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The Maternal Plasma Proteome Changes as a Function of Gestational Age in Normal Pregnancy: a Longitudinal Study

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Abstract

Objective—Pregnancy is accompanied by dramatic physiologic changes in maternal plasma proteins. Characterization of the maternal plasma proteome in normal pregnancy is an essential step for understanding changes to predict pregnancy outcome. The objective of this study was to describe maternal plasma proteins that change in abundance with advancing gestational age, and determine biological processes that are perturbed in normal pregnancy.

Materials and methods—A longitudinal study included 43 normal pregnancies that had a term delivery of an infant who was appropriate for gestational age (AGA) without maternal or neonatal complications. For each pregnancy, 3 to 6 maternal plasma samples (median=5,) were profiled to measure the abundance of 1,125 proteins using multiplex assays. Linear mixed effects models with polynomial splines were used to model protein abundance as a function of gestational age, and significance of the association was inferred via likelihood ratio tests. Proteins considered to be significantly changed were defined as having: 1) more than 1.5 fold change between 8 and 40 weeks of gestation; and 2) a false discovery rate (FDR) adjusted p-value <0.1. Gene ontology

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enrichment analysis was employed to identify biological processes over-represented among the proteins that changed with advancing gestation.

Results—1) Ten percent (112/1,125) of the profiled proteins changed in abundance as a function of gestational age; 2) of the 1,125 proteins analyzed Glypican-3, sialic acid-binding immunoglobulin-type lectins (Siglec)-6, placental growth factor (PlGF), C-C motif (CCL)-28, carbonic anhydrase 6, Prolactin (PRL), interleukin-1 receptor 4 (IL-1 R4), dual specificity mitogen-activated protein kinase 4 (MP2K4) and pregnancy-associated plasma protein-A (PAPP-A) had more than 5 fold change in abundance across gestation. These 9 proteins are known to be involved in a wide range of both physiologic and pathologic processes, such as growth regulation, embryogenesis, angiogenesis immunoregulation, inflammation etc.; and 3) biological processes associated with protein changes in normal pregnancy included defense response, defense response to bacteria, proteolysis and leukocyte migration (FDR=10%).

Conclusions—The plasma proteome of normal pregnancy demonstrates dramatic changes in both magnitude of changes and the fraction of the proteins involved. Such information is important to understand the physiology of pregnancy, development of biomarkers to differentiate normal vs. abnormal pregnancy, and determine the response to interventions.

Keywords

aptamer; biomarker; C-C motif (CCL)-28; carbonic anhydrase 6; dual specificity mitogen-activated protein kinase kinase 4 (MP2K4); high-throughput biology; glypican-3; placental growth factor (PlGF); prolactin (PRL); interleukin-1 receptor 4 (IL-1 R4); pregnancy-associated plasma protein A (PAPP-A); proteins; sialic acid-binding immunoglobulin-type lectins (Siglec)-6

Introduction

Viviparity requires major adaptations in the mother to sustain the establishment, development, growth, and eventual separation of a fetal semi-allograft at the time of parturition. Pregnancy in placental mammals is a unique biological phenomenon, perhaps unmatched in the history of evolutionary biology. Reproductive success depends on an intensive and rich dialogue among mother, placenta, and fetus. The adaptations required for pregnancy appear to involve virtually all maternal organs/systems, including the metabolic/endocrine (1–6), cardiovascular (7–12), respiratory (13), gastrointestinal (12, 14–21), hematologic (22, 23), and central nervous systems (24–26).

The study of the physiology of pregnancy has spanned decades, and has largely depended upon available technological capabilities to study parameters that change with gestational age. Such parameters have been studied with relatively simple methods, such as hormonal determinations (20, 21, 27), concentrations of nutrients/fuels (glucose(17–19), lipids(28–33), proteins(34–40), amino acids(41–43)), blood pressure (8, 44), cardiovascular function (9, 11), spirometry (13, 16), immunological assays (14, 24, 45), and different tests of central nervous system function (25, 26). These studies have been essential to understand changes in body composition (4, 18), physiologic adaptation (10, 13), pathophysiology of selected pregnancy complications (17, 46–48). One of the domains of interest that has proven to be extraordinarily successful is the study of changes in plasma protein concentrations in maternal blood. For example, the detection of human chorionic gonadotrophin (hCG) in

human blood has allowed the early detection of pregnancy, even before the first signs of amenorrhea(56–58). Monitoring early pregnancy with serial determinations of hCG has also allowed identification of patients at risk for ectopic gestation(59–70), and other forms of pregnancy failure (60, 62, 63, 65, 67). In the case of ectopic pregnancy, this has allowed not only earlier diagnosis, but even the introduction of minimally invasive surgical techniques (e.g. laparoscopic resection and medical treatment) (71–79). The monitoring of gestational trophoblastic disease has also been dramatically changed with the use of serial hCG determinations in maternal serum/plasma (80, 81). However, these examples only represent the first of many diagnostic advances made possible by monitoring concentrations of a single protein during pregnancy.

