REVIEW

Translational Systems Pharmacology Studies in Pregnant Women

Sara K. Quinney[1,2]*, Rakesh Gullapelli[3] and David M. Haas[1,2]

Pregnancy involves rapid physiological adaptation and complex interplay between mother and fetus. New analytic technologies provide large amounts of genomic, proteomic, and metabolomics data. The integration of these data through bioinformatics, statistical, and systems pharmacology techniques can improve our understanding of the mechanisms of normal maternal physiologic changes and fetal development. New insights into the mechanisms of pregnancy-related disorders, such as preterm birth (PTB), may lead to the development of new therapeutic interventions and novel biomarkers.
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Bioinformatics and systems pharmacology approaches are enabling us to understand the mechanisms of alterations in maternal physiology, diseases of pregnancy, fetal development, and fetal origins of disease. Pregnancy is a period of rapid growth and transformation. The integration of data from genomic, transcriptomic, proteomic, metabolomic, and other data sources is leading to increased understanding of the complex interplay among the maternal, fetal, and placental organs. In addition to exploring normal fetal growth and development, systems pharmacology techniques can be used to explore conditions unique to pregnancy, such as preterm labor and pre-eclampsia. Additionally, system models can help us better understand the links between disorders that occur during pregnancy, such as gestational diabetes, and the development of future maternal and neonatal morbidities.

The ultimate aim of systems pharmacology research in pregnancy is to improve clinical outcomes of both mother and baby through enhanced clinical care. Many diseases of pregnancy, including preterm birth (PTB), pre-eclampsia, and gestational diabetes, are multifactorial but seem to have a genetic component.[1] It is clear that no single biomarker can predict the population level risk of these diseases due to their complex etiology. Translational systems pharmacology provides the means to integrate data from a large number of studies and variety of data types; spanning multiple physiological scales (e.g., organ-level, cellular-level, protein-level, and genomic-level data).[2] Systems pharmacology approaches link biological connections in a system using mathematical approaches, such as differential equations and statistical models. Pregnancy creates additional complexities in the analysis of data, as maternal, fetal, and even paternal sources of variability must be considered. Additionally, longitudinal models are needed to account for the rapid physiologic changes that occur during pregnancy. Consequently, more sophisticated algorithms are required when developing systems models of pregnancy and pregnancy disorders.

The potential impact of translational systems pharmacology models in understanding the maternal-fetal interplay and improvement of therapeutic opportunities to improve pregnancy outcomes is great. However, as with other areas of research, the incorporation of large data analysis in obstetrics lags behind other fields (**Figure 1**). Less than 2% of human genomic and proteomic studies and 3.5% of metabolomic studies recorded in PubMed are related to obstetrics. Using preterm labor as an example, this review explores the need for translational systems pharmacology modeling in obstetrics, challenges faced by researchers, and opportunities for future advancement.

Physiologic changes in pregnancy

Women who develop either acute or chronic disease may continue to take a variety of medications throughout gestation. In addition, pharmaceutical intervention may be required for pregnancy-induced disorders. However, information on pregnancy-induced changes in the pharmacokinetics and pharmacodynamics is unavailable for many drugs.

Pregnancy brings a time of rapid physiologic change, not only for the developing fetus but also to the mother. These physiological changes usually begin in early weeks of gestation are amplified in the third trimester of pregnancy and impact nearly all maternal systems. Adaptations in the cardiovascular system, respiratory system, hepatic metabolism, renal excretion, and gastrointestinal system can affect drug pharmacokinetics. The activities of cytochrome P450 (CYP)3A4, CYP2D6, and CYP2C9 increase during pregnancy, leading to higher clearance of substrates of these enzymes.[3,4] On the other hand, drugs metabolized by CYP1A2 and CYP2C19 may require lower doses during pregnancy due to the reduced activity of these isoenzymes.[3,4] The decreased concentrations of albumin and α1-acid glycoprotein during pregnancy can alter the protein binding of drugs.[5] Glomerular filtration rate begins to

[1]Department of Obstetrics and Gynecology, Indiana University School of Medicine, Indianapolis, Indiana, USA; [2]Division of Clinical Pharmacology, Department of Medicine, Indiana University School of Medicine, Indianapolis, Indiana, USA; [3]School of Informatics and Computing, Indiana University Purdue University Indianapolis, Indianapolis, Indiana, USA. *Correspondence: S. Quinney (squinney@iu.edu)
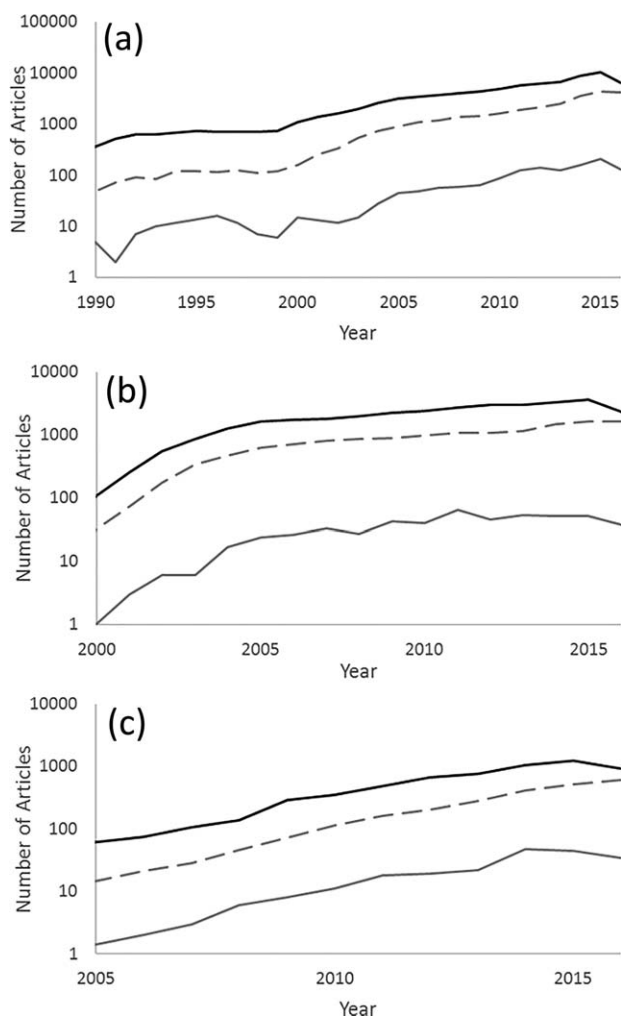
Figure 1 PubMed citations per year relating to (**a**) genomic, (**b**) proteomic, and (**c**) metabolomic studies in pregnancy (gray line). For comparison, the black line represents all genomic, proteomic, or metabolomics studies in PubMed and the dashed line indicates those studies relating to oncology.

increase as early as 6 weeks gestation, and leads to increased creatinine clearance and renal clearance of drugs. Furthermore, increased cardiac output beginning at 8–10 weeks' gestation causes an increase in blood flow to the uterus and kidneys, which may also increase the renal clearance of drugs. A pregnancy-induced increase in plasma and blood volume starting at 6–10 weeks' gestation leads to hemodilution, which can alter the distribution of drugs to red blood cells.[4] Increased progesterone, estrogen, cortisol, placental lactogen, and other hormones during pregnancy also lead to physiologic changes that can also affect the pharmacodynamics of drugs. For instance, changes occur in coagulation and fibrinolytic pathways leading to hypercoagulation, increased insulin resistance can lead to carbohydrate intolerance, leptin production increases, and thyroid hormone control is challenged by estrogenic increases in thyroid binding globulin.[6]

Physiologically based pharmacokinetic models of pregnancy

To date, systems pharmacology modeling in pregnancy has largely been limited to the use of physiologically based pharmacokinetic (PBPK) modeling to estimate changes in drug disposition. Olanoff & Anderson[7] were among the first to use PBPK modeling in pregnancy to describe tetracycline disposition in rats. Initial use of PBPK models in pregnancy was for the prediction of fetal risk assessment.[8] More recently, PBPK models have been used to describe changes in the pharmacokinetics of therapeutic agents during pregnancy.

