Biology of Reproduction, 2018, 99(6), 1255–1265
doi:10.1093/biolre/ioy148
Research Article
Advance Access Publication Date: 23 June 2018

Research Article

Temporal transcriptomic analysis of metabolic genes in maternal organs and placenta during murine pregnancy†

Alison Paquette[1], Priyanka Baloni[1], Anisa B. Holloman[1], Sanjay Nigam[2], Theo Bammler[3], Qingcheng Mao[4] and Nathan D. Price[1,*]

[1]Institute for Systems Biology, Seattle, Washington, USA; [2]Departments of Pediatrics and Medicine, University of California San Diego, San Diego, California, USA; [3]Department of Environmental and Occupational Health Science, School of Public Health, University of Washington, Seattle, Washington, USA and [4]Department of Pharmaceutics, School of Pharmacy, University of Washington, Seattle, Washington, USA

*__Correspondence:__ Institute for Systems Biology, 401 Terry Avenue N, Seattle, WA 98119, USA. Tel: (+206) 732-1204; E-mail: Nathan.Price@systemsbiology.org

†__Grant Support:__ Bill and Melinda Gates Foundation: Systems Biology Approaches to Birth Timing and Preterm Birth Risk, Eunice Kennedy Shriver National Institute of Child Health & Human Development of the National Institutes of Health: Grant R01HD091527 and U54HD090259, National Institutes of Health: National Institute for Drug Abuse Grant DA032507, National Institutes of Health: Center for Exposures, Diseases, Genomics, and Environment - Grant P30 ES007033.
Conference Presentation: This work was presented in part at the 2017 Keystone Conference "Maternal Fetal Crosstalk; Harmony vs. Conflict", October 4–8, Washington DC, USA.
Edited by Dr. Romana Nowak, PhD, University of Illinois Urbana-Champaign.

Received 5 April 2018; Revised 22 May 2018; Accepted 22 June 2018

Abstract

Maternal pregnancy adaptation is crucial for fetal development and long-term health. Complex interactions occur between maternal digestive and excretory systems as they interface with the developing fetus through the placenta, and transcriptomic regulation in these organs throughout pregnancy is poorly understood. Our objective is to characterize transcriptomic changes across gestation in maternal organs and placenta. Gene expression was quantified in the kidney, liver, and small intestine harvested from nonpregnant and pregnant FVB mice at four time points and placenta at three time points (N = 5/time point) using Affymetrix Mouse Gene 1.0 ST arrays. In maternal organs, we identified 476 genes in the liver, 207 genes in the kidney, and 27 genes in the small intestine that were differentially expressed across gestation (False Discovery Rate [FDR] adjusted $q < 0.05$). The placenta had a total of 1576 differentially expressed genes between the placenta at either/gd15 or gd19 compared to gd10. We identified a number of pathways enriched for genes differentially expressed across gestation, including 5 pathways in the placenta, 9 pathways in the kidney, and 28 pathways in the liver, including the citrate cycle, retinol metabolism, bile acid synthesis, and steroid bile synthesis, which play functional roles in fetal development and pregnancy maintenance. Characterization of normal longitudinal changes that occur in pregnancy provides context to understand how perturbations in these biochemical pathways and perturbations in nutrient signaling may impact pregnancy.

Summary Sentence

We have characterized differences in metabolic genes and pathways in maternal organs (kidney, liver, small intestine) and placenta of mice over the course of gestation to understand metabolic adaptation to pregnancy.

Key words: gene regulation, gene expression, metabolism, placental transport, pregnancy, rodents.

Introduction

During pregnancy, women must adapt to the increasing energy supplies required for the exponential growth and development of their fetus. To sustain fetal development, the mother must provide glucose, amino acids, and fatty acids, which are transported across the placenta [1]. Pregnancy is a dynamic process, with different metabolic requirements as fetal development progresses. In the first and second trimesters, the mother stores nutrients and fetal growth is limited; and in the third trimester rapid fetal growth occurs and the maternal physiology must shift to adapt to this growth, entering a catabolic state and increasing lipid breakdown [2]. The placenta is the master regulator of fetal–maternal interactions and must undergo similar dynamic changes to adapt to fetal growth across trimesters by transporting more nutrients across the placenta in late gestation. This process involves changes in transporter gene expression and pathways related to nutrient transport, and production of hormones required for pregnancy maintenance and fetal growth [3]. Changes in placental nutrient transporters are associated with pregnancy conditions including intrauterine growth restriction, gestational diabetes, and maternal obesity [1]. Biochemical processes which are responsible for generation and transportation of crucial nutrients have significant impacts on fetal development, pregnancy progression, and maternal and fetal health outcomes.

The generation and transport of metabolites (including nutrients and hormones) is regulated by a series of cellular metabolite transport mechanisms, which have been extensively characterized through genome-scale metabolic network reconstructions [4, 5]. These models provide crucial insight into precursor molecules and biochemical processes required for specific metabolic events, and characterize specific genes which are involved in biosynthesis and metabolism of nutrients and hormones [6]. Longitudinal changes in the maternal metabolome have been observed across gestation in maternal plasma captured using mass spectrometry [7], which provides evidence that profound metabolic changes occur during gestation which are reflected in metabolite concentration differences detectable in plasma. However, maternal plasma measurements do not directly capture the organ within which the changes are occurring.

The small intestine chemically digests food and absorbs nutrients, including gut microbiome metabolites, which pass through intestinal mucosal barrier to enter blood circulation. During pregnancy, histomorphometric changes occur in components of the small intestinal wall as a response to increasing nutrient intake, including increased villi length and changes in muscular thickness [8]. Concurrently, the small intestine undergoes positional changes and decreased motility as it is shifted during rapid fetal growth that occurs in late gestation [9]. The nutrients absorbed from the intestine lumen are metabolized by the liver, a multifaceted organ that is responsible for lipid and carbohydrate metabolism, xenobiotic metabolism, and biosynthesis and breakdown of hormones and other bioproducts to metabolites. The liver also adapts to increasing nutrient intake and provides adequate building blocks for fetal growth. Hepatic glucose production increases by 16–30% in order to provide energy for the increasing needs of placenta and fetus [10]. Pregnancy-related changes in other liver-related proteins are detectable in serum, including albumin, bilirubin, alanine transaminase, and alkaline phosphatase [11]. The kidneys re-absorb nutrients including glucose, amino acids, and sodium from the blood; regulate homeostasis; and remove waste produced by metabolic processes from the body. During pregnancy, renal plasma flow increases up to 80% [12], the glomerular filtration rate increases by 50%, and the maternal kidneys increase in size [13]. These adaptations allow the mother to provide the fetus with the necessary nutrients and hormones required for fetal growth. From a systems physiological perspective, these processes also provide highly dynamic remote inter-organ (gut-liver-kidney) and interorganismal (e, g. maternal-fetal, gut microbiome-mother) communication. Along with neuroendocrine and growth factor-cytokine mechanisms, this communication involves the flow of small organic molecules central to signaling and metabolism between tissues, organisms and body fluid compartments [14, 15].

The placenta is solely responsible for the transport of nutrients and metabolites from maternal blood to the developing fetus, and undergoes structural and functional changes throughout pregnancy to adapt to fetal needs. In humans, 25% of the placental transcriptome is differentially expressed between the first and third trimester, with genes upregulated in the first trimester responsible for angiogenesis and cellular proliferation, and genes in the third trimester involved with signal transduction, ion transport, and signaling [16]. These changes in gene expression have the potential to influence metabolic processes. Concurrently, nontargeted metabolomics analysis has identified 156 unique metabolomic signatures different between the first and third trimester placentae, including changes in di- and triglycerides, phospholipids, sphingolipids, fatty acids, and fatty acid carnitines [17]. These changes are thought to reflect increased utilization of fatty acids for energy production, and increased lipid signaling between the maternal and fetal interface. The placenta is also a source of hormone production during pregnancy which is essential for maintaining normal pregnancy, and the majority of progesterone is made by the placenta in late gestation in humans [18], although in mice, progesterone is produced by maternal ovaries and from fetal tissues. More work, as well as denser longitudinal sampling, is needed to understand the transcriptional processes that regulate these metabolic changes.