The observation that a fetus with a neural tube defect had been born to a mother with an idiopathic elevation of maternal serum alpha fetoprotein eventually led to the discovery that such elevations could be used to screen for the presence of neural tube defects in the mid-trimester of pregnancy(56, 82–86), and continue to be the basis – more than 20 years later – for the biochemical screening of these congenital anomalies(84, 87–93). Further advances occurred with the introduction of the triple (followed by the quadruple) biochemical screening, including three proteins (alpha fetoprotein, hCG, inhibin), which were subsequently combined with a steroid hormone, estriol, to assess the likelihood of trisomies 18 and 21 at the end of the first trimester (88, 89, 93–100). Collectively, this evidence, which is now in routine clinical practice, provides compelling evidence that examination of the plasma/serum composition can provide important insight into the biology of pregnancy, fetal health and disease, and has changed the practice of obstetrics and medicine.

The most recent entry into the protein biomarker discovery panel is the determination of angiogenic/anti-angiogenic factors for the early prediction of preterm preeclampsia(47, 101–110), fetal death(111, 112), late preeclampsia(47, 101–110), small-for-gestational-age (SGA) (113), and maternal floor infarction(114). This set of biomarkers can now be readily determined using commercially-available tests, and has the potential to identify patients at risk for pregnancy complications in the long subclinical phase of the “great obstetrical syndromes”, and open the door for prevention, which has been a challenge in obstetrics for decades(105, 115–124).

High-dimensional biology techniques allow characterization of the genome (54, 55, 125, 126), transcriptome(55, 127–137), proteome(55, 125, 127, 138–148), lipidome(149–155), glucome(156, 157), metabolome(6, 55, 128, 158–163), and cytome(164–169). Major trust of the new biology and medicine has been to gather the information derived from these platforms to optimize diagnosis, prognosis, and treatment (170, 171). Although each of these techniques has a particular informational domain (DNA, RNA, proteins, metabolites, lipids, etc.), they can be used independently or in combination (172, 173). Proteins are considered to be important executors of biological functions, as they include enzymes (80), structural components of tissues/cells (174), the coagulation cascade (175, 176), and the inflammatory network (177).

There have been substantial attempts to characterize the human plasma proteome in non-pregnant subjects, which have been made possible by the development of sophisticated

mass-spectrometry techniques, using a top-down approach (81, 140, 178). These efforts have been extended to the maternal plasma proteome and amniotic fluid(138–140, 145, 147, 148, 179–203); however, technological complexities continue to challenge the goal of developing a comprehensive map of the entire human plasma proteome. The total estimated number of proteins in the human body ranges from 20,000 to one million(78) – of these, plasma proteome studies have been able to detect and semi-quantify a number between 16,500–20,000 (79, 204–206). Immunoassays and related methods continue to be the "gold standard" for the sensitive determination of proteins in peripheral plasma, guide diagnosis, monitoring of disease, and treatment; in addition, they are more sensitive than 2D gels or mass spectrometry, and can detect analytes in very small quantities (below nM range). In addition, technologies like enzyme-linked immunosorbent assay (ELISA) require two antibodies to the same protein to elicit a signal. Such immunoassays cannot be multiplexed above a few tens of simultaneous measurement, largely due to the cross-reactivity of the secondary antibodies to surface-immobilized proteins (207–210).

To enlarge the number of proteins that can be detected simultaneously with a high degree of sensitivity and dynamic range, a new technology has been developed which is not based on conventional antigen/antibody reaction. The aptamer-based method uses single-strand DNA or RNA molecules that bind, with high affinity and specificity, to proteins, peptides, or other pre-defined molecules. Recent publications have emphasized the extraordinary potential of aptamer technology in biomarker discovery for cardiovascular disease (211),and other important biomedical disorders (212–218).

The objective of this study was to use aptamer-based technology to characterize the maternal plasma proteome of normal pregnancy as a function of gestational age. This study is important for the description of the simultaneous changes in plasma protein composition, and will serve as the basis to detect departures of abnormalities, before and during the time of diagnosis of major obstetrical complications.

Methods

Study design

We conducted a prospective longitudinal study that enrolled normal pregnant women attending the Center for Advanced Obstetrical Care and Research of the Perinatology Research Branch, NICHD, and the Detroit Medical Center / Wayne State University. A retrospective cohort study was conducted to include 43 who delivered at term, and each one of them had 3 to 6 plasma samples obtained during pregnancy, before the spontaneous onset of labor (median number of samples=5),]. Plasma samples were collected at the time of a prenatal visit, scheduled at four-week intervals from the first or early second trimester until delivery. Each patient had at least three samples collected during the following gestational age intervals (8-<16 weeks, 16-<24 weeks, 24-<28 weeks, 28-<32 weeks, 32-<37 weeks and >37 weeks). All patients had the placenta collected at the time of delivery, transported to the laboratory, and sectioned for histological examination, following criteria of the Society for Perinatal Pathology. Lesions were diagnosed using previously established criteria(71–73). Only patients without acute inflammatory lesions of the placenta were included in this study because of the potential to confound the relative abundance of the maternal plasma

proteome. All patients provided written informed consent and the use of biological specimens, as well as clinical and ultrasound data for research purposes were approved by the Wayne State University and Institutional Review Boards of NICHD.