A variety of structural models, demonstrating a wide range of complexity, have been used for pregnancy PBPK models. The majority of models have used a full maternal PBPK structural model, although some have used reduced models that isolate organs of interest (e.g., liver and placenta/fetalunits).[9,10] The structures applied to the fetal-placental unit are more varied and include combined placental-fetal compartment[11]; the separation of fetus and placenta into two distinct compartments[10,12]; addition of an amniotic fluid compartment[13]; and full fetal PBPK models with separate placental compartments.[14]

In addition to the inclusion of fetal/placental compartments, these models are also parameterized to describe the physiological changes that occur during pregnancy. Because pregnancy is a fluid state with rapid changes in maternal, placental, and fetal physiology, it is important to account for the longitudinal changes in blood flows, organ composition, and functional activity during pregnancy. Initially, PBPK models of pregnancy focused on a snapshot of gestation, often describing a few hours or days of exposure.[9] These models typically used static parameter models, describing the gestational age of interest. In more recent years, pregnancy PBPK models have incorporated dynamic changes in maternal and fetal physiology to estimate exposure across gestation.[11,15] Although the majority of published pregnancy PBPK models have been user-built, the commercial PBPK modeling software Simcyp (Certera) has released a pregnancy population model that utilizes a combined placental-fetal compartment and allows for longitudinal changes in number of key physiologic parameters across gestation.[11,15]

One limitation in the development of pregnancy PBPK models is the availability of data regarding changes in physiological parameters affecting drug disposition and clinical pharmacokinetic data for model verification. Several groups have cataloged a number of physiological changes across gestation.[4,16,17] However, these data often come from a variety of literature sources, which may have been obtained using inaccurate methods.[17] In some cases, these data are based on studies in nonpregnant women or animals. Some parameters may be impacted by study design, leading to inconsistencies in the literature. For instance, hepatic blood flow in pregnancy has been reported to increase in some studies but remain constant in others.[18,19] In addition, these studies often do not account for correlations among parameters, such as the relationship between albumin concentration and blood volume, or include covariates, such as

gravity.[17] Physiologic data from pregnancy, especially with respect to fetal and placental makeup, are often limited by the gestational age range. For instance, fetal blood volume is well-defined only from 21–35 weeks of gestation.[17]

Although PBPK modeling is useful to predict the pharmacokinetics of drugs in pregnancy, it can also aid in understanding the physiologic changes that occur during pregnancy. This approach has been used to better understand the fluctuations in drug metabolizing activity during pregnancy. For instance, although it has long been recognized that the clearance of CYP3A substrates is increased in pregnancy,[3] PBPK modeling allows for the incorporation of changes in plasma protein binding, tissue volumes, blood flow, and renal clearance to more accurately estimate the change in CYP3A activity during pregnancy. Interestingly, we and others have demonstrated that hepatic, but not gut wall, CYP3A activity seems to be increased during pregnancy.[10,11] This approach has also been used to estimate the altered activity of other CYP450 enzymes during pregnancy.[20] However, verification of pharmacokinetic models in pregnancy is complicated by a lack of clinical drug concentration data from pregnant women. Pharmacokinetic studies in pregnancy are restricted due to ethical or logistic issues. Fetal concentrations are often only available from umbilical venous blood collected at delivery. Although the maternal plasma to umbilical vein concentration of drugs has often been used to estimate fetal drug exposure, this ratio is affected by other disposition (i.e., ADMET) properties of the drug and time of sample collection postdose. Obtaining high-quality clinical pharmacokinetic data on drugs from women throughout all stages of gestation is necessary to improve therapeutic outcomes in pregnant women.

Expanding systems pharmacology in pregnancy beyond PBPK

Although PBPK models have provided a means to evaluate longitudinal changes in drug exposure during pregnancy, virtually no group has extended systems pharmacology approaches to improve our understanding of pregnancy-associated diseases. During pregnancy, the maternal body undergoes immense adaptation to sustain fetal development, growth, and parturition. This requires remarkable integration and communication between the fetus, placenta, and mother. As described above, nearly every aspect of maternal physiology is impacted by pregnancy, leading to major changes in expression and function of genes, proteins, and organ systems. In the past, many of these changes have been studied in isolation, based on available technological capabilities.[21] However, advanced technology now enables high-dimensional study of genes, proteins, and other analytes. Systems pharmacology models are needed to integratively characterize the longitudinal changes in the genome, transcriptome, proteome, metabolome, lipidome, etc., throughout pregnancy and directly relate these molecular-level data with organ-level changes and outcomes, such as PTB. A first step in developing systems pharmacology models of pregnancy-related diseases is understanding the mechanisms of normal fetal development and parturition.

One challenge faced by obstetrics researchers is the analysis of large, longitudinally collected datasets. The majority of omics studies compare data obtained at a single time from an individual. However, especially with the rapid developmental changes occurring during gestation, it is important to understand the longitudinal changes in omics data across pregnancy. Many studies have evaluated longitudinal changes of a single factor (e.g., organ volume, blood flow, or gene expression) in maternal and fetal physiology across pregnancy. A number of meta-analyses are available to inform parameterization of quantitative models across pregnancy.[4,16,17] Yet, few studies have evaluated longitudinal changes in large datasets throughout gestation. In part, this is due to lack of informatics and statistical tools to evaluate such data.[22]

Romero *et al.*[21] recently conducted a proteomics study that sampled plasma at 3–6 time points across gestation from 43 women with normal deliveries. A multiplex Slow-Off Rate Modified APtamers (SOMAmer) platform (Somalogic, Boulder, CO) was used to evaluate the abundance of >1,125 plasma proteins. They identified 112 proteins that exhibited >1.5-fold change between 8 and 40 weeks of gestation that had false discovery rate-adjusted values (q-values) of $P < 0.1$. Longitudinal changes in protein abundance were modeled using a cubic spline function and hierarchical clustering demonstrated six distinct pattern changes: increasing abundance at increasing rate ($n = 21$), constant rate ($n = 23$), and decreasing rate ($n = 27$) across gestation and decreasing abundance at increasing rate ($n = 8$), constant rate ($n = 16$), and decreasing rate ($n = 17$) across gestation. The highest-fold changes were observed for placental growth factor; pregnancy-associated plasma protein A (PAPP-A); sialic acid-binding immunoglobulin type lectins (Siglec-6); glypican-3; CC-motif (CCL); carbonic anhydrase 6; prolactin (PRL); interleuken-1 receptor 4 (IL-1 R4); and dual-specificity mitogen-activated protein kinase 4 (MP2K4; **Table 1**[21]). Many of these proteins are involved in angiogenesis, embryogenesis, and immune regulation. Protein-protein interaction network mappings and functional analyses identified the proteins with the greatest change across gestation involved in general defense response; defense response to bacteria and fungi; germ cell migration; proteolysis; and leukocyte migration.[21]

Preterm birth

PTB affects 9.6% of pregnancies annually and is a major cause of neonatal morbidity and mortality worldwide.[23] Although environmental factors, such as smoking, obesity, and maternal stress increase the risk of PTB, maternal genomics have been linked to 15–25% of PTBs and fetal genomics with around 10% of PTBs.[24] The etiology of PTB is multifactorial and complex owing to variations in the physiology of pregnancy women, gestational age, interactions between maternal and fetal genome, and environmental factors. Currently, the best clinical approach for prediction of PTB is the medical history, in which the recurrence risk may increase up to 15% with each previous PTB.[25] Moreover, available data suggest that the increased

Table 1 Proteins exhibiting the greatest fold-change across gestation in normal pregnancy

Protein	Gene symbol	Fold-change	Direction of change	Trend across pregnancy
Glypican 3	GPC3	26.04	Increasing	Decreasing rate
Siglec-6	SIGLEC6	16.92	Increasing	Increasing rate
PlGF	PGF	14.46	Increasing	Decreasing rate
CCL28	CCL28	6.25	Increasing	Decreasing rate
Carbonic anhydrase 6	CA6	5.79	Increasing	Decreasing rate
PRL	PRL	5.75	Increasing	Constant rate
IL-1 R4	IL1RL1	5.49	Increasing	Increasing rate
MP2K4	MAP2K4	5.23	Decreasing	Constant rate
PAPP-A	PAPPA	5.18	Increasing	Decreasing rate
LEAP-1	HAMP	4.32	Decreasing	Decreasing rate
RET	RET	4.15	Increasing	Decreasing rate
TFF3	TFF3	4.31	Increasing	Decreasing rate

CCL, CC-motif; IL-1, interleukin; LEAP-1, liver-expressed antimicrobial peptide 1; MP2K4, mitogen-activated protein kinase 4; PAPP-A, pregnancy-associated plasma protein A; PlGF, placental growth factor; PRL, prolactin; RET, ret proto-oncogene; Siglec, sialic acid-binding immunoglobulin type lectins; TFF3, trefoil factor 3.
Adapted from Romero *et al.*,[21] *Am. J. Obstet. Gynecol.* (2017).

risk of PTB is often inherited across generations.[1] Genomic, transcriptomic, proteomic, and metabolomic studies of PTB are providing enhanced insight into the various mechanisms underlying the pathophysiology of PTB. It is hoped that these studies will lead to the identification of early biomarkers of PTB that would enable earlier intervention and development of novel therapies to prevent and treat PTB. Below, we review the status of genomic, proteomic, and metabolomic studies in PTB.

Genomic studies of PTB

The majority of genomic studies of PTB have used targeted approaches, often based on results of previous studies, pathway analyses, or biological plausibility. Inflammatory factors have been most commonly associated with increased risk of PTB, including single nucleotide polymorphisms (SNPs) in IL-1, IL-4, matrix metalloproteinases, natural killer cells, and toll-like receptors. However, none of these factors consistently predicts the occurrence of PTB.[26] This deficiency of targeted studies is anticipated for a disorder such as PTB, where there are complex patterns of inheritance and multiple factors contribute to its pathophysiology.[27,28] Limiting investigations to just a few targeted genes, proteins, or metabolites restricts the information obtained to select pathways and not the system as a whole. Therefore, candidate gene studies are likely to miss important contributors outside of the known pathways, as well as the potential for interaction among different genes, proteins, or environmental factors.

Although a number of genomewide association studies (GWAS) have been conducted evaluating the timing of delivery, none have identified SNPs that are robustly associated with preterm delivery (**Table 2**[29–34]). A number of factors may contribute to the lack of association observed in these studies, including limited sample size, ethnic differences, and heterogeneity in the phenotype definition. Although PTB is a multifactorial process, with distinct phenotypes, such as infection/inflammation, uterine overdistention,

hemorrhage, and cervical dysfunction,[35] the majority of genomic studies only classify PTB by gestational age at delivery. Even this cutoff point can vary among and even within studies. Standardized definitions of PTB phenotypes and more precise categorization of subphenotypes will lead to improved understanding of the various etiologies of PTB. Capece *et al.*[36] recently conducted a meta-analysis to differentiate pathways associated with PTB due to preterm premature rupture of membranes (PPROMs) or spontaneous PTB (sPTB) with intact membranes. They included data from 15 studies of 3,600 women on 2,175 SNPs in 274 genes and found 248 SNPs of 102 genes that were statistically associated with sPTB with intact membranes, and 39 SNPs in 32 genes statistically significantly associated with PPROM. Although ingenuity pathway analysis identified that the top four molecular and cellular functions (cell-to-cell signaling and interaction, cellular movement, lipid metabolism, and small molecule biochemistry) were common to both groups, more detailed interrogation identified distinct differences. Spontaneous PTB was found to be more associated with autoimmune/hormonal regulation, including the glucocorticoid signaling pathway, whereas etiologies of PPROM included hematologic/coagulation function disorder, collagen metabolism, matrix degradation, and local inflammation.[36] Although overlap was observed in pathways associated with PPROM and sPTB, this study demonstrated that they also have distinct pathophysiologies. These effects may be diluted when studies of both phenotypes are grouped together.

Proteomic studies of PTB

Proteomics could be one of the most reliable "omics" studies and serves as an alternative, unbiased approach for biomarker discovery. As proteins play a pivotal role in understanding the etiology and pathogenesis of PTB and spontaneous preterm deliveries, rich information on the biological mechanisms underlying PTB can be gleaned from proteomic analyses. The majority of the proteomic studies

Table 2 Genomewide studies of PTB or gestational age at delivery

Subjects	Platform	Findings	Replication	Reference
Boston Birth Cohort; 698 PTB; 1,035 term controls	Illumina HumanOmni2.5-4v1 or 8v1 arrays; 2,160,368 SNPs analyzed	Rs11161721 (COL24A1) in normal weight African American mothers associated with an increased risk of PTB (AA genotype 1.8–2.0 times higher risk) but risk of PTB tended to decrease in overweight mothers	GWAS of prematurity and its complication (dbGaP #phs000353.v1.p1), and the NICHD Genomic and Proteomic Network for PTB Research (dbGaP #phs000714.v1.p1). Results replicated in African American subjects but not white subjects	29
Norwegian Mother and Child Cohort (MoBa); 1,743 maternal and 1,109 fetal samples	a) 513,273 autosomal; 12,304 X chromosome SNPs b) Gene-set enrichment 1,541 genes	a) No significant SNPs identified b) Maternal genes in labor-initiated labor were enriched for pregnancy related gene sets (infection, inflammation, immunity)	Not conducted	30
GPN for PTB research; 1,025 sPTB <34 weeks; 1,015 controls	Affymetrix SNP array 6.0; maternal and fetal DNA analyzed	No maternal SNPs reached statistical significance; fetal rs17527054 (major histocompatibility complex, $OR = 0.39$; $P = 2.7E{-}12$) and rs3777722 (RNASET2, $OR = 0.57$; $P = 1.4E{-}10$) were significant	293 cases, 200 controls; did not validate fetal SNPs. No overlap in the top 10 maternal and fetal gene sets	31
Norwegian Mother and Child Cohort (MoBa) and Danish National Birth Cohort; 1,535 PTBs; 1,487 controls	Illumina Human660W-Quad BeadChip analysis of X-chromosome	No maternal or fetal SNPs reached significance after correction for multiple comparisons	SNPs approaching significance were not replicated in validation cohort from United States, Argentina, and Denmark	32
Norwegian Mother and Child Cohort (MoBa) and Danish National Birth Cohort; maternal: 2,128 PTBs; 1,868 controls; fetal: 1,763 PTBs; 1,543 controls	Illumina Human660W-Quadv1_A including 135 mitochondrial SNPs	No SNPs were significant after correction for multiple comparison	88 SNPs were included in a meta-analysis of both cohorts. None found significant	33
Finnish cohorts; 165 PTBs; 163 controls	Affymetrix SNP array 6.0; 150 genes selected for analysis based on evolutionary mapping	91 SNPs, including 8 of 10 SNPs in FSHR were significant ($P < 0.01$) before correcting for multiple comparisons	299 cases and 620 controls from European American, African American, or Hispanic (Mexican) American cohorts genotyped for FSHR. 3 SNPs significant in African Americans after correcting for multiple comparisons	34

FSHR, follicle stimulating hormone receptor; GPN, Genomic and Proteomic Network; GWAS, genomewide association study; NICHD, National Institute of Child Health and Human Development; OR, odds ratio; PTB, preterm birth; SNPs, single nucleotide polymorphisms; sPTB, spontaneous preterm birth.

in PTB are based on either changes in the concentration of a protein/peptide or in their differential expression between the preterm and control groups indicating a diagnostic and or predictive factor for PTB.[37] Studies have explored the proteomes of PTB in maternal serum, plasma, fetal and amniotic fluids with or without intra-amniotic inflammation/infection, and preterm rupture of membranes at an early stage of pregnancy before the onset of symptoms, during the second and third trimesters of pregnancy (**Table 3**[38–45]). Several high-throughput analytical techniques (e.g., gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), [1]hydrogen nuclear magnetic resonance (^{1}H-NMR), ultra-performance liquid chromatography (UPLC-MS), immunosorbent assays, iTRAQ matrix-assisted desorption/ionization-mass spectrometry (MALDI-MS)) have been used in proteomic studies to identify as well as measure the biomarkers indiscriminately.

Although sampling of amniotic fluid carries inherent risks to the fetus, it provides the opportunity to evaluate the fetal environment during pregnancy. Proteomic profiling of amniotic fluids in patients with PTB with or without intra-amniotic infection/inflammation (IAI) using liquid chromatography-tandem mass spectrometry (LC-MS/MS) was studied by Romero *et al.*[46] The study identified 309 unique high-confidence proteins that were associated with PTB with IAI. Many proteins, including thymosin-like 3, leukocyte elastase precursor, and 14-3-3 protein isoforms, were upregulated in patients with PTB and IAI. They also identified 82 differently expressed proteins in the amniotic fluid of pregnant patients who delivered preterm without IAI, including latent-transforming growth factor beta-binding protein isoform 1L precursor, mimecan precursor, and resistin. This study provides further evidence of unique mechanisms responsible for different phenotypes of PTB. Other studies have evaluated potential

Table 3 Proteomic studies of preterm birth

Sample	Proteomic analysis	Key proteins affected (OR)	Key pathways	Reference
Plasma collected 10–15 weeks GA in 41 women experiencing PTB and 88 term controls with uncomplicated spontaneous vaginal delivery in the Denver Complement Study	SOMAscan proteomic assay; 1,129 proteins	Coagulation factors IX ab (158 OR) and IX (281); factor B (41); PECAM-1 (40.4); complement factor H (26); SAP-component (17.8); VEGF SR2 (10.5); cathepsin Z (6.6); GHR (6.4); ficolin-3 (5.6); ATS15 (4.9); P-cadherin (4.8); etc	Complement cascade; immune system; clotting cascade	38
Serum circulating microparticles at 15–17 weeks GA from 14 sPTB and 12 term control primigravida and 10 sPTB and 12 term second pregnancies	LC-MS analysis generating ~500,000 peptide signals	α1-antitrypsin (1.33 OR); antithrombin III (1.36); α2-macroglobulin (2.07), α1B-glycoprotein (1.33); albumin (1.34); apolipoproteins L1 (3.12) & D (1.32); AZGP1 (1.44); IGG kappa (0.48); serotransferrin (1.37); IGHM2 (2.22); complement factors C1R (0.76) C3 (0.8),C4-B (0.74), & H (0.7), etc	Antigen presentation, humoral immune, and inflammatory pathways	39
Serum from 19–24 weeks ($n = 35$), or 28–32 weeks ($n = 16$) GA from 51 PTBs and 51 matched controls in the Genomic and Proteomic Research Network for Preterm Birth Research	Targeted proteomics using SILAP; shotgun proteomics using LC-MS/MS on pooled samples	Serpin B7 concentrations 1.5-fold higher in women with subsequent PTBs	Matrix degradation	40
Serum obtained at 24 weeks GA from 40 subjects with sPTB and 40 uncomplicated controls	Capillary liquid chromatography electrospray ionization time-of-flight mass spectrometry of low molecular weight proteins	Inter-alpha-trypsin inhibitor heavy chain 4 protein (OR 2.4–8.76)	Not performed	41
Circulating microparticles isolated from plasma collected 10–12 weeks gestation from 25 singleton PTBs ≤34 weeks and 50 matched controls Brigham and Women's Hospital, LIFECODES cohort	Targeted LC-MRM of 132 proteins (Biogenesis AG)	62 proteins "robust power of detecting" sPTB	Inflammation, wound healing, coagulation cascade, steroid metabolism	42
Serum from 5 nonpregnant women; 5 women with preterm labor leading & preterm delivery; 5 with preterm labor with term delivery; 5 with term labor resulting in delivery; 5 at term with contractions University Hospital, Cincinnati, OH	SELDI; Matrix-assisted laser desorption ionization (MALDI); 2-dimensional electrophoresis	All proteomic techniques identified differentially expressed between groups. However, proteins were not identified	Not performed	43
Serum collected from 48 women with sPTB <34 weeks; 62 women who experienced preterm labor with delivery ≥34 weeks (PTL) at the University of Washington	MALDI-TOF-MS and 2D-LC MS/MS analysis of glycoproteins	52 proteins differentially expressed between PTL and PTB (e.g., coagulation factor VII (5.4 fold-change)); serotransferrin (3.7); protein S100-A9 (5.6); alpha-enolase (-6.5); serum amyloid-P component (4.5); tenascin C (-4.89); cell adhesion molecule L1-like protein (-5.6)	Complement and coagulation cascade; inflammation and immune response; fetal-placental development; extracellular matrix	44
Serum collected 16–17 weeks EGA from 10 women with PTB at 34–37 weeks and 10 term controls; Rostov Research Institute of Obstetrics and Pediatrics, Rostov-on-Don, Russia	MALDI-MS following fractionation of serum samples using magnetic beads with reverse phase (MB-HIC C8), metal-affine (MB-IMAC Cu), and weak cation-exchange (MB-WCX) surfaces according	25 proteins showed differential expression (presence/absence), including transgelin-2, β2-glycoprotein-1, SOD1, gelsolin, VEGF-A, prolactin-inducible protein, E-cadherin, endoplasmin, bikunin, fibrinopeptide B, lipocalin-1	Antioxidant enzymes, chaperons, cytoskeleton proteins, cell adhesion molecules, angiogenesis, proteolysis, transcription, and inflammation	45

2D-LC MS/MS, 2-dimentional liquid chromatography tandem mass spectrometry; EGA, estimated gestational age; GA, gestational age; GHR, growth hormone receptor; LC/MRM, liquid chromatography/multiple reaction monitoring; LC-MS/MS, liquid chromatography tandem mass spectrometry; MALDI, matrix-assisted laser desorption ionization; MALDI-MS, matrix-assisted desorption/ionization-mass spectrometry; MALDI-TOF-MS, matrix assisted laser desorption/ionization-time of flight-mass spectrometry; MS, mass spectrometry; OR, odds ratio; PTB, preterm birth; PTL, preterm labor; SAP, serum amyloid P; SELDI, surface-enhanced laser desorption ionization; SILAP, stable isotope-labeled proteome; VEGF, vascular endothelial growth factor.

proteomic biomarkers in cervical vaginal fluid (CVF). Pereira *et al.*[47] used multidimensional LC-MS/MS, multidimensional protein identification technology, and fluorescence two-dimensional differential in-gel electrophoresis techniques to identify and quantify the novel protein biomarkers of PTB in the CVF. Using multidimensional protein identification technology analysis, they discovered 205 proteins in CVF, whereas two-dimensional differential in-gel electrophoresis identified 17 proteins in PTB and spontaneous PTB.[47]

Identification of biomarkers in maternal serum or plasma provides a less invasive approach to identify women at risk for PTB. Gunko *et al.*[48] used mass spectrometric profiling of maternal serum to identify the proteomic predictors of PTB. Their findings detected changes in the production of 25 proteins with various regulatory functions namely proteolysis, angiogenesis, inflammation processes, transcription, and binding and transportation of various ligands that can predict the PTB as early as during the second trimester of pregnancy.[45] Lynch *et al.*[38] performed a longitudinal cohort study using SOMAscan proteomic assay method to measure the protein pathways in plasma among preterm deliveries and controls. The study identified complement cascade, the immune system, and the clotting cascade as top pathways to be associated with PTB, as coagulation factors IX and IX ab, Factor H, and Factor B were associated with increased risk of preterm delivery. Despite having heterogeneous study population and lack of validation of the findings, the authors concluded that plasma protein profiling at 10–15 weeks of gestation can predict the PTB later in pregnancy. Parry *et al.*[40] used shotgun and targeted proteomic techniques combined with stable isotope labeling of amino acids in cell culture to identify proteins in maternal serum. They found that higher serpin B7 concentrations in maternal serum obtained 28–32 weeks gestation were associated with shorter interval to delivery and lower gestational age at delivery.

Metabolomic studies of PTB

Like other omic approaches, metabolomics does not depend on a specific hypothesis but provides an unbiased analysis of the phenotypic makeup of a typical cell, tissue, or organ by virtue of its ability to quantify multiple metabolites. Metabolomic studies profile low molecular weight metabolites in a given biological sample to generate an all-inclusive spectrum. The majority of metabolomic studies for PTB have utilized maternal serum, plasma, amniotic fluid, urine, and CVF using both targeted and nontargeted approaches. A large variety of analytical technologies have been utilized, including ^{1}H-NMR spectroscopy,[48,49] GC-MS,[50] LC-MS,[51] gas and liquid chromatography and mass spectroscopy (GC-LC-MS),[52] UPLC-MS,[53] and ultra-high performance liquid chromatography coupled to mass spectrometry (UHPLC-MS).[54] Each analytic method may target different metabolites. In addition, data may be reported in a variety of formats and any number of downstream informatics and statistical analyses may be utilized. Thus, it may be difficult to compare data between studies.

Graça *et al.*[55] used NMR to analyze amniotic fluid and an untargeted UPLC-MS to analyze the second trimester amniotic fluid and maternal urine to identify the maternal biomarkers and their effects on fetal malformations, PTB, and gestational diabetes and compared women with PTB and controls. Despite the limitations of sample size and technology they used, their findings revealed the existence of different levels of amino acids between the two groups. Heazell *et al.*[53] used UPLC-MS to identify the differences in maternal and fetoplacental metabolites in maternal serum in third trimester pregnancies compared with controls. The study found that 98 metabolites, including free fatty acids, sterol lipids, glycerolipids, sphingolipids, progesterone, and vitamin D metabolites have differed between normal and poor pregnancy outcomes. Their findings are helpful in predicting the potential molecular mechanisms associated with pregnancy complications linked with placental dysfunction in the third trimester.[53] Romero *et al.*[52] used mass spectrometry (MS), using both gas and liquid chromatography data for a retrospective study, to identify the potential predictor metabolites for PTB in amniotic fluid collected between 22 and 35 weeks gestation, regardless of the presence or absence of intra-amniotic infection/inflammation. Although lacking confirmatory studies, the researchers reported that carbohydrates, amino acids, and xenobiotic compounds among the top predictors to identify the patient at risk for PTB. **Table 4**[48–52,54] provides information on these and other serum metabolomics studies relating to PTB.

Although metabolomic studies have provided generalized approaches to identify the high-risk pregnant women through various biochemical risk factors, several limitations still exist that limit their utility as predictive biomarkers of PTB. High intra-individual variabilities, biomarker redundancy, overlapping biochemical and metabolic pathways, and the lack of qualitative and quantitative confirmatory studies are hindering the accurate prediction of PTB. A systematic review revealed that 116 biomarkers have been identified for their role in PTB, but no single biomarker is able to accurately and reliably predict the risk of PTB.[56]

Pharmacogenomic studies of drugs used in PTB

Studies of drug therapies in pregnant women are often limited due to logistic and ethical concerns. Only a handful of studies have evaluated the role of pharmacogenomics in interindividual variability in response. We have previously reviewed the status of personalized medicine and pharmacogenomic data available for drugs used in a number of pregnancy conditions.[57,58] Below, we summarize the data available for drugs commonly administered to women experiencing preterm labor.

Antenatal corticosteroids have been prescribed to induce fetal lung maturation in women with threatened preterm delivery for decades.[59] The dosing regimen of betamethasone used today is identical to that administered by Liggins & Howie[59] in their 1972 clinical study. Although shown to improve neonatal outcomes, including respiratory distress syndrome (RDS), there is still wide variability in response.[60] Systems pharmacology studies may provide insight into

Table 4 Metabolomic studies of PTB

Sample	Metabolomic analysis	Key findings	Reference
Amniotic fluid and maternal serum 35 PTBs; 35 term controls	UHPLC-TOF-MS and UHPLC-MS-MS	Differences detected among groups in 13 lipids in maternal serum; differences in a number of biomarkers, including pyruvate, glutamic acid, inositol in amniotic fluid and hypoxanthine, tryptophan, and pyroglutamic acid in serum	54
Amniotic fluid from African American women; 25 PTBs; 25 controls	GC-MS; LC-MS/MS performed by Metabolon	116 metabolites were significantly different among groups; common pathways involved in liver function, fatty acid, and coenzyme A metabolism, and histidine metabolism	51
CVF in 82 women with threatened, but not confirmed PTL	^{1}H-NMR and enzyme-based spectrophotometry	Elevated CVF acetate was predictive of PTB but did not add predictive accuracy over ultrasound cervical length and fetal fibronectin	48
CVF collected at 20 weeks from 30 sPTB cases and 30 controls	GC-MS of 112 compounds	No significant differences between PTB cases and normal term controls	50
88 sPTB and 275 controls urine samples from late first trimester	^{1}H-NMR spectroscopy of 34 metabolites	sPTB associated with elevated urinary lysine and lower urinary formate	49
Amniotic fluid from 33 women who experienced PTL without IAI; 40 women who experienced PTL with IAI; 40 women with control delivery	GC-LC-MS	Metabolomic profiles differed among all groups. Patients delivering preterm without IAI had relative decrease in carbohydrates and amino acids, whereas those with IAI had decreased carbohydrates but increased amino acids.	52

CVF, cervical vaginal fluid; GC, gas chromatography; H-NMR, hydrogen nuclear magnetic resonance; IAI, intra-amniotic inflammation; LC, liquid chromatography; LC-MS/MS, liquid chromatography tandem mass spectrometry; MS, mass spectrometry; NMR, nuclear magnetic resonance spectroscopy; PTB, preterm birth; PTL, preterm labor; sPTB, spontaneous preterm birth; TOF, time of flight; UHPLC, ultra-high performance liquid chromatography.

individuals who may benefit from increased or decreased dosing of betamethasone. We have conducted a series of studies evaluating pharmacogenomic determinants of neonatal respiratory response to antenatal corticosteroids, reporting results from a study of 109 women who delivered 117 infants of whom 64 were diagnosed with RDS. Maternal CYP3A5, maternal NR3C1 (rs4142347), fetal ADCY9 (rs2230739), and fetal CYP3A7*1E (rs28451617) were significantly associated with neonatal RDS in a multivariable analysis that adjusted for maternal age, maternal and paternal race, estimated gestational age (EGA) at delivery, birthweight, infant sex, cesarean delivery (vs. vaginal delivery), EGA at first dose of betamethasone, and the presence of chorioamnionitis.[61] Subsequently, we have reported that betamethasone clearance is increased in women who are CYP3A5 expressers.[62] In a separate report, 867 SNPs in 68 glucocorticoid response genes were evaluated for associations with neonatal RDS. Interestingly, the most influential SNPs on RDS outcomes seemed to be involved in a variety of physiological pathways. Maternal SNPs were related to maintaining chromosome stability (CENPE), cell cycle regulation and progression (AURKA), cell adhesion, motility, and differentiation (CD9), and signaling (GLXR). Significant fetal SNPs included structural collagen (COL4A3); regulation of hematopoietic cell proteoglycan (SRGN); chromosome stability maintenance (CENP1); and cell differentiation (BHLHE40).[63] Ongoing studies will further probe the relationships among pharmacokinetics, pharmacogenomics, and clinical outcome in neonates exposed to antenatal corticosteroids.

Antitocolytic therapy to stop preterm labor is common but success varies and no agent has emerged as a gold standard.[64,65] Many commonly used tocolytics are substrates of polymorphic enzymes or target polymorphic receptors. However, there is a paucity of research evaluating factors contributing to the interindividual variability in response to antitocolytic agents.

The β2 adrenergic receptor agonist ritodrine is the only US Food and Drug Administration-approved drug for preterm labor tocolysis. However, side effects and limited efficacy of ritodrine and other β2 adrenergic receptor agonists, such as terbutaline and hexoprenaline, have led the US Food and Drug Administration and the European Medicines Agency to issue warnings and limit prolonged use of these agents in recent years.[66,67] Although results are not consistent, some β2 adrenergic receptor genotypes may be protective against preterm delivery.[68,69] Women who are homozygous for Arg16 in β2 adrenergic receptor had improved pregnancy outcomes following hexoprenaline in one study.[70]

Nifedipine and other calcium channel blockers have been shown to have favorable efficacy and safety profiles for the acute treatment of preterm labor.[71] Nifedipine undergoes metabolism by CYP3A enzymes in the liver and gastrointestinal tract. Nifedipine clearance is increased in pregnancy, due to increased CYP3A.[3,10,11,72,73] Plasma concentrations of nifedipine also demonstrate high interindividual variability (30–70%) among pregnant women. Studies of nifedipine in pregnant women have found that CYP3A5 genotype and concomitant administration of

CYP3A inhibitors, such as clarithromycin, erythromycin, and fluoxetine, are associated with altered nifedipine pharmacokinetics.[72,73] Genetic polymorphisms in the L-type calcium channel (*CANC1C* and *CACN1D*) and the large-conductance calcium and voltage-dependent potassium channel ß1 subunit gene (*KCNMB1*) have been associated with responsiveness and risk of cardiovascular side effects in patients taking calcium channel blockers for hypertension.[74,75] However, to our knowledge, these variants have not been studied in pregnant women.

Prostaglandin inhibitors, such as indomethacin, are also effective for the acute treatment of preterm labor.[71] Indomethacin is primarily metabolized by the CYP2C9 enzyme. The CYP2C9*2 and *3 poor metabolizer haplotypes may lead to decreased indomethacin clearance in 10–20% of white patients and up to 6% of black patients.[76] In addition, the activity of CYP2C9 is increased during pregnancy,[3] leading to increased clearance of indomethacin (14.5 ± 5.5 L/h vs. 6.5–9.8 L/h in nonpregnant subjects).[77] Although it has an increased risk of adverse maternal events,[71] magnesium sulfate is often used for tocolysis due to its neuroprotective effect.[78,79] To our knowledge, personalization of magnesium sulfate therapy based on clinical, demographic, or pharmacogenomics has not been investigated.

The 17-alpha hydroxyprogesterone caproate (17-OHPC) is indicated for the prevention of PTB in women with a history of singlet sPTB. As with other progestins, 17-OHPC is metabolized by CYP3A enzymes.[80] Only a few clinical studies with limited sample size have investigated the pharmacogenetics of 17-OHPC.[81–84] Common polymorphisms in CYP3A genes have not been associated with outcomes to 17-OHPC.[81] Studies examining polymorphisms in the progesterone receptor have produced conflicting results.[81,82] A recent exome-wide study by the NICHD's Genomics and Proteomics Network for Preterm Birth Research group has identified a number of genetic differences associated with response to 17-OHPC, including genes associated with breast cancer estrogen signaling and oxidoreductase activity.[84] Additional, larger scaled studies are needed to further identify factors influencing response to 17-OHPC.

Systems approaches to understanding PTB

As indicated above, single-level "omics" approaches have been largely unsuccessful in identifying biomarkers and drug targets associated with PTB. Systems pharmacology approaches that integrate a variety of data types, provide novel tools to probe the pathophysiology of PTB, and other pregnancy-associated diseases. As shown in **Figure 2**, the genomic, proteomic, and metabolomic changes observed in various studies of PTB (**Tables 2–4**) can be combined to detect pathways that are altered in PTB, such as the complement cascade, inflammatory pathways, and coagulation pathways. To fully elucidate the mechanism of PTB, more detailed mechanistic pathway analyses are required. Although some of these pathways can be adopted from other therapeutic areas (e.g., inflammatory cascade), additional details may be needed to fully understand the connection of these pathways to PTB pathogenesis. To this

end, data from animal or *in vitro* studies may prove central to the development of systems models. For instance, *ex vivo* mouse studies provide evidence that progesterone may work, in part, to directly modulate uterine contractility.[85] Additionally, impact of environmental or other exogenous stressors on PTB may be incorporated (e.g., to investigate mechanisms associated with increased risk of PTB in women who smoke).

Challenges of translational pharmacology in obstetrics

Although systems pharmacology holds great promise in helping understand complex diseases of pregnancy, a number of challenges must be overcome to fully realize its worth. As in other areas of research, systems pharmacology approaches in obstetrics requires comprehensive clinical data from electronic medical records, data management resources, and bioinformatics and statistical tools to catalog, integrate, and analyze data obtained from various sources and reported in numerous formats. As illustrated above, appropriate study design is essential to identifying and understanding factors associated with PTB and other disorders of pregnancy. Conde-Agudelo *et al.*[86] conducted a meta-analysis evaluating the accuracy of proteomic and metabolomics biomarkers to predict asymptomatic sPTB in women with singleton pregnancies. None of the 30 novel biomarkers identified in their meta-analysis of 72 studies, including 89,768 women, were deemed clinically useful to predict sPTB in asymptomatic singleton pregnancies. Heterogeneity among studies makes validation and integration of results difficult. For instance, although higher amniotic fluid concentrations of IL-6 have been found by a number of studies to be a predictor of PTB, variability among study methods has made it difficult to establish a quantitative cut-off point.[56]

Overcoming sample size limitations: Biobanks

One challenge in analyzing high-dimensional data is the number of subjects required to detect a statistically significant difference. In addition, the Institute of Medicine guidelines recommend that a potential biomarker should be studied in separate training, test, and validation cohorts.[87] It is impracticable to recruit women prospectively into studies of this magnitude. The recent explosion of perinatal biobanks, which collect biological specimens across pregnancy, enables access to the longitudinal samples required for these studies. These biobanks, especially when linked to comprehensive clinical data, provide a rich data source for understanding changes in gene and protein expression and function across pregnancy.

Perinatal biobanks vary drastically in size and scope of individuals enrolled. A survey of North American perinatal biobanks from 2013–2015 evaluated the scope, clinical documentation, and biospecimens collected. Seven pregnancy biobanks, including the Indiana University Building Blocks of Pregnancy Biobank, the Mount Sinai School of Medicine Pregnancy Biobank, the Global Alliance to Prevent Prematurity and
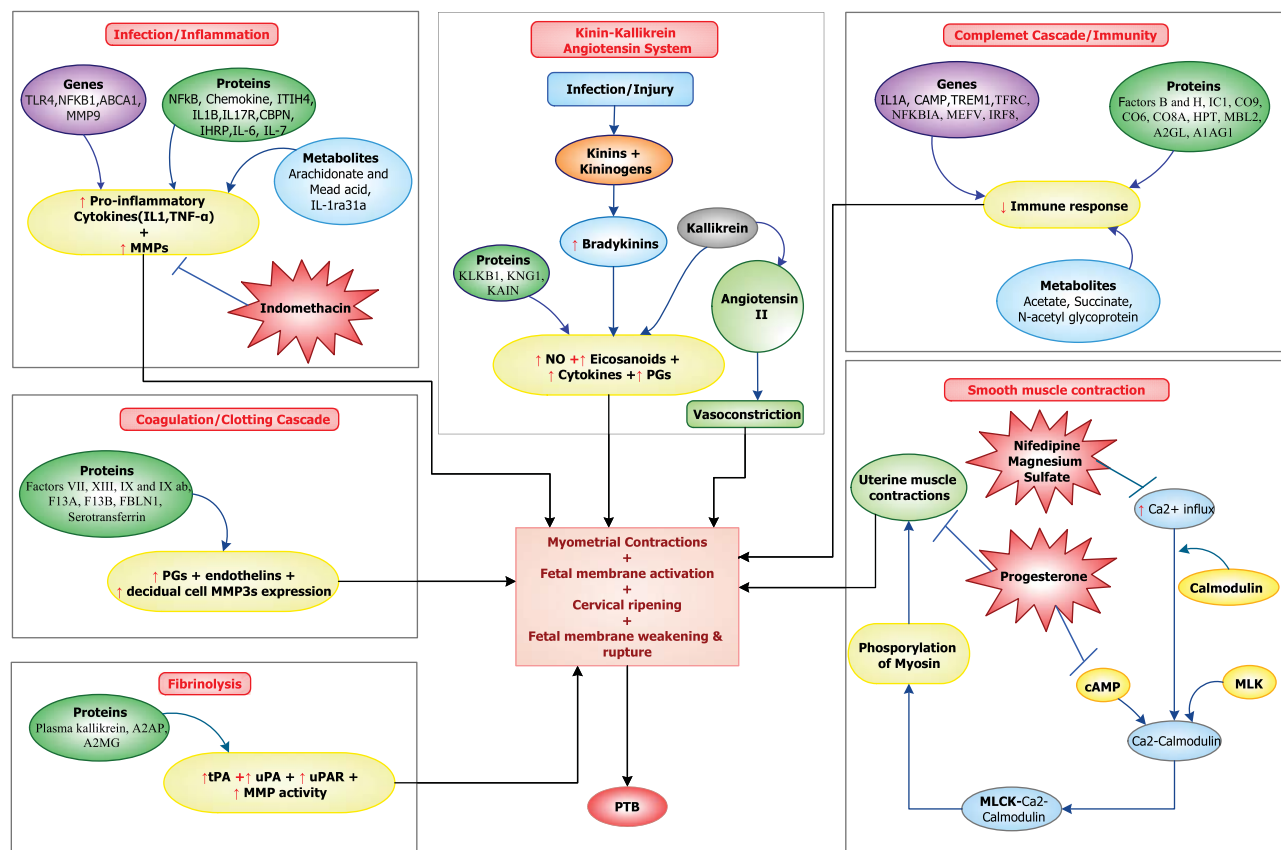
Figure 2 Complex pathophysiology of preterm birth (PTB). Systems pharmacology approaches can integrate data from genomic, proteomic, metabolomic, clinical, *in vitro*, and animal studies to improve understanding and optimize therapy for PTB.

Stillbirth (GAPPS) repository, Baylor College of Medicine, Washington University St. Louis (WIHSC), University of Hawaii, and Lunenfeld-Tanenbaum Research Institute Sinai Health System Research Centre for Women's and Infants' Health (RCWIH) BioBank responded to the survey.[88] All of the biobanks collected cord blood, placental specimens, and maternal plasma at delivery. Several collect maternal whole blood, serum, and/or plasma samples throughout pregnancy. Two collected urine and CVF. Women who had adverse pregnancy outcomes were well represented in the biobanks surveyed, with PTB, pre-eclampsia, and gestational diabetes each occurring in about 10% of enrollees.

Although these localized biobank efforts provide a resource for translational research, the discordance among methods used to collect samples and information complicates efforts to combine data from various groups. For instance, placental samples may be collected in RNALater, formalin fixed, fresh frozen, or snap frozen in liquid nitrogen and time to delivery may vary depending on the protocol.[88] These variations may impact the stability of analytes, leading to discrepancies in downstream analyses. Additionally, clinical and demographic variables collected by individual biobanks, as well as the informatics infrastructure used to store the data, are not uniform. Thus, the informatic requirements for combining the data from these samples may be overwhelming.

In an effort to overcome these difficulties, a number of biobank consortia have been established. For instance, the Preterm Birth Genome Project was established by the World Health Organization in 2007 to pool resources from GWAS studies of PTB.[89] The Preterm Birth International Collaborative (PREBIC) also has worked to collate data on studies of PTB and to develop standard definitions and practices for future studies.[29,89,90] The Global Pregnancy Collaboration (CoLab) is an international collaboration of over 35 centers with data on over 300,000 pregnancies, including biobanks containing 20,000 plasma, serum, and DNA.[91] The technological, organizational, and legal challenges of developing these consortia are burdensome. However, large insight into the pathogenesis of disorders of therapies can be gleaned by the increased access to data and biological samples provided by these consortia.

Phenotype harmonization

The PTB is defined as birth before 37-weeks' gestation. This definition, based on a time point, encompasses a wide variety of clinical syndromes with numerous pathophysiologies. The PTB may be due to multifetal gestation (12%), severe fetal malformations, or death in utero (9%), iatrogenic causes (20%), or sPTB (~60%).[89] Iatrogenic PTB

are medically indicated due to other comorbidities, including pre-eclampsia, gestational diabetes, and fetal growth restriction. Spontaneous PTB can be further classified into PPROMs, in which membranes rupture at least 1 hour before onset of labor at <37-weeks' gestation, or spontaneous preterm labor, defined as regular contractions with cervical changes <37-weeks' gestation. Although PPROM and spontaneous preterm labor have distinct pathophysiologies and clinical presentations, studies of PTB often group them into a single cohort. This may reduce the power of a study to detect significant associations or prevent results from being replicated. Thus, there a critical need for large, generalizable studies of women with well-defined phenotypes to develop specific cutoff points of validated biomarker panels. Consortia, such as PREBIC, are leading efforts to develop minimal datasets for "omics" studies of PTB and to mitigate issues surrounding the assimilation of data from multiple studies.[89,90] Clear definition of clinical phenotype is also necessary to define the pharmacodynamic outcomes of systems pharmacology models, which may incorporate a variety of endpoints (e.g., uterine contraction, cervical dilation, time to delivery, and gestational age at delivery).

Informatic and biostatistical resources

The progress in the application of omics technologies (e.g., genomics, proteomics, and metabolomics) has enabled the high-throughput monitoring of a variety of biochemical systems and characterization of the pathophysiology of several conditions to develop efficient prediction models for diagnostic application. However, the advancement is hindered by challenges within and in between omics-domain data integration where combining the experimental results on molecular profiling from different omics platforms could be the major concern for interpretation of changes and prediction models.

A variety of approaches to combining metabolomics with genomics and proteomics data have been developed based on the biochemical pathway, network, ontology, and empirical-correlation methods. Biochemical pathway analysis provides the users a way to explore whether molecular pathways or biochemical processes are associated with a phenotype study based on the omics data. A novel pathway analytical approach gene set enrichment analysis (GSEA) that combines transcriptomics data with metabonomics data was developed for interpreting genome-wide expression profile data.[92] However, pathway analysis of genome-wide profiling assume that genes and pathways are independent of each other often suffers from missing cell-specific information and incomplete annotations.[93] Genetic analysis of PTB was studied using pathway-based analysis where curated genes and the genomewide approach were used to identify the gene-gene interactions from a validated set of genes.[94]

A number of bioinformatic tools have been developed to combine data from genomic, proteomic, and metabolomic studies to describe biological pathways or networks. SAM-NetWeb, a web-based analytical tool, uses proteomics and transcriptomics to identify the distinct and common pathways among multiple experiments and visualize them in a single interaction network.[95] Another rapidly growing web-based tool, Metscape2, also links metabolite data with other omics data, such as proteomics and transcriptomics. Users can enter the experimental data (collected from different pathways of metabolites, proteins, and genes) stored in distinct Kyoto Encyclopedia of Genes and Genomes and Edinburgh Human Metabolic Network databases and identify the activated or expressed profiling data, build, and analyze the data into a common network.[96] Empirical-correlation methods are helpful to integrate biochemical with other omics data when domain knowledge is unavailable. Weighted gene co-expression network analysis can be used to identify the clusters of highly correlated genes based on various gene screening methods that have a potential to recognize candidate biomarkers.[97] Although these system biology tools have been used in other domains, we are unaware of examples in which multiple types of omics data from preterm labor studies have been systematically integrated.

Interpretation of high-dimensional data in pregnancy is made even more complex by the need to integrate maternal, fetal, and placental data. The majority of bioinformatic tools is developed for studies of conditions, such as cancer and neurodegenerative diseases, and is thus biased to adult diseases. Therefore, they may not encompass processes of normal fetal development and parturition. This bias toward functional classification was demonstrated by Edlow *et al.*[98] through the evaluation of transcriptomic datasets of cell-free RNA in amniotic fluid obtained from second trimester fetuses with aneuploidy (trisomy 21), hemodynamic (twin to twin transfusion syndrome), and metabolic (maternal obesity) complications and controls. Functional analyses conducted by ingenuity pathway analysis (IPA) and GSEA through the gene ontology database augmented with fetal-specific gene sets cataloged by the Developmental Functional Annotation at Tufts (DFLAT),[1] which catalogs human fetal gene functions.[99] Edlow *et al.*[98] found that IPA and GSEA/DFLAT analyses provided different, but largely complementary, interpretations of the data. The IPA, a proprietary software, provided a preferred user interface and unique analysis tools, such as the Canonical Pathway and Upstream Regulator Analysis, that allowed for improved mechanistic insight. However, compared to GSEA/DFLAT, IPA was more biased toward adult diseases, misclassifying fetal cell proliferation assays as cancer pathways.[98]

CONCLUSIONS

Systems pharmacology provides a means to integrate changes in the physiologic factors that affect drug disposition and the complex interplay among maternal, placental, and fetal systems and improve our understanding of the pathophysiology and molecular mechanisms of disorders of pregnancy, such as PTB. However, there are many challenges that need to be overcome to fully harness the potential of systems pharmacology in obstetrics, including harmonization of phenotype definitions and standardized methods of annotation and analysis. As clinical studies in pregnant women are often limited by ethical and practical constraints,

other sources of data are important to consider (e.g., studies in other therapeutic areas, *in vitro*, or animal models). Systems pharmacology approaches to integrate knowledge of the physiologic and molecular mechanisms responsible for normal and adverse outcomes of pregnancy may detect new therapeutic targets for PTB and other pregnancy-associated disorders. In addition, systems pharmacology approaches that integrate the effect of pregnancy on pharmacokinetics (e.g., through PBPK modeling) and pharmacodynamic effects of drugs will better inform therapeutic approaches and optimize dosing regimens in pregnancy.

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