These types of longitudinal analyses of specific transcriptomic changes that occur in maternal organs across development are not feasible to perform in humans, but important knowledge can be gained using mouse models. Key components of pregnancy and partition are retained in both mice and humans, including the proteins that regulate birth timing [19], and both mouse and humans have hemochorial, discoid placentas [20]. However, there are dramatic differences in the molecular timing of early pregnancy that differ between mice and humans. Mice undergo a rapid eccentric implantation, where the luminal epithelium forms an invagination to surround the trophoblast [21]. In contrast, human implantation occurs interstitially, where the trophoblast passes through the luminal epithelium to invade the endometrial stroma [21]. The human placenta has more density of spiral arteries and the invasion is deeper [20], and

is characterized by a number of specific molecular features including HLA genes, microRNA clusters, and syncytians [22]. As there are drastic differences in the mechanisms of implantation, source of progesterone during pregnancy, and placental physiology, mouse pregnancy is an inaccurate reflection of human implantation. However, mouse models can shed insight into physiological changes that occur during pregnancy, and have been successfully used to measure changes in pregnancy related to endogenous compounds [23], xenobiotics [24], or toxin exposure [22] which can be recapitulated in humans. In this way, mouse models of pregnancy provide an important experimental system to capture temporal changes in pregnancy.

Previously, a mouse model of pregnancy was generated with the goal of performing longitudinal analysis of changes in maternal organs and placenta of mice during the course of pregnancy. This previous study identified significant changes in expression of genes related to xenobiotics, bile acid, and steroid hormone metabolism, and transport in maternal organs and placenta throughout the course of gestation [25]. However, expression changes of genes involved in metabolism of other substances within maternal organs and the placenta remains unclear, and these metabolic changes may have important implications for maternal adaption to pregnancy. The goal of the study described herein is to characterize transcriptomic changes in metabolic genes across gestation in maternal organs and placenta using the previously generated mouse model. This type of longitudinal, organ-specific analysis is not feasible to perform in humans. By understanding metabolic pathways that change during pregnancy, we will gain additional insight into which pathways are important for fetal growth.

Materials and methods

Sample generation

Transcriptomic data were generated from wild-type female FVB mice aged 7–10 weeks which were mated with male mice of the same age. Pregnancy progression was monitored by visual inspection and body weight increase, and gd1 was defined as the presence of a sperm plug after an overnight housing. Nonpregnant controls were derived from a population of female mice which had no exposure to males, and were defined as gestational day 0 (gd0). On gd 0, 7.5, 10, 15, and 19, female mice ($n = 5$ per gestational age) were sacrificed under anesthesia (isoflurane) by cardiac puncture, and the maternal liver, kidney, and small intestine were collected. Placentas were collected on gd 10, 15, and 19. All tissues were rinsed with phosphate-buffered saline, snap-frozen in liquid nitrogen, and stored at −80°C until use. Detailed description of the study design was previously described [25].

RNA isolation and microarray hybridization

Total RNA was isolated from organs using the miRNeasy mini kit (Qiagen, Valencia, CA). RNA integrity was assessed using the Agilent 2100 Bioanalyzer (Agilent Technologies Inc., Santa Clara, CA), and only samples with an RNA integrity number higher than 9 were considered acceptable for further processing. Processing of RNA samples was carried out according to the AffymetrixGeneChip Whole Transcript Sense Target labeling protocol. RNA was hybridized to the Affymetrix Mouse Gene 1.0 ST array. Arrays were scanned with the AffymetrixGeneChip 3000 scanner, and image generation and feature extraction were performed using the AffymetrixGeneChip Command Console software as previously described [25], and data are publicly available on the Gene Expression Omnibus [26] as GSE41438.

Multivariate gene level analyses

Raw data were summarized at the transcript level using Robust Multi-array average Method using the Bioconductor "oligo" package [27], and normalized using quantile normalization within the Linear Model for Microarray Analysis (LIMMA) Bioconductor package [28]. For genes which mapped to multiple probes, probe level expression values were averaged to generate gene level expression values. Prior to analysis, genes were mapped to human orthologs using biomaRt [29] and filtered to include only metabolomic genes as defined from the latest human metabolic reconstruction (Version 3) [5]. This expression data contained 2993 of the 3697 genes contained within the human metabolic reconstruction. Data variability was assessed using principal components analysis. Differences in gene expression over gestational time within maternal organs were calculated using linear regression implemented within LIMMA, with gene expression as the dependent variable and time point as a continuous independent variable. Genes were considered significantly different with an False Discovery Rate (FDR) adjusted $q < 0.05$. We elected not to perform continuous analysis of gene expression changes within the placenta as the time point data was not sufficiently dense (three time points).

Time series pathway enrichment analysis

Differences in gene expression at each time point (gd 7.5, 10, 15, or 19) compared to baseline (gd 0) within each tissue were determined using univariate comparisons within LIMMA, except in the placenta where gene expression at gd15 and gd19 was compared to gd10. Changes in gene expression were considered statistically significant with an FDR adjusted $q < 0.05$. These lists of differentially expressed genes (DEGs) were used to perform pathway enrichment analysis through a series of Fisher's exact tests implemented within R using the ClusterProfiler Package [30]. Gene sets were defined using Kyoto Encyclopedia of Genes and Genomes (KEGG) metabolic pathways, which included 90 gene sets with 5 or more genes. Pathways were considered statistically significant using a q value cutoff of 0.05. All data were analyzed in R, version 3.3.1. Pathways were visualized within Cytoscape [31] using the "Metscape" package for KEGG terms [32].

Results

Descriptive characteristics

In this study, we captured changes in gene expression in maternal organs (kidney, liver, small intestine) of pregnant mice at gestational time points gd7.5, gd10, gd15, and gd19, which are analogous to mid first trimester, beginning of second trimester, beginning of third trimester, and end of third trimester respectively, as well nonpregnant control mice which were considered gd0, or the absolute start of pregnancy. Placental tissues were collected at time points gd10, gd15, and gd19, and in this case we considered gd10 reflective of the start of pregnancy, as this is approximately when the placenta is formed in the mouse and when nutrient interactions between mother and fetus begin [33].

We assessed contributions to variation in our data across and between tissues using principal components analysis. In all tissues, 91% of the variation in the data was explained by the first three principal components, which in turn were all significantly associated

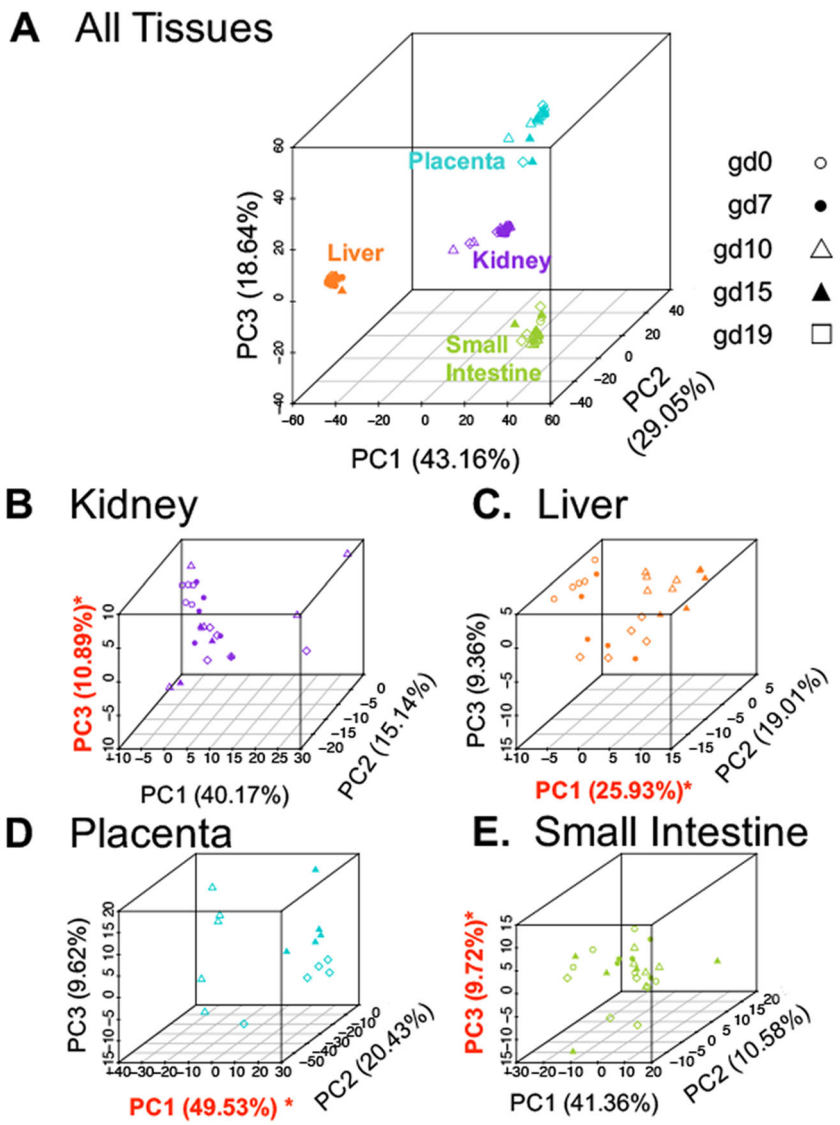


Figure 1. Principal component analysis of expression data. We assessed data variability and evaluated the first three principal components in the complete gene expression matrix (A) and individual tissue-specific matrixes (B–E). The data points are colored by tissue (liver = orange, kidney = purple, small intestine = green, placenta = cyan), and shape represents the gestational day. * on axis and red text represents significant association between principal component and gestational age ($P < 0.05$, analysis of variance). The percentages reflected in the X, Y, and Z axes reflect the percent variation of each principal component.

with tissue of origin ($P < 0.05$, analysis of variance), and not gestational day (Figure 1A, Table 1). We conclude that the data must be analyzed in a tissue-specific manner in order to identify changes related to gestational age. In the liver, the first principal component explained 26% of the variation in the data and was strongly associated with gestational day ($P = 4.8 \times 10^{-6}$), and in the placenta the first principal component explained 50% of the data and was also significantly associated with gestational day ($P = 3.14 \times 10^{-5}$) (Figure 1, Table 1). This indicates that the majority of variation in expression of metabolic genes is related to gestational age in this dataset. In both the kidney and small intestine, the third principal component (PC3) was significantly associated with gestational age ($P < 0.05$), but was responsible for very little of the variation in the data (11% and 10% respectively). This indicates that less variation in expression in metabolic genes is related to gestational age within these tissues (kidney and small intestine).

Transcriptomic changes across gestational time

We performed a series of linear regression models to model the changes in expression of metabolic genes in maternal organs across gestational time using LIMMA, with a significance cutoff of FDR adjusted $q < 0.05$. We identified the most differences in expression in the liver, including 322 genes which decreased across pregnancy and 154 genes which increased across gestation. In the kidney, we observed 108 genes which increased across gestation and 99 genes which decreased across pregnancy. We saw differential gene expression of only three genes in the small intestine (Figure 2A). We identified 13 genes in both the liver and kidney which were increased as pregnancy advanced, and 14 genes whose expression decreased as pregnancy advanced (Figure 2B and C). There were no overlapping DEGs between the small intestine and other organs. This indicates tissue-specific regulation of metabolic gene expression across gestation.

Table 1. Principal components analysis of metabolic gene expression data.

	% Variance	P (Time)*	P (Tissue)*
All data			
PC1	43.16	0.42	4.72E-92
PC2	29.05	0.98	7.28E-92
PC3	18.64	0.02	3.01E-76
Kidney			
PC1	40.17	0.11	–
PC2	15.14	0.87	–
PC3	10.89	1.33E-04	–
Liver			
PC1	25.93	4.80E-06	–
PC2	19.01	0.18	–
PC3	9.36	0.13	–
Placenta			
PC1	49.53	3.14E-05	–
PC2	20.43	0.86	–
PC3	9.62	0.21	–
Small intestine			
PC1	41.36	0.97	–
PC2	10.58	0.77	–
PC3	9.72	0.01	–

*Analysis of variance: Principal component compared to gestational day or tissue.

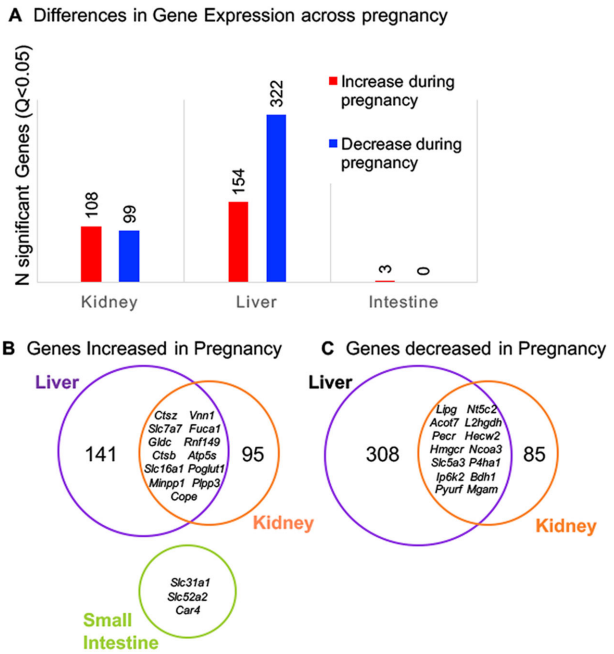


Figure 2. Linear regression analysis. Differences in gene expression at each time point compared to baseline within each tissue were determined using univariate comparisons within LIMMA, and genes were considered statistically significant with an FDR adjusted q < 0.05. (A) Barplots of the number of genes which significantly increased (positive estimate) and decreased (negative estimate) in each tissue in the linear models. (B) Venn diagram of the intersecting genes which were significantly increased in the kidney, liver, and small intestine. (C) Venn diagram of the intersecting genes which were significantly decreased in the kidney, liver, and small intestine.

The changes in expression over gestational time of top and bottom four significant genes sorted by logfold change in each tissue are shown in Table 2. In the liver, *Scd1*, *Elovl3*, *Lipg,* and *Lipc* decreased the most over the course of pregnancy, and *Slc41a2*, *Vnn1*, *Gpx3,* and *Cyp17a1* increased the most over pregnancy (Supplemental Figure S1A). In the kidney, *Bcat1, Hpd, Akr1d*, and *Hmgcr* decreased the most over the course of pregnancy, and *Rbp1, Hmgcs2, Alb*, and *Hdc* increased the most over pregnancy (Supplemental Figure S1B). In the small intestine, *Car4*, *Slc52a2*, and *Slc31a1* were the only genes significantly different, which increased during pregnancy (Supplemental Figure S1C). Overall, the liver had the greatest magnitude of changes in gene expression related to gestational age, and the small intestine minimal changes in gene expression related to gestational age.

Univariate changes in gene expression

We performed univariate analysis to compare gene expression in the liver, kidney, or placenta of pregnant mice at each gestational time point to gene expression in the same organ of nonpregnant mice (gd0). In the placenta, we compared expression on gd15 and gd19 to gd10 to generate gene lists for pathway enrichment analysis. Overall, we identified the highest differences in expression in the placenta, where 1576 genes were significantly differentially expressed in the placenta on gd15 and gd19 compared to gd10. Fifty-seven percent of these genes were differentially expressed at both time points, and 33% of these genes were differentially expressed only on gd15 (Figure 3A). We identified 891 genes which were differentially expressed in the liver, with the highest number of DEGs occurring specifically at gd15 (Figure 3B). We observed no significant changes on gd7 compared to baseline, indicating that changes in expression of metabolic genes occur during mid-gestation in the liver. We observed changes in expression of 533 genes in the kidneys, including changes in expression of genes on gd7 and gd10 (Figure 3C). We observed changes in expression for nine genes within the small intestine, mostly in late gestation (Figure 3D), which was insufficient to perform subsequent pathway analysis. Overall, we saw dramatic differences in the magnitude and timing of differential gene expression within maternal liver and kidney compared to nonpregnant mice as well as placental tissues of mice during different gestational periods.

Pathway analysis

We performed enrichment analysis to determine which metabolic pathways were enriched for the DEGs at each time point, and to compare these pathways across gestational time and across tissues. The complete results of our pathway enrichment analysis across all metabolic pathways for every time point and tissue are available in Supplemental Table S1. Pathways considered statistically significant ($P < 0.05$) are shown in Figure 4. Overall, we saw the highest number of statistically significant pathways in the liver (N = 28), with the highest number of pathways changing in mid-late gestation (gd15–gd19). The kidney had nine pathways enriched with DEGs, but with more changes in metabolic pathways occurred in early to mid-gestation (gd7–gd10). While the placenta had a higher magnitude of DEGs, there were only five pathways enriched for these DEGs, and these pathways were markedly distinct across gestational time and different from the pathways significant in other tissues. We performed supplemental pathway analysis using a more stringent statistical cutoff to generate the input gene list (FDR adjusted $q < 0.001$), and identified eight pathways which were enriched for DEGs, including three at GD15 and five at GD19

Table 2. Top differentially expressed genes identified in linear regression analyses.