Proteomics technique

Maternal plasma protein abundance was determined using the SOMAmer (Slow Off-rate Modified Aptamers) platform and its reagents. This platform allows measurement of over 1,125 proteins in maternal plasma samples (210, 219, 220). Proteomics profiling was performed by Somalogic, Inc. (Boulder, CO).

The serum samples were diluted and then incubated with the respective SOMAmer mixes pre-immobilized onto streptavidin-coated beads. The beads were washed in order to remove all non-specifically bound proteins and other matrix constituents. Proteins that remained specifically bound to their cognate SOMAmer reagents were tagged using an NHS-biotin reagent. After the labeling reaction, the beads were exposed to an anionic competitor solution that prevents non-specific interactions from reforming after disruption.

Using this approach, pure cognate-SOMAmer complexes and unbound (free) SOMAmer reagents are released from the streptavidin beads using ultraviolet light that cleaves the photo-cleavable linker used to quantitate protein. The photo-cleavage eluate, which contains all SOMAmer reagents (some bound to a biotin-labeled protein and some free), was separated from the beads and then incubated with a second streptavidin-coated bead that binds the biotin-labeled proteins and the biotin-labeled protein-SOMAmer complexes. The free SOMAmer reagents were then removed during subsequent washing steps. In the final elution step, protein-bound SOMAmer reagents were released from their cognate proteins using denaturing conditions. These SOMAmer reagents were then quantified by hybridization to custom DNA microarrays. The Cyanine-3 signal from the SOMAmer reagent was detected on microarrays (210, 219, 220).

Statistical analysis

Demographics data analysis—Clinical characteristics of the patient population were summarized as median and inter-quartile ranges (IQR) for continuous variables, or percentages for categorical variables, using SPSS software (version 19; IBM Corporation, Armonk, NY)

Differential abundance analysis—Protein abundance expressed as fluorescence units was log (base 2) transformed to improve normality. Linear mixed-effects models with cubic splines (number of knots = 3) were used to model protein abundance as a function of gestational age using *lme4* package (221) under the R statistical language and environment (www.r-project.org). Inference about statistical significance of associations was calculated using likelihood ratio tests between a model that included the gestational age terms (fixed and corresponding random effects) and a simpler random intercept linear mixed-effects model without gestational age terms.

Protein abundance was considered to have changed significantly with gestational age if it met the following criteria: 1) the magnitude of abundance of change was >1.5 fold between

8 and 40 weeks of gestation; and 2) false discovery rate (FDR) (222) adjusted p-values (q-values) <0.1. Similar or less stringent criteria have been extensively used in high-dimensional biology and have shown good validation by alternative techniques and/or in independent sets of samples(223–226).

Fold change was defined as the ratio of protein abundance (in relative fluorescence units) between the highest and the lowest mean value across gestational age. The median (50^{th} quantile) and 10^{th} and 90^{th} quantiles of the protein abundance were also determined using quantile regression modeling (227, 228). In these models, the relationship between protein abundance was assumed to be linear within a narrow moving window of gestational age, and this allowed obtaining a non-linear smooth estimate of the quantiles as previously described (107).

Clustering proteins by average profile—To identify groups of proteins based on their pattern of change across gestation, we have used unsupervised hierarchical clustering. The input in this analysis was the mean protein abundance across gestation (longitudinal patterns) computed from linear mixed-effects models for each gestational week from 8 to 40 weeks. Average profiles were scaled between 0 and 1 prior to applying hierarchical clustering with Euclidean distance, so that proteins with similarity longitudinal patterns (e.g. monotonically increasing) are grouped together despite eventual differences in the magnitude of change (e.g. rate of increase) or overall protein abundance. With hierarchical clustering, each protein is considered a cluster by itself and iteratively, clusters are merged so that the distance between the farthest apart members of the clusters (complete linkage) is minimized. To determine the optimal number of clusters, we have used a goodness of clustering measure (the gap statistic) which compares the change in within-cluster dispersion (sum of squared distances between cluster members) with that expected under the null distribution (simulated by bootstrap)(229).

Gene ontology enrichment analysis—Having identified proteins which change in abundance as a function of gestational age, the next step was to gain an understanding of the potential functional roles of these proteins in human pregnancy. To accomplish this, we relied on the information deposited for the genes encoding for each protein, and which have been organized in publicly-available databases (i.e. gene ontology). We focused on the biological processes in which these proteins have been implicated. Each protein was mapped to an identifier in the Entrez gene database.(230) based on Somalogic, Inc. protein annotation system, and then to gene ontology (231). Biological processes over-represented among the proteins that changing in abundance with advancing gestational age. We used a Fisher's exact test and odds ratios to estimate enrichment. Gene ontology terms with three or more hits and an adjusted p-value <0.1 were considered significantly enriched in gestational age modulated proteins.

Protein-protein interaction network analysis—To explore the potential impact of gestation on human plasma proteins not profiled in this study, we conducted an in-silico analysis for which we retrieved the known protein-protein map interactions from the publicly available Protein Reference Database (HPRD, release 9) (232) using the *NCBI2R* package. For each of the 1,125 proteins profiled, we determined the number of protein-

protein interactions documented in the database. For visualization purposes, a graph was constructed linking proteins with known interactions with the 112 proteins changing with gestational age.

Results

Clinical characteristic of the study population

Forty-three women with normal pregnancies met the criteria for inclusion in this study. The median (interquartile range) maternal age was 25 (21–28) years old, and 88.4% of patients were of African-American origin by self-reporting. All patients delivered at term without any obstetrical complications and had neonates with an appropriate weight for gestational age(233). The median gestational age at delivery was 39.4 [interquatile range (IQR) 39.0–40.1].

Maternal Plasma Proteome in Normal Pregnancy Characterized by SomaSCAN

Ten percent (112/1,125) of the proteins profiled changed in abundance as a function of gestational age (fold change >1.5 and q-value <0.1) (see Table 2). Figure 1 shows longitudinal protein abundance for two selected proteins together with the fitted mean (by linear-mixed effects models) and median (by quantile regression) as a function of gestation. Similar plots for all 112 significant proteins are displayed in supp. File 1

Thirty-six percent (41/112) of proteins decrease in abundance and 64% (71/112) increase with gestational age. Hierarchical cluster analysis of average protein profiles across gestation (see Figure 2) demonstrated among the increasing abundant proteins three patterns in the change of the relative fluorescence reading (increasing rate: n=21, constant rate: n=23, and decreasing rate: n=27). Similar patterns were observed among proteins with decreasing abundance during gestation (increasing rate: n=8, constant rate: n=16, and decreasing rate: n=17).

The most highly modulated proteins (highest fold change) among the significant ones were: 1) placental growth factor (PlGF); 2) pregnancy-associated plasma protein A (PAPP-A) (>5 fold change in abundance across gestation,; 3) sialic acid-binding immunoglobulin-type lectins (Siglec)-6; 4) glypican-3; 5) C-C motif (CCL)-28; 6) carbonic anhydrase 6; 7) prolactin (PRL); 8) interleukin-1 receptor 4 (IL-1 R4); and 9) dual specificity mitogen-activated protein kinase kinase 4 (MP2K4)(see Figure 3).

Biological Processes Modulated During Gestation

Gene ontology analysis of the corresponding proteins that changed with gestational age revealed fourteen biological processes that are impacted with advancing gestation. These biological processes included: general defense response, defense response to bacteria, defense response to fungi, germ cell migration, proteolysis and leukocyte migration (FDR=10%) (see Table 3). Out of the fourteen processes, defense response to fungus, germ cell migration, and defense response to bacterium had all decreased abundance of the protein associated with these biological processes with advancing gestation. In contrast, only the

biological process of smooth muscle cell migration has an increase abundance of the proteins involved in it.

Protein-protein Interaction network

An analysis was performed to retrieve the known protein-protein interactions from the Human Protein Reference Database to assess the connections with proteins changing with gestational age of this study (see Figure 4). Proto-oncogene tyrosine-protein kinase Src (SRCN1) and Tyrosine-protein kinase Fyn (FYN) had the largest number of documented interactions (260 and 196, respectively) of all proteins changing during pregnancy suggesting that they have important roles in the interactome. Other well-known proteins such as MMP-9, VEGF and PlGF had 38, 26, and 5 documented interactions, respectively.

Discussion

Principal findings of the study

I) Ten percent (112/1,125) of the 1,125 proteins assessed in this study in maternal plasma change with advancing gestational age; II) the concentration of nine proteins [1) placental growth factor (PlGF); 2) pregnancy-associated plasma protein A (PAPP-A) 3) sialic acid-binding immunoglobulin-type lectins (Siglec)-6; 4) glypican-3; 5) C-C motif (CCL)-28; 6) carbonic anhydrase 6; 7) prolactin (PRL); 8) interleukin-1 receptor 4 (IL-1 R4); and 9) dual specificity mitogen-activated protein kinase kinase 4 (MP2K4)] increased dramatically in abundance (fold change >5); III) proteins demonstrated either increased or decreased in abundance with advancing gestational age following at least 3 distinct patters of change (increasing rate, constant rate, and decreasing rate of change in abundance); and IV) functional analysis revealed that the proteins that were identified as changing with gestational age belonged to the following biological processes (as determined by gene ontology-derived analysis methods): a) general defense response, b) defense response to bacteria, c) defense response to fungi, d) germ cell migration, e) proteolysis, and f) leukocyte migration. This communication reports changes in a large number of proteins in the maternal plasma proteome in normal pregnancy using a state-of-the-art proteomic multiplex platform. The results in normal pregnancy reported herein can serve as the basis for the identification of biomarkers for the prediction, monitoring of disease, and diagnosis of obstetrical disorders.

Meaning of the Study

The reasons to characterize the maternal plasma proteome in normal pregnancy have been outlined in the introduction of this manuscript, and the information derived from measuring protein concentrations has had great clinical value for the care of pregnant women.

We found nine proteins which showed a dramatic increase in concentration, defined as more than five-fold. The magnitude of change of Placental growth factor (PlGF) was 14.5 fold, and this protein plays an important role in the control of angiogenesis, which is a key process in placental development. Low concentrations of PlGF have already been reported in patients who subsequently developed early onset preeclampsia(104–107, 234–237), fetal death of unknown origin(111, 112), small-for-gestational-age infants (with and without

Doppler abnormalities) (104–107), maternal floor infarction(109, 238, 239), mirror syndrome(114, 123, 240–244), and some forms of twin-to-twin transfusion syndrome(74). The identification of PlGF as a protein that undergoes dramatic changes in maternal plasma concentration in this study (and using current technologies) is reassuring, given its physiologic importance, as well as the prognostic value in measuring this concentration in maternal plasma to identify patients at risk for adverse pregnancy outcome.

The same applies to Pappalysin-1 (PAPP-A), one of the top nine proteins whose concentration increased more than five-fold in this study. PAPP-A is mainly produced by the placenta, and its concentration is low in the first trimester of pregnancies complicated by either chromosomal abnormalities (Trisomies 21, 18 and 13, and monosomy X) (93, 97–100, 245–248) or a subset destined to develop placenta-mediated obstetrical syndromes such as SGA and preeclampsia (119, 249–261) and preterm birth (262). In this study, we also report the longitudinal changes in the plasma concentrations of PAPP-A as a function of gestational age. The concentration of PAPP-A increased steadily until about 20 weeks of gestation, when it plateaued and remained relatively stable until term. Our observations are consistent with those of a previous report, and provide further strength to the validity of our findings (263).

Maternal plasma Siglec-6 and Glypican-3 were also among the top-ranked proteins which increase in concentration with advancing gestational age 17 and 26 fold, respectively (Table 2). Although these proteins were previously described in *in vitro* studies and non-pregnant subjects, there is no systematic study describing the changes in the concentrations of these proteins in maternal plasma in normal or abnormal pregnancy.

Sialic-acid-binding immunoglobulin-like lectins (Siglec)-6 is a CD33rSiglec that has been implicated in the regulation of two major biological functions: cell-to-cell interactions and regulation, through glycan recognition, of the innate and adaptive immune systems (264–267). In addition, this molecule can also serve as a leptin receptor (OB-BP1), which has high specificity and affinity to this molecule. Importantly, Siglec-6 is expressed only in cyto- and syncytiotrophoblasts of the human placenta (268–270), and this may explain how it gains access to the maternal circulation and increases with gestational age as the volume of the villous tree increases (270). A recent report has documented an increase in Siglec-6 expression in the placenta following spontaneous labor and delivery in comparison to those following elective cesarean section without labor; therefore, it has been proposed that this molecule may play a role in the process of parturition (270, 271). In addition, increased Siglec-6 trophoblast expression was reported in women with gestational trophoblastic disease and those with preterm preeclampsia (124, 269, 272–274). The findings in Siglec-6 reported herein provide further support that an increase in abundance of this protein, detected with current technology in the maternal circulation, may have biological and clinical implications.

There is a paucity of information about Glypican-3 in pregnancy. This protein is a heparan-sulfate proteoglycan which acts as a co-receptor for heparin-binding growth factors, such as insulin-like growth factor (275–281), which is expressed in the syncytiotrophoblast of term placentas, and has been reported to be downregulated in trophoblasts of patients with

preeclampsia (282, 283). One study reported a link between Glypican-3 and one of the major anticoagulation proteins of the trophoblast-placenta protein 5/tissue factor pathway inhibitor 2: this anticoagulation protein is specific to pregnancy and has been implicated in the maintenance of placenta hemostasis; the placental expression of the latter is reduced in preeclampsia(282). Glypican-3 has been proposed to serve as the anchoring protein of placenta protein 5/tissue factor pathway inhibitor 2; and that the decrease in Glypican-3 in preeclampsia leads to the release of placenta protein 5/tissue factor pathway inhibitor 2 into the maternal circulation, promoting a higher concentration in this obstetrical syndrome. Our study reports the first changes in the concentrations of Glypican-3 in the maternal circulation, and it is noteworthy that this protein increases 26-fold during pregnancy in a pattern of decreasing slope (Figure 3) with advancing gestational age.

Several of the findings reported herein are consistent with those described in maternal plasma/serum for other proteins: this lends reassurance to the validity of our findings derived with the use of a novel aptamer-based technology. Specific examples include plasminogen activator inhibitor type 1 (PAI-1) (284), insulin-like growth factor binding protein (IGFBP)-1 (285–290), hepcidin (291) and thyroid stimulating hormone (292). The current study is the first to examine and analyze longitudinally the profile of the maternal plasma proteome of more than 1,000 proteins simultaneously using a high-dimensional biology platform.

Biological processes modulated during gestation

Gene ontology analysis revealed that the defense response, proteolysis and cellular response to hormone stimulus were the most enriched biological processes among proteins that changed with gestational age. The host defense response is a protective mechanism against pathogens and “danger signals” such as alarmins, and involves activation of both the innate and adaptive immune response (293–300). Tolerance of a semi-allograft (fetus and placenta) represents a major biological challenge imposed by viviparity. While the fundamental mechanisms responsible for this remain to be elucidated, a general proposal is that there is a down-regulation of the specific limb of the adaptive immune response aimed at paternal antigens, with an up-regulation of the innate immune response (116–118, 149–154, 301, 302). Down-regulation of specific immune responses includes not only paternal/fetal antigens, but also microbial products; therefore, the physiologic modulation of the immune response to enhance tolerance of the semi-allograft could expose the mother to infection, and the infectious process may be more severe (165, 169). Evidence in support of this is that patients with pyelonephritis during pregnancy are more likely to develop acute respiratory distress syndrome and other complications,(155–157, 166–168) and those with viral diseases during pregnancy (e.g. influenza, varicella, H1N1, SARS) are more likely to develop serious complications, and even die during pregnancy ((179–186, 188, 189). The production of antimicrobial peptides (AMP) is a mechanism to enhance host immunoprotection, and indeed, the production of a broad range of antimicrobial peptides (antibacterial and antiviral) is enhanced during pregnancy (303–308). Such products have been detected in both the amniotic cavity and systemic circulation ((187, 190, 309, 310).

The amniotic fluid is known to have anti-microbial properties (310–316) and this could be due to the presence of naturally occurring AMPs, such as bactericidal/permeability-

increasing protein (307, 309), lysozyme (141, 317–320) lactoferrin (320–322), calprotectin (MRP8/14) (309), LL37 (319) and neutrophil defensins (305, 309, 310, 319, 323, 324). Moreover, we reported that increased AMP concentrations are associated with pregnancy complications such as intra-amniotic infection, preterm labor and preterm prelabor rupture of membranes (309, 310). The nature of the host immune response to microorganisms during pregnancy may not be uniform, and may be context-dependent. For example, there is some evidence that the host defense against fungi is not as robust as that against bacteria, and this may explain the increased predisposition to vaginal candidiasis during pregnancy ((191, 192, 325–327). In addition, patients who conceived with an intrauterine contraceptive device are particularly susceptible to infections with fungi in the amniotic cavity, and this may reflect unique features of the complex immune response to different microorganisms during pregnancy(193–202, 328).

One of the findings of this study is that proteins related to smooth muscle cell migration are increased in abundance in maternal plasma with advancing gestational age – this finding is entirely consistent with the fact that the uterus must grow during pregnancy to accommodate the increased size of the fetus, placenta, and amniotic fluid. Importantly, there has not been a good biomarker to monitor smooth muscle function in maternal plasma, and the findings in this study open the door for the identification of such proteins.

Protein-protein interaction network

Proteins function in concert as a part of larger protein complexes within a cell; therefore, an important aspect of proteomic analysis lies in the elucidation of interacting proteins and mapping the corresponding binding sites (329, 330). By identifying proteins interacting with those modulated during normal pregnancy, we can hypothesize on the importance of the change in protein abundance with gestation. These inferences rely on the basis of their degree (number of direct protein-protein interactions) in the *interactome*. Of note, the average number of interacting proteins in the Human Protein Reference Database is ~3.7 per protein (330). Proto-oncogene tyrosine-protein kinase Src (SRCN1) and Tyrosine-protein kinase Fyn (FYN) had 260 and 196 documented interactions in the Human Protein Reference Database; thus, these two proteins appear to play a central role in the interactome.

SRCN1 is part of the Src signaling system involved in cellular signaling of proteinase-activated receptors (PAR) 2, that is, a G-protein coupled receptor that modulates activation induces vascular endothelial growth factor receptor (VEGFR)-1 promoter activity and sVEGFR-1 release from endothelial cells. Given the importance of VEGFR-1 in the regulation of endothelial function during pregnancy and its pathogenesis in preeclampsia, SGA, fetal death, maternal floor infarction, and some forms of spontaneous preterm labor, our findings strengthen the case for the importance of discovery techniques in the elucidation of fundamental mechanisms of disease in obstetrics.

Tyrosine-protein kinase Fyn (FYN) is also a member of the Src family. In contrast to SRCN1, FYN is involved in immune system activity, especially in T-cell signaling and animal models, suggesting that it is associated with decreased fetal maternal tolerance through the regulation of Th17. In addition, animal experimentation has demonstrated that

this protein is also involved in neuronal signaling, migration, and cortical development cell physiology (331, 332).

Strengths and Limitations

The major strength of this study is the characterization of the maternal plasma proteome in longitudinal samples of patients who had a normal pregnancy outcome defined by clinical, neonatal, and pathologic examination of the placenta. We have examined a large number of proteins simultaneously (over 1,000), using sensitive techniques which have been rigorously validated. This study represents the largest examination of the maternal plasma proteome during pregnancy. Another strength of this study is that we have identified proteins that have been found previously to be dramatically up-regulated during pregnancy, such as PlGF and PAPP-A, but also discovered novel proteins which were not known to be drastically changed in concentration. The identification of three broad patterns of protein changes with a large number of proteins is also important, as these observations were not known with specificity prior to this study.

One limitation of this study is that the concentrations are expressed in relative fluorescence units, rather than absolute concentrations, and hence batch effects do not allow a direct comparison of the normal pregnancy reference intervals estimated in this study with data obtained in future studies. However, departures of longitudinal patterns across gestation from the references intervals we have determined can enable discovery of biomarkers unhindered by possible experimental biases. Such discoveries can be followed by quantification by alternative protein measurement techniques such as immunoassays or mass-spectrometry-based assays. In addition, the large majority of the patients included in this study were of African American descent, and future studies will be required to determine whether the changes reported herein occur in populations of other ethnic groups.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. King JC. Physiology of pregnancy and nutrient metabolism. Am J Clin Nutr. 2000; 71(5 Suppl): 1218S–25S. [PubMed: 10799394]
2. Herrera E. Metabolic adaptations in pregnancy and their implications for the availability of substrates to the fetus. Eur J Clin Nutr. 2000; 54(Suppl 1):S47–51. [PubMed: 10805038]
3. Herrera E. Lipid metabolism in pregnancy and its consequences in the fetus and newborn. Endocrine. 2002; 19(1):43–55. [PubMed: 12583601]
4. Lof M, Olausson H, Bostrom K, Janerot-Sjoberg B, Sohlstrom A, Forsum E. Changes in basal metabolic rate during pregnancy in relation to changes in body weight and composition, cardiac

output, insulin-like growth factor I, and thyroid hormones and in relation to fetal growth. Am J Clin Nutr. 2005; 81(3):678–85. [PubMed: 15755839]

5. Luan H, Meng N, Liu P, Feng Q, Lin S, Fu J, et al. Pregnancy-induced metabolic phenotype variations in maternal plasma. J Proteome Res. 2014; 13(3):1527–36. [PubMed: 24450375]
6. Lindsay KL, Hellmuth C, Uhl O, Buss C, Wadhwa PD, Koletzko B, et al. Longitudinal Metabolomic Profiling of Amino Acids and Lipids across Healthy Pregnancy. PLoS One. 2015; 10(12):e0145794. [PubMed: 26716698]
7. Rubler S, Damani PM, Pinto ER. Cardiac size and performance during pregnancy estimated with echocardiography. Am J Cardiol. 1977; 40(4):534–40. [PubMed: 910718]
8. Clapp JF 3rd, Seaward BL, Sleamaker RH, Hiser J. Maternal physiologic adaptations to early human pregnancy. American journal of obstetrics and gynecology. 1988; 159(6):1456–60. [PubMed: 3207124]
9. Clapp JF 3rd, Capeless E. Cardiovascular function before, during, and after the first and subsequent pregnancies. Am J Cardiol. 1997; 80(11):1469–73. [PubMed: 9399724]
10. Savu O, Jurcut R, Giusca S, van Mieghem T, Gussi I, Popescu BA, et al. Morphological and functional adaptation of the maternal heart during pregnancy. Circ Cardiovasc Imaging. 2012; 5(3):289–97. [PubMed: 22455877]
11. Chung E, Leinwand LA. Pregnancy as a cardiac stress model. Cardiovasc Res. 2014; 101(4):561–70. [PubMed: 24448313]
12. Costantine MM. Physiologic and pharmacokinetic changes in pregnancy. Front Pharmacol. 2014; 5:65. [PubMed: 24772083]
13. Hegewald MJ, Crapo RO. Respiratory physiology in pregnancy. Clin Chest Med. 2011; 32(1):1–13. vii. [PubMed: 21277444]
14. Bonney EA. Immune Regulation in Pregnancy: A Matter of Perspective? Obstet Gynecol Clin North Am. 2016; 43(4):679–98. [PubMed: 27816154]
15. Carlin A, Alfirevic Z. Physiological changes of pregnancy and monitoring. Best practice & research Clinical obstetrics & gynaecology. 2008; 22(5):801–23. [PubMed: 18760680]
16. Rees GB, Broughton Pipkin F, Symonds EM, Patrick JM. A longitudinal study of respiratory changes in normal human pregnancy with cross-sectional data on subjects with pregnancy-induced hypertension. American journal of obstetrics and gynecology. 1990; 162(3):826–30. [PubMed: 2316594]
17. Buchanan TA, Metzger BE, Freinkel N, Bergman RN. Insulin sensitivity and B-cell responsiveness to glucose during late pregnancy in lean and moderately obese women with normal glucose tolerance or mild gestational diabetes. American journal of obstetrics and gynecology. 1990; 162(4):1008–14. [PubMed: 2183610]
18. Baeyens L, Hindi S, Sorenson RL, German MS. beta-Cell adaptation in pregnancy. Diabetes Obes Metab. 2016; 18(Suppl 1):63–70. [PubMed: 27615133]
19. Catalano PM, Huston L, Amini SB, Kalhan SC. Longitudinal changes in glucose metabolism during pregnancy in obese women with normal glucose tolerance and gestational diabetes mellitus. American journal of obstetrics and gynecology. 1999; 180(4):903–16. [PubMed: 10203659]
20. Tulchinsky D, Hobel CJ. Plasma human chorionic gonadotropin, estrone, estradiol, estriol, progesterone, and 17 alpha-hydroxyprogesterone in human pregnancy. 3. Early normal pregnancy. American journal of obstetrics and gynecology. 1973; 117(7):884–93. [PubMed: 4759826]
21. Frankenne F, Closset J, Gomez F, Scippo ML, Smal J, Hennen G. The physiology of growth hormones (GHs) in pregnant women and partial characterization of the placental GH variant. J Clin Endocrinol Metab. 1988; 66(6):1171–80. [PubMed: 3372680]
22. Peck TM, Arias F. Hematologic changes associated with pregnancy. Clin Obstet Gynecol. 1979; 22(4):785–98. [PubMed: 535196]
23. Letsky EA. Erythropoiesis in pregnancy. Journal of perinatal medicine. 1995; 23(1–2):39–45. [PubMed: 7658318]
24. Fragiadakis GK, Baca QJ, Gherardini PF, Ganio EA, Gaudilliere DK, Tingle M, et al. Mapping the Fetomaternal Peripheral Immune System at Term Pregnancy. J Immunol. 2016; 197(11):4482–92. [PubMed: 27793998]

25. Christensen H, Leach LS, Mackinnon A. Cognition in pregnancy and motherhood: prospective cohort study. Br J Psychiatry. 2010; 196(2):126–32. [PubMed: 20118458]
26. Henry JD, Rendell PG. A review of the impact of pregnancy on memory function. J Clin Exp Neuropsychol. 2007; 29(8):793–803. [PubMed: 18030631]
27. Feldt-Rasmussen U, Mathiesen ER. Endocrine disorders in pregnancy: physiological and hormonal aspects of pregnancy. Best Pract Res Clin Endocrinol Metab. 2011; 25(6):875–84. [PubMed: 22115163]
28. Edstrom K, Persson B, Cerasi E, Luft R. Patterns of free fatty acids, glycerol, D-beta-hydroxybutyrate and insulin in pregnant women and their newborn infants. Effects of a low and a high insulin response to glucose in the mothers. Acta obstetricia et gynecologica Scandinavica. 1975; 54(4):347–56. [PubMed: 1199704]
29. Herrera E, Lasuncion MA, Gomez-Coronado D, Aranda P, Lopez-Luna P, Maier I. Role of lipoprotein lipase activity on lipoprotein metabolism and the fate of circulating triglycerides in pregnancy. American journal of obstetrics and gynecology. 1988; 158(6 Pt 2):1575–83. [PubMed: 3287929]
30. Kashyap ML, Sivasamboo R, Sothy SP, Cheah JS, Gartside PS. Carbohydrate and lipid metabolism during human labor: free fatty acids, glucose, insulin, and lactic acid metabolism during normal and oxytocin-induced labor for postmaturity. Metabolism: clinical and experimental. 1976; 25(8): 865–75. [PubMed: 940472]
31. Knopp RH, Warth MR, Charles D, Childs M, Li JR, Mabuchi H, et al. Lipoprotein metabolism in pregnancy, fat transport to the fetus, and the effects of diabetes. Biology of the neonate. 1986; 50(6):297–317. [PubMed: 3542067]
32. Potter JM. Perinatal plasma lipid concentrations. Australian and New Zealand journal of medicine. 1977; 7(2):155–60. [PubMed: 196585]
33. Potter JM, Nestel PJ. The hyperlipidemia of pregnancy in normal and complicated pregnancies. American journal of obstetrics and gynecology. 1979; 133(2):165–70. [PubMed: 217273]
34. Joseph JC, Baker C, Sprang ML, Bermes EW. Changes in plasma proteins during pregnancy. Annals of clinical and laboratory science. 1978; 8(2):130–41. [PubMed: 76457]
35. Belo L, Santos-Silva A, Rocha S, Caslake M, Cooney J, Pereira-Leite L, et al. Fluctuations in C-reactive protein concentration and neutrophil activation during normal human pregnancy. European journal of obstetrics, gynecology, and reproductive biology. 2005; 123(1):46–51.
36. Del Priore G, Chatterton R Jr, Lee C, Silver R, Berg L, Lee MJ. Comparison of mononuclear cell proteins and plasma proteins before and during parturition by two dimensional electrophoresis. Journal of perinatal medicine. 1991; 19(5):373–7. [PubMed: 1804947]
37. Malm J, Laurell M, Dahlback B. Changes in the plasma levels of vitamin K-dependent proteins C and S and of C4b-binding protein during pregnancy and oral contraception. British journal of haematology. 1988; 68(4):437–43. [PubMed: 2967717]
38. Walker MC, Smith GN, Perkins SL, Keely EJ, Garner PR. Changes in homocysteine levels during normal pregnancy. American journal of obstetrics and gynecology. 1999; 180(3 Pt 1):660–4. [PubMed: 10076144]
39. Whittaker PG, Lind T. The intravascular mass of albumin during human pregnancy: a serial study in normal and diabetic women. British journal of obstetrics and gynaecology. 1993; 100(6):587–92. [PubMed: 8334096]
40. Yoneyama Y, Suzuki S, Sawa R, Takeuchi T, Kobayashi H, Takei R, et al. Changes in plasma adenosine concentrations during normal pregnancy. Gynecologic and obstetric investigation. 2000; 50(3):145–8. [PubMed: 11014943]
41. Cetin I, Ronzoni S, Marconi AM, Perugino G, Corbetta C, Battaglia FC, et al. Maternal concentrations and fetal-maternal concentration differences of plasma amino acids in normal and intrauterine growth-restricted pregnancