducted Experiments: Liao, Gao, Bhatt

Performed Data Analysis: Liao, Bhatt

Wrote or contributed to the writing of the manuscript: Liao, Gao, Bhatt, Prasad, Mao

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Animal studies were approved by the Institutional Animal Care and Use Committee of the University of Washington. Animals were cared for in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Research Council. Wild-type FVB mice, 7-10 weeks old, were purchased from Taconic Farms (Germantown, NY).

HUMAN AND ANIMAL RIGHTS

No humans were involved in this study, the reported experiments on animals were in accordance with the standards set forth in the 8th Edition of Guide for the Care and Use of Laboratory Animals (http:// grants.nih.gov/grants/olaw/ Guide-for-thecare-and-use-of-laboratory-animals.pdf) published by the National Academy of Sciences.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

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