	Gene name	Estimate	FDR adjusted Q
Liver			
Scd1	stearoyl-Coenzyme A desaturase 1	-0.11	3.77E-08
Elovl3	elongation of very long chain fatty acids (FEN1/Elo2, SUR4/Elo3, yeast)-like 3	-0.10	4.18E-04
Lipg	lipase, endothelial	-0.09	6.05E-06
Lipc	lipase, hepatic	-0.09	1.54E-04
Slc41a2	solute carrier family 41, member 2	0.12	2.29E-03
Vnn1	vanin 1	0.13	1.00E-05
Gpx3	glutathione peroxidase 3	0.15	6.28E-06
Cyp17a1	cytochrome P450, family 17, subfamily a, polypeptide 1	0.17	4.07E-08
Kidney			
Bcat1	branched chain aminotransferase 1, cytosolic	-0.08	1.56E-02
Hpd	4-hydroxyphenylpyruvic acid dioxygenase	-0.07	5.01E-03
Akr1d1	aldo-keto reductase family 1, member D1	-0.07	2.76E-02
Hmgcr	3-hydroxy-3-methylglutaryl-Coenzyme A reductase	-0.05	9.16E-03
Rbp1	retinol binding protein 1, cellular	0.06	1.59E-04
Hmgcs2	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2	0.11	1.39E-03
Alb	albumin	0.14	1.37E-02
Hdc	histidine decarboxylase	0.15	1.23E-03

compared to gd0 (Supplemental Table S2). This includes pathways that were also enriched in the placenta generated using less stringent criteria ($q < 0.05$), including glycosaminoglycan biosynthesis keratan sulfate, and glycosphingolipid biosynthesis—ganglio series, as well as some pathways that were also differentially expressed within other tissues, including pyruvate metabolism and steroid biosynthesis. While the placenta had a higher number of DEGs, we observed less enrichment of these changes within specific metabolic pathways. This indicates that the large number of DEGs were not primarily localized a specific set of pathways, but rather represented global differences across metabolism.

In the kidney, we observed different metabolic pathways that were enriched at different gestational time points, with limited pathway overlap, as shown in Figure 4. In early and mid-gestation, there was enrichment of genes involved in tryptophan metabolism and retinol metabolism (gd7) as well as arginine metabolism and steroid biosynthesis (gd10), which were unique from the pathways observed in late gestation (gd15–gd9). Here we observed differences in five additional pathways, including significant differences in genes involving biosynthesis of unsaturated fatty acids (gd15 and gd19), which was the only pathway significant in late gestation.

In the liver, we observed differences in nicotinate and nicotinamide metabolism and steroid biosynthesis at all time points across mid-late gestation (gd10–gd19). We observed a strong enrichment of genes involved in glycine, serine, and threonine metabolism at gd10, which trended toward significance ($P = 0.06$) at gd15. We saw a higher number of pathways that were significantly different in late gestation, including eight pathways that were significant at gd15, six pathways that were significant at gd19, and six pathways that were significant at both gestational time points. Pathways which were significant after adjustment for multiple comparisons (FDR adjusted $q < 0.05$) include alanine, aspartate, and glutamate metabolism, and TCA cycle (gd15), and 2-oxocarboxylic acid metabolism (gd15 and gd19), and steroid biosynthesis.

We observed six pathways which were enriched for DEGs in both the kidney and the liver, including cysteine and methionine metabolism, retinol metabolism, 2-oxocarboxylic acid metabolism, biosynthesis of amino acids, biosynthesis of unsaturated fatty acids, and steroid biosynthesis. The steroid biosynthesis pathway was also enriched for DEGs in the placenta at GD19 as well, using a more stringent statistical cutoff of $q < 0.001$.The 2-oxocarboxylic acid metabolism pathway was significant in the kidney at gd15 and significant in the liver at both gd15 and gd19, and the biosynthesis of unsaturated fatty acids significant in both liver and kidney at gd15 and g19. This indicates shared changes in metabolic genes in these tissues in late gestation, indicating shifts in biological mechanisms.

We elected to perform an in-depth analysis on changes in expression of genes within the 2-oxocarboxylic-acid metabolism pathway (mmu01210) in the liver, as this pathway had the most enrichment of genes that were differentially expressed in late gestation (gd15 and gd19) compared to gd0. mmu01210 is a KEGG metabolic pathway which includes 19 genes; 8 of which were significantly different on gd15 and 7 of which were significantly different at gd19 compared to baseline. The directionality and significance of these genes at each time point is presented in Supplemental Table S3. The directionality of the change in expression for all the genes in this pathway was consistent between gd19 and gd15 compared to baseline, and four of these genes were significantly different ($P < 0.05$) on both gd15 and gd19, including *Aadat*, *Got1*, *Gpt*, and *Idh3b*, while *Idh2* was significantly increased on gd15 and gd19 compared to base. The 10 genes which were different at both gd15 and gd19 are visualized in Supplemental Figure S2. The 2- oxocarboxylic-acid metabolism pathway consists of seven subpathways, including the glycine, serine, and threonine metabolism, which was significantly enriched for differential genes in the liver on gd10 ($P = 3.57 \times 10^{-4}$) but not at later time points, and the citrate cycle, which was significantly enriched for DEGs at both time points as well ($P < 0.05$). Overall, we saw decreases in expression of genes in this pathway, including *Got1*, which converts oxaloacetate to aspartate, as well as Cs, *Idh2*, *Gpt2*, and *Acy1*, which overall are responsible for the conversion of oxaloacetate to its intermediate compounds, and eventually to glutamate and ornithine. This pathway diagram is indicative of general suppression of genes involved in the citrate acid cycle in the maternal liver that happens across gestation.

We also analyzed changes in genes involved in the retinol metabolism, primary bile acid biosynthesis, and steroid biosynthesis pathways in the liver and kidney based on a priori hypotheses. The retinol metabolism pathway (mmu00830) was significantly enriched

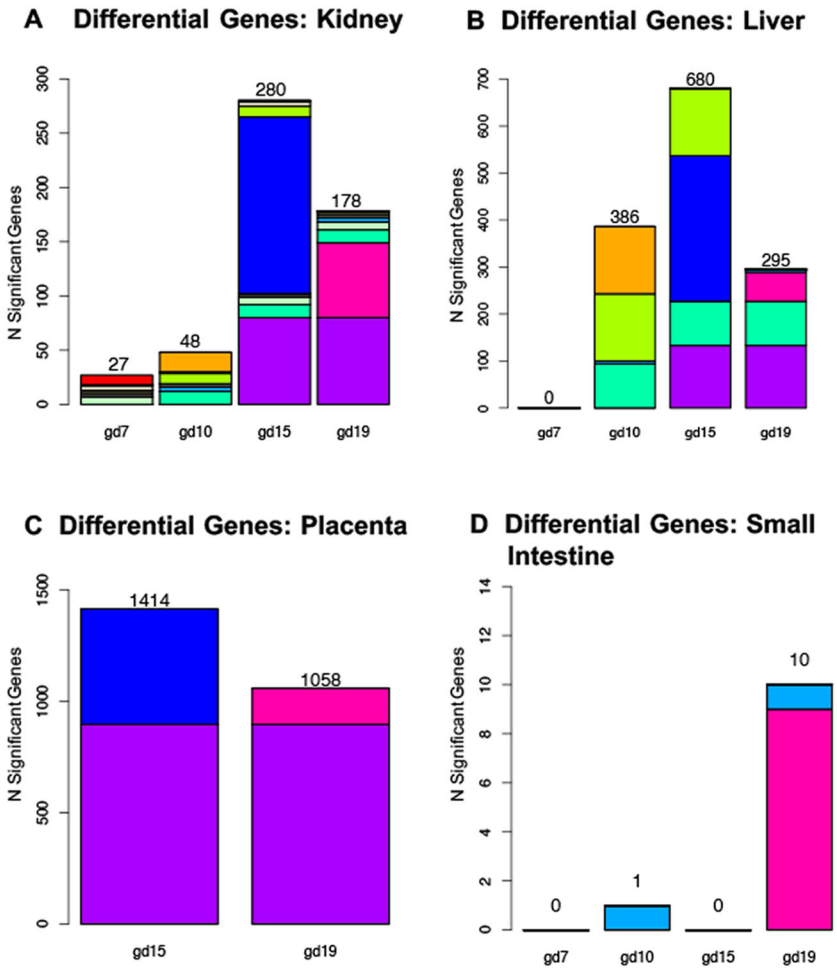


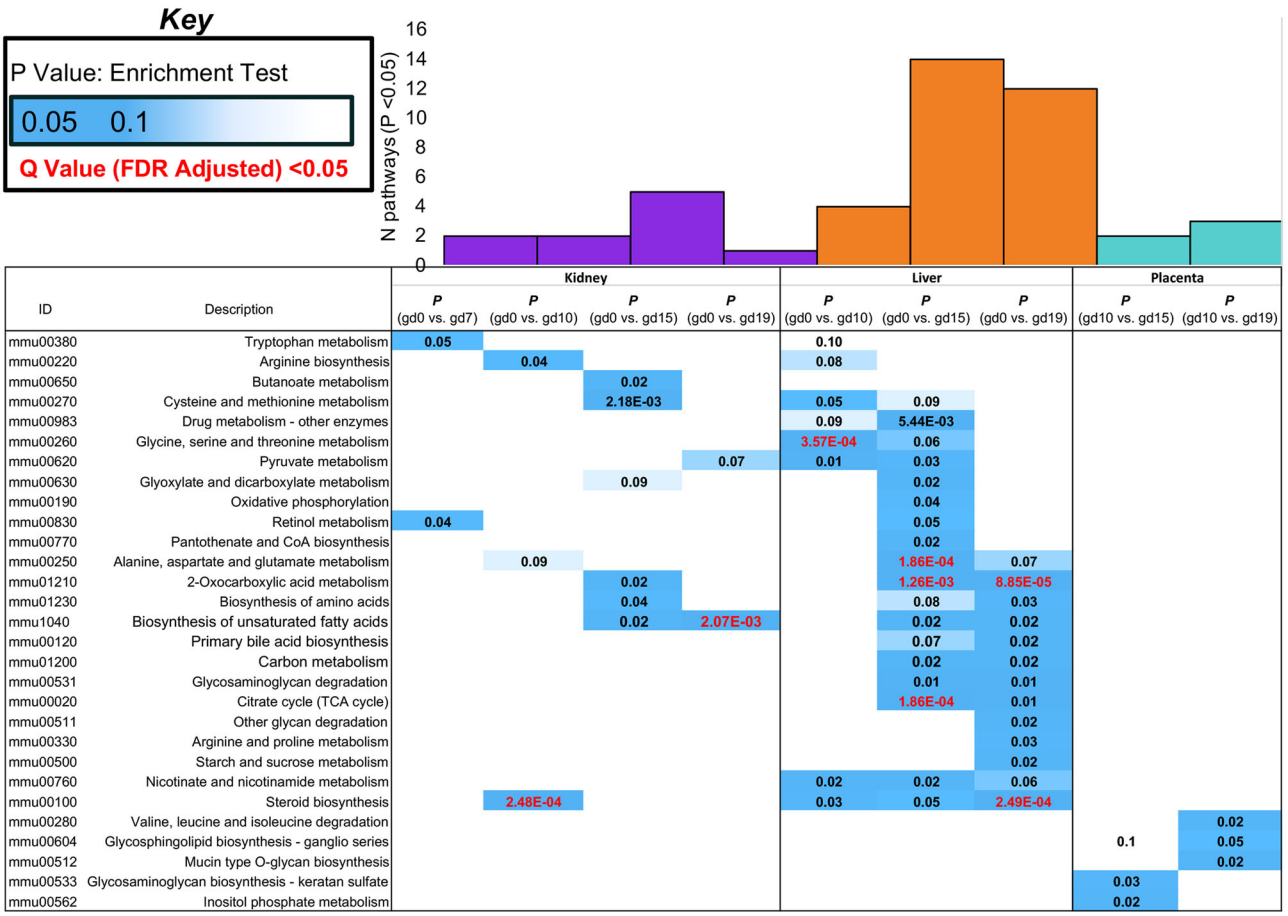
Figure 3. Univariate gene expression analysis. We performed univariate analysis to compare gene expression at each time point to gene expression in nonpregnant mice (gd0), or in the placenta expression at gd10. Barplots show the number of significant genes (FDR adjusted $q < 0.05$) at each time point in the (A) placenta, (B) liver, (C) kidney, and (D) small intestine. Bars are stacked by the genes that are in common at each time point, so that bars that share the same color the same genes which were significant at multiple time points (for example, in the small intestine 1 gene was differentially expressed at time point 10 and 19, and it is shown in blue).

for DEGs on gd7 compared to baseline in the kidney ($P = 0.04$), based on increases in expression of *Lrat* and *Cyp2s1* (Supplemental Table S4, Supplemental Figure S3). The liver was also enriched for DEGs in this pathway ($P = 0.05$), and had decreased expression of 11 genes and increased expression in 1 gene on gestational day 15 compared to baseline.

The primary bile acid biosynthesis pathway (mmu00120) was enriched for DEGs in late gestation (gd 15 $P = 0.07$, and gd 19 $P = 0.02$) in the liver, although this enrichment was only considered statistically significant at gd19. At gd15, five genes exhibited decreased expression and two genes exhibited increased expression, and at gd19, four genes exhibited decreased expression and one gene was increased compared to gd0 (Supplemental Table S5, Supplemental Figure S4). The KEGG steroid metabolism pathway was significantly enriched for differential gene expression in the liver in both mid and late gestation (gd10 $P = 0.02$, gd15 $P = 0.05$, gd 19 $P = 2.49 \times 10^{-4}$) which corresponded to nine genes which were decreased at these time points compared to gd 0, and one gene that was increased on gd10 compared to gd0 (*Soat1*), as shown in Supplemental Table S6 and Supplemental Figure S5. In contrast, the steroid metabolism pathway was enriched for genes which were increased (four genes) in the kidney on gd7 compared to gd0 (Supplemental Table S6).

Discussion

In this study, we characterized transcriptomic changes in metabolic genes across gestation in maternal organs and placenta at a genome scale, expanding our understanding of maternal metabolic adaptions to pregnancy. Key findings of this study were as follows. (1) We identified a number of distinct genes and pathways which were differentially expressed at different gestational time points in the maternal liver and kidneys as well as in the placenta which roughly corresponded to early, mid, and late gestation. (2) The changes in expression of metabolic genes was more pronounced in the placenta than any of the other organs tested. (3) There was a distinct lack of transcriptomic changes quantifiable within the small intestine across pregnancy. (4) We observed the most significant changes in biological pathways across gestation in the liver including genes within the citrate cycle, retinol metabolism, bile acid synthesis, and steroid bile synthesis, which play functional roles in fetal development and



ID	Description	Kidney				Liver			Placenta	
		P (gd0 vs. gd7)	*P* (gd0 vs. gd10)	*P* (gd0 vs. gd15)	*P* (gd0 vs. gd19)	*P* (gd0 vs. gd10)	*P* (gd0 vs. gd15)	*P* (gd0 vs. gd19)	*P* (gd10 vs. gd15)	*P* (gd10 vs. gd19)
mmu00380	Tryptophan metabolism	0.05				0.10				
mmu00220	Arginine biosynthesis		0.04			0.08				
mmu00650	Butanoate metabolism			0.02						
mmu00270	Cysteine and methionine metabolism			2.18E-03		0.05	0.09			
mmu00983	Drug metabolism - other enzymes					0.09	5.44E-03			
mmu00260	Glycine, serine and threonine metabolism					3.57E-04	0.06			
mmu00620	Pyruvate metabolism				0.07	0.01	0.03			
mmu00630	Glyoxylate and dicarboxylate metabolism			0.09			0.02			
mmu00190	Oxidative phosphorylation						0.04			
mmu00830	Retinol metabolism	0.04					0.05			
mmu00770	Pantothenate and CoA biosynthesis						0.02			
mmu00250	Alanine, aspartate and glutamate metabolism		0.09				1.86E-04	0.07		
mmu01210	2-Oxocarboxylic acid metabolism			0.02			1.26E-03	8.85E-05		
mmu01230	Biosynthesis of amino acids			0.04			0.08	0.03		
mmu1040	Biosynthesis of unsaturated fatty acids			0.02	2.07E-03		0.02	0.02		
mmu00120	Primary bile acid biosynthesis						0.07	0.02		
mmu01200	Carbon metabolism						0.02	0.02		
mmu00531	Glycosaminoglycan degradation						0.01	0.01		
mmu00020	Citrate cycle (TCA cycle)						1.86E-04	0.01		
mmu00511	Other glycan degradation							0.02		
mmu00330	Arginine and proline metabolism							0.03		
mmu00500	Starch and sucrose metabolism							0.02		
mmu00760	Nicotinate and nicotinamide metabolism					0.02	0.02	0.06		
mmu00100	Steroid biosynthesis		2.48E-04			0.03	0.05	2.49E-04		
mmu00280	Valine, leucine and isoleucine degradation									0.02
mmu00604	Glycosphingolipid biosynthesis - ganglio series								0.1	0.05
mmu00512	Mucin type O-glycan biosynthesis									0.02
mmu00533	Glycosaminoglycan biosynthesis - keratan sulfate								0.03	
mmu00562	Inositol phosphate metabolism								0.02	

Figure 4. Pathway enrichment analysis results. Pathway enrichment analysis was performed on the differentially expressed genes from the kidney, liver, and placenta, and the number of significant pathways ($P < 0.05$) is shown in the barplot, with the table listing each significant pathway. In this figure, only significant pathways are shown. Any other time point with a *P* value <0.1 is also shown to highlight trends, and cells are shaded based on *P* value. *P* values in red were significant after adjustment for multiple comparisons (FDR adjusted $q < 0.05$).

pregnancy maintenance. These analyses provide insight into tissue-specific changes that occur during adaptation to pregnancy.

This comprehensive analysis of changes in gene expression in multiple organs across gestation can be interpreted in the context of the remote sensing and signaling hypothesis. These organs interface with various body and body fluid compartments (gut lumen, blood, bile, urine, amniotic fluid), as well as other "organisms" of the same species (fetus) or different species (gut microbes) [11, 12]. This theory emphasizes the critical role of small organic molecules—handled by transporters and drug metabolizing enzymes—that function as rate-limiting metabolites, signaling molecules, antioxidants, and nutrients in interorgan and inter-organismal cross-talk. The apparent "objective" is to optimize levels of such molecules so as to meet the physiological needs of multiple tissues and, in the case of pregnancy, two organisms (mother, fetus). In pregnancy, there is a requirement for such cross-talk to be optimized at multiple levels to meet the needs of changing maternal systemic and organ physiological demands, fetal organ development and growth, maternal–fetal communication and (probably) maternal–gut microbiome communication. Our study of longitudinal gene expression in the intestine, liver, kidney, and placenta and accompanying changes in specific pathways throughout gestation may reflect the cross-talk between mother and fetus as these energy needs are balanced.

Through our analysis, we identified a number of tissue-specific changes in metabolic genes across gestation which were enriched for distinct KEGG metabolic pathways within the liver, kidney, and placenta. Notably, we observed some overlaps in metabolic pathways which were enriched for genes that changed across gestation in the liver and kidney, but a completely unique set of pathways which were enriched for specific genes in the placenta. The placenta modulates fetal development by regulation of nutrient transport [34]. The human placenta undergoes major shifts in gene expression throughout the course of pregnancy, with 25% of genes found to be differentially expressed between the first and third trimester in a previous study [16]. Our work is concordant with these findings, as we also observed changes in 1576 metabolic genes in the placenta which were significant at either gd15 and/or gd19 compared to gd10, representing 53% of the metabolic genes. The number of genes changed in the placenta was 1.75-fold more than the liver and 2.94-fold more than the kidney. This large number of differentially expressed metabolic genes suggests global shifts in metabolism that are not localized to a few specific pathways (that could be captured through enrichment analysis). As the placenta had many more statistically significant genes using a cutoff of $q < 0.05$, we performed supplemental enrichment analysis using a higher statistical cutoff ($q < 0.001$) which produced a more targeted gene list more appropriate for pathway

enrichment analysis. However, this analysis only nominally changed the number of enriched pathways (8 total vs. 5 total). Overall, this work reveals that expression of metabolic genes is highly tissue specific and changes throughout gestation, and may suggest that there is a degree of tissue and time-specific transcriptional regulation that occurs to control this process. More work is needed to understand the mechanisms of this transcriptional regulation.

We observed minimal changes in expression of metabolic genes across gestation in the small intestine, which may suggest that there is minimal metabolic adaptation to pregnancy that occurs at the transcriptomic level in the small intestine. These changes may be undetectable in laboratory mice, which are housed in a tightly regulated environment, may be species specific, or occur in other parts of the digestive system (such as the large intestine or stomach). Alternatively, changes in metabolic function in the small intestine related to pregnancy may not occur within the intestinal cells, but rather in the microbiome. Human studies have shown dramatic changes in the human gut microbiome between the first and third trimester, which were attributable to host–microbial interactions [35]. These changes are related to metabolic and immunological adaptations that are needed for a healthy pregnancy [36]. Alternatively, these changes may be related to cellular heterogeneity present within this dataset, which may confound results, as this transcriptomics data reflect a mix of different cell types, and only a small number of specific cells may undergo transcriptomic changes in response to labor [37]. More analysis is required to identify the scope of metabolomic changes that occur within the tissue itself and compare this to the metabolic changes that occur in the microbiome.

There was a significant enrichment of DEGs involved in the 2-oxocarboxylic acid metabolism and citrate cycle in the livers of mice at the end of pregnancy compared to nonpregnant mice. The liver is a key metabolic organ which governs body energy metabolism, and generates energy within mitochondria through the TCA cycle [38]. The citrate cycle is the mechanism by which energy stored in carbohydrates, fat, and proteins is released into chemical energy through ATP. During pregnancy, the liver increases energy production to provide for the increasing requirements of the developing fetus [10]. Detectable changes in energy consumption have been related to the metabolites produced in the citric acid cycle. Increased concentrations of citrate cycle intermediates in maternal plasma were detected using a targeted metabolomics approach [7], which the authors suggest shows conversion of fatty acid oxidation to acetyl-CoA. In our study, the genes responsible for producing these metabolites exhibited decreased expression, so we would predict to observe changes in concentration of metabolites which are produced by these enzymes, which may present as increased concentration of intermediates or decreased concentration of final products, which is in alignment with the targeted metabolomics results [7]. This shift away from the TCA cycle may reflect a shift toward faster, more anerobic metabolic processes, which are well established to occur in other highly proliferative physiological conditions such as cancer [39], as well as in acute stress states in animal models [40], and highly glycolytic states have been observed in the mouse decidua and play a critical role during decidualization. Further work is needed to confirm these findings using metabolomics analysis, which was beyond the scope of this current study, as well as to explore changes in glucose metabolism across pregnancy.

In the liver of mice in mid to late pregnancy, there was decreased expression of genes involved in retinol metabolism compared to the livers of nonpregnant controls. Retinoid homeostasis is crucial for proper embryogenesis and development, as embryos cannot synthesize retinoic acid and must derive it from maternal sources, with abnormalities in this processing resulting in fetal abnormalities [41]. Decreased retinoic acid levels have been identified in maternal plasma as reserves are shifted to the fetus during embryogenesis, with subsequent increases in the placenta during mid-gestation [40]. Thus, the decreased expression of retinoic acid metabolism genes could reflect a decreased metabolic response as a result of decreased retinoic acid in maternal circulation as this is being shuttled to the fetus instead.

We observed decreased expression of genes involved in bile acid synthesis, and steroid biosynthesis in the liver of mice in mid to late gestation compared to nonpregnant mice, and increases in expression of genes involved in steroid biosynthesis in the kidney in mice in early gestation compared to nonpregnant mice, and within the placenta at late gestation (using a more stringent statistical cutoff than that of the liver and kidney). Analysis of genes involved in bile acid synthesis may provide a physiological basis for understanding complications that arise during pregnancy such as intrahepatic cholestasis of pregnancy. Characterization of genes involved in steroid hormone production during pregnancy may shed light on mechanisms responsible for gestational age-dependent induction or downregulation of enzymes or transporters important for drug/xenobiotic disposition. Previously, targeted analysis of genes involved in bile acid and steroid synthesis in mice over gestational age was performed in this dataset, and changes to key enzymes or transporters involved in bile acid and steroid synthesis and disposition were observed within the Cyp, Abc, and Slc families of metabolic genes [37]. Through our unsupervised, genome-scale approach, we identified the same changes in genes in these pathways, including upregulation of *Cyp7b1* and downregulation of *Cyp27a1* in the liver during gestational day 15, confirming the strength and significance of these changes in the liver during pregnancy. Changes in other cytochrome P450 genes have been observed in hepatic tissue of mice during pregnancy quantified using real-time qPCR [42]. Decreases in these metabolic genes may be a compensatory mechanism to manage increased bile acid concentrations and steroid concentrations that occur throughout pregnancy [37]. Changes in expression of transporters and metabolic genes observed in the liver and kidney need to be directly linked to metabolic measurements to understand the consequences in changes of these metabolic pathway changes on maternal liver and kidney function.

Comprehensive gene analysis in multiple organs across gestation is clearly not feasible in humans, and thus needs to be done in an animal model. We chose the pregnant mouse to conduct the gene analysis because a subset of us has previously shown that the pharmacokinetic changes of various drugs in pregnant women can be replicated in the pregnant mouse model [24, 37, 43, 44], suggesting that the overall patterns of pregnancy-induced changes in gene expression across gestation might be similar in mice and humans. In this analysis, we generated a list of metabolic genes from the latest version of the human metabolic network reconstruction (RECON3) [5], which was mapped to the mouse transcriptome as a representation of global metabolic pathways. Genome sequence analysis has revealed 85–92% homology between human and mouse [45, 46]. An earlier version of the human metabolic model (Recon 1) was used as a template to build a mouse mode correlation with experimental gene knockout study [47]. Mouse orthologs have been successfully used to characterize functional activities of enzymes involved in human metabolism [47, 48], suggesting it is feasible to translate observations generated in mouse models to human systems.

This study provides novel insights into changes that occur within the transcriptome of mice throughout different points of their pregnancy, but caution should be taken to extrapolate our findings to

humans due to species-specific physiological differences in pregnancy [49]. Particularly, there are dramatic differences in the mouse and human placenta at the anatomical, functional, and molecular level, making it challenging to translate these findings. Additionally, the shorter gestational length of the mouse pregnancy provided only limited opportunities to capture time series data, limiting the resolution of our analyses. Specifically, this study was missing transcriptomic data from the early time points of placental development as the placenta was collected at gestational day 10, limiting our ability to perform continuous time series analysis in the placenta. Thus, we did not include a linear model for the placenta in this study since there are only three time points. More work is required to generate placental transcriptomics data from earlier time points and with denser longitudinal data. Transcriptomic data provide only a snapshot of genes expressed during a moment in time, and enhanced insight from proteomics and/or metabolomics data would provide more insight into metabolomic changes. This analysis is also limited by the small sample size. However, this study provides a novel and comprehensive profiling of transcriptional changes in metabolic genes in organs which are crucial to pregnancy. Samples are collected in organs throughout pregnancy, in a way that could not be captured with human data. This analysis has high coverage of human metabolic pathways (96% of metabolic genes analyzed), and we have implemented targeted pathway analysis which uses appropriate background gene lists and reduces the overall number of comparisons.

This analysis and the accompanying data presented here serve as a valuable resource for researchers and clinicians to understand changes in metabolic pathways that occur in various organs throughout pregnancy. This study represents the first step in addressing the knowledge gap in understanding transcriptomic, proteomic, and metabolomic changes during pregnancy. Further work is needed to better extrapolate this data to a human population, as well as to link these changes to secreted metabolites across the same gestational time points. Through a better understanding of normal longitudinal changes that occur in pregnancy, we can better understand how perturbations in these biochemical pathways and perturbations in nutrient signaling in pregnancy may result in pregnancy disorders related to nutrient imbalances, such as in utero growth restriction, gestational diabetes, and pregnancies complicated by maternal obesity.

Supplementary data

Supplementary data are available at *BIOLRE* online.

Supplemental Figure S1. Top differentially expressed genes with relation to gestational day. Line plots across gestational age of significant genes (FDR adjusted $q < 0.05$, LIMMA analysis) with four highest and four lowest estimate values, representing the genes with the most significant changes in the (A) liver, (B) small intestine, and (C) kidney.

Supplemental Figure S2. Differentially expressed genes within the 2-oxocarboxylic-acid metabolism pathway: The KEGG 2-oxocarboxylic-acid metabolism pathway (MMU 0210) pathway map was downloaded from KEGG (http://www.genome.jp/kegg-bin/show_pathway?mmu01210) and uploaded to metscape for data visualization. Genes which significantly upregulated are highlighted in red, and genes which decrease are highlighted in green.

Supplemental Figure S3. Differentially expressed genes in KEGG retinol metabolism pathway (mmu00830).

Supplemental Figure S4. Differentially expressed genes in the KEGG primary bile acid biosynthesis pathway (mmu00120) in the liver.

Supplemental Figure S5. Differentially expressed genes in KEGG steroid biosynthesis pathway (mmu00100).

Supplemental Table S1. Complete pathway enrichment analysis for all KEGG metabolic pathways.

Supplemental Table S2. Enriched pathways for genes highly significant in the placenta at gd19 and gd 15 compared to gd10 (FDR cutoff for genes $q < 0.001$).

Supplemental Table S3. Differentially expressed genes in KEGG 2-oxocarboxyclic acid metabolism pathway (mmu01210).

Supplemental Table S4. Differentially expressed genes in KEGG retinol metabolism pathway (mmu00830).

Supplemental Table S5. Differentially expressed genes in KEGG primary bile acid biosynthesis pathway (mmu00120).

Supplemental Table S6. Differentially expressed genes in KEGG steroid metabolism pathway (mmu00100).

Acknowledgments

The authors would like to thank the members of the Mao and Price Labs who contributed to this manuscript, as well as Theresa Fitzgerald for her help with the coordination of this manuscript.

Author contributions

AGP: Conception of study design, performed final analyses and primary interpretation of results. PB: Assisted with data analysis, visualization, and interpretation of metabolic pathways. ABH: Performed preliminary analysis and interpretation of results. SN: Provided guidance with data analysis and interpretation. TB: Assisted with data preprocessing and analysis of results. QM: Conception of study design, provided guidance with data analysis and interpretation. NDP: Conception of study design, provided guidance with data analysis and interpretation.

References

1. Brett KE, Ferraro ZM, Yockell-Lelievre J, Gruslin A, Adamo KB. Maternal–fetal nutrient transport in pregnancy pathologies: the role of the placenta. *Int J Mol Sci* 2014; **15**(9):16153–16185.
2. Herrera E. Metabolic adaptations in pregnancy and their implications for the availability of substrates to the fetus. *Eur J Clin Nutr* 2000; **54**(S1):S47–S51.
3. Mesiano S. CHAPTER 11 - the endocrinology of human pregnancy and fetoplacental neuroendocrine development A2 - Strauss, Jerome F. In: Barbieri RL, editor. *Yen & Jaffe's Reproductive Endocrinology (Sixth Edition)*. Philadelphia: W.B. Saunders; 2009: 249–281. https://www.sciencedirect.com/science/article/pii/B9781416049074000115.
4. Thiele I, Swainston N, Fleming RMT, Hoppe A, Sahoo S, Aurich MK, Haraldsdottir H, Mo ML, Rolfsson O, Stobbe MD, Thorleifsson SG, Agren R et al. A community-driven global reconstruction of human metabolism. *Nat Biotechnol* 2013; **31**(5):419–425.
5. Brunk E, Sahoo S, Zielinski DC, Altunkaya A, Drager A, Mih N, Gatto F, Nilsson A, Preciat Gonzalez GA, Aurich MK, Prlić A, Sastry A et al. Recon3D enables a three-dimensional view of gene variation in human metabolism. *Nat Biotechnol* 2018; **36**(3):272–281.
6. Zhang C, Hua Q. Applications of Genome-Scale metabolic models in biotechnology and systems medicine. *Front Physiol* 2015; **6**:272–281.
7. Lindsay KL, Hellmuth C, Uhl O, Buss C, Wadhwa PD, Koletzko B, Entringer S. Longitudinal metabolomic profiling of amino acids and lipids across healthy pregnancy. *PLoS One* 2016; **10**(12):e0145794.

8. Sabet Sarvestani F, Rahmanifar F, Tamadon A. Histomorphometric changes of small intestine in pregnant rat. *Vet Res Forum* 2015; **6**(1):69–73.
9. Lawson M, Kern FJ, Everson GT. Gastrointestinal transit time in human pregnancy: prolongation in the second and third trimesters followed by postpartum normalization. *Gastroenterology* 1985; **89**(5):996–999.
10. Butte NF. Carbohydrate and lipid metabolism in pregnancy: normal compared with gestational diabetes mellitus. *Am J Clin Nutr* 2000; **71**(5):1256S–1261S.
11. Bacq Y, Zarka O, Brechot JF, Mariotte N, Vol S, Tichet J, Weill J. Liver function tests in normal pregnancy: a prospective study of 103 pregnant women and 103 matched controls. *Hepatology* 1996; **23**(5):1030–1034.
12. Dunlop W. Serial changes in renal haemodynamics during normal human pregnancy. *Br J Obstet Gynaecol* 1981; **88**(1):1–9.
13. Cheung KL, Lafayette RA. Renal physiology of pregnancy. *Adv Chronic Kidney Dis* 2013; **20**(3):209–214.
14. Ahn S-Y, Nigam SK. Toward a systems level understanding of organic anion and other multispecific drug transporters: a remote sensing and signaling hypothesis. *Mol Pharmacol* 2009; **76**(3):481–490.
15. Nigam SK. What do drug transporters really do? *Nat Rev Drug Discov* 2015; **14**(1):29–44.
16. Sitras V, Fenton C, Paulssen R, Vårtun Å, Acharya G. Differences in gene expression between first and third trimester human placenta: A microarray study. *PLoS One* 2012; **7**(3):e33294.
17. Dunn WB, Brown M, Worton SA, Davies K, Jones RL, Kell DB, Heazell AEP. The metabolome of human placental tissue: investigation of first trimester tissue and changes related to preeclampsia in late pregnancy. *Metabolomics* 2012; **8**(4):579–597.
18. Raunig JM, Yamauchi Y, Ward MA, Collier AC. Assisted reproduction technologies alter steroid delivery to the mouse fetus during pregnancy. *J Steroid Biochem Mol Biol* 2011; **126**(1-2):26–34.
19. Wang H, Dey SK. Roadmap to embryo implantation: clues from mouse models. *Nat Rev Genet* 2006; **7**(3):185–199.
20. Grigsby PL. Animal models to study placental development and function throughout normal and dysfunctional human pregnancy. *Semin Reprod Med* 2016; **34**(1): 11–16.
21. Lee KY, DeMayo FJ. Animal models of implantation. *Reproduction* 2004; **128**(6):679–695.
22. Schmidt A, Morales-Prieto DM, Pastuschek J, Frohlich K, Markert UR. Only humans have human placentas: molecular differences between mice and humans. *J Reprod Immunol* 2015; **108**:65–71.
23. Satre MA, Ugen KE, Kochhar DM. Developmental changes in endogenous retinoids during pregnancy and embryogenesis in the mouse. *Biol Reprod* 1992; **46**(5):802–810.
24. Zhang Y, Zhou L, Unadkat JD, Mao Q. Effect of pregnancy on nitrofurantoin disposition in mice. *J Pharm Sci* 2009; **98**(11):4306–4315.
25. Shuster DL, Bammler TK, Beyer RP, Macdonald JW, Tsai JM, Farin FM, Hebert MF, Thummel KE, Mao Q.. Gestational age-dependent changes in gene expression of metabolic enzymes and transporters in pregnant mice. *Drug Metab Dispos* 2013; **41**(2):332–342.
26. Edgar R, Domrachev M, Lash AE. Gene Expression Omnibus: NCBI gene expression and hybridization array data repository. *Nucleic Acids Res [Internet]* 2002; **30**. http://dx.doi.org/10.1093/nar/30.1.207. Accessed January 4, 2018.
27. Carvalho BS, Irizarry RA. A framework for oligonucleotide microarray preprocessing. *Bioinformatics* 2010; **26**(19):2363–2367.
28. Smyth GK. limma: Linear Models for Microarray Data. In: Gentleman R, Carey VJ, Huber W, Irizarry RA, Dudoit S (eds). *Bioinformatics and Computational Biology Solutions Using R and Bioconductor [Internet]*. New York, NY: Springer New York; 2005: 397–420. https://doi.org/10.1007/0-387-29362-0_23. Accessed January 4, 2018.
29. Durinck S, Moreau Y, Kasprzyk A, Davis S, De Moor B, Brazma A, Huber W. BioMart and Bioconductor: a powerful link between biological databases and microarray data analysis. *Bioinformatics* 2005; **21**(16):3439–3440.
30. Yu G, Wang L-G, Han Y, He Q-Y. clusterProfiler: an R package for comparing biological themes among gene clusters. *OMICS* 2012; **16**(5):284–287.
31. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res* 2003; **13**(11):2498–2504.
32. Karnovsky A, Weymouth T, Hull T, Tarcea VG, Scardoni G, Laudanna C, Sartor MA, Stringer KA, Jagadish HV, Burant C, Athey B, Omenn GS. Metscape 2 bioinformatics tool for the analysis and visualization of metabolomics and gene expression data. *Bioinformatics* 2012; **28**(3):373–380.
33. Watson ED, Cross JC. Development of structures and transport functions in the mouse placenta. *Physiology* 2005; **20**(3):180–193.
34. Lager S, Powell TL. Regulation of nutrient transport across the placenta. *J Pregnancy* 2012; **2012**:1–14.
35. Koren O, Goodrich JK, Cullender TC, Spor A, Laitinen K, Bäckhed HK, Gonzalez A, Werner JJ, Angenent LT, Knight R, Bäckhed F, Isolauri E et al. Host remodeling of the gut microbiome and metabolic changes during pregnancy. *Cell* 2012; **150**(3):470–480.
36. Konstantinov SR, van der Woude CJ, Peppelenbosch MP. Do pregnancy-related changes in the microbiome stimulate innate immunity? *Trends Mol Med* 2013; **19**(8):454–459.
37. Shuster DL, Risler LJ, Liang C-KJ, Rice KM, Shen DD, Hebert MF, Thummel KE, Mao Q. Maternal-Fetal disposition of glyburide in pregnant mice is dependent on gestational age. *J Pharmacol Exp Ther* 2014; **350**(2):425–434.
38. Rui L. Energy metabolism in the liver. *Compr Physiol* 2014; **4**(1):177–197.
39. Vander Heiden MG, Cantley LC, Thompson CB. Understanding the warburg effect: The metabolic requirements of cell proliferation. *Science* 2009; **324**(5930):1029–1033.
40. Chandrasekaran S, Ament SA, Eddy JA, Rodriguez-Zas SL, Schatz BR, Price ND, Robinson GE. Behavior-specific changes in transcriptional modules lead to distinct and predictable neurogenomic states. *Proc Natl Acad Sci USA* 2011; **108**(44):18020–18025.
41. Qin F, Shen Z, Peng L, Wu R, Hu X, Zhang G, Tang S. Metabolic characterization of all-trans-retinoic acid (ATRA)-induced craniofacial development of murine embryos using in vivo proton magnetic resonance spectroscopy. *PLoS One* 2014; **9**(5):e96010.
42. Zhang H, Wu X, Wang H, Mikheev AM, Mao Q, Unadkat JD. Effect of pregnancy on cytochrome P450 3a and P-glycoprotein expression and activity in the mouse: mechanisms, tissue specificity, and time course. *Mol Pharmacol* 2008; **74**(3):714–723.
43. Zhou L, Zhang Y, Hebert MF, Unadkat JD, Mao Q. Increased glyburide clearance in the pregnant mouse model. *Drug Metab Dispos* 2010; **38**(9):1403–1406.
44. Liao MZ, Gao C, Phillips BR, Neradugomma NK, Han LW, Bhatt DK, Prasad B, Shen DD, Mao Q. Pregnancy increases norbuprenorphine clearance in mice by induction of hepatic glucuronidation. *Drug Metab Dispos* 2018; **46**(2):100–108.
45. Blatt C, DePamphilis ML. Striking homology between mouse and human transcription enhancer factor-1 (TEF-1). *Nucleic Acids Res* 1993; **21**(3):747–748.
46. Zhu L, Swergold GD, Seldin MF. Examination of sequence homology between human chromosome 20 and the mouse genome: intense conservation of many genomic elements. *Hum Genet* 2003; **113**(1):60–70.
47. Sigurdsson MI, Jamshidi N, Steingrimsson E, Thiele I, Palsson BO. A detailed genome-wide reconstruction of mouse metabolism based on human Recon 1. *BMC Syst Biol* 2010; **4**(1):140.
48. Hrycay EG, Bandiera SM. Expression, function and regulation of mouse cytochrome P450 enzymes: comparison with human cytochrome P450 enzymes. *Curr Drug Metab* 2009; **10**(10):1151–1183.
49. Chaouat G, Clark DA. Are animal models useful or confusing in understanding the human feto-maternal relationship? A debate. *J Reprod Immunol*2015; **108**(Supplement C):56–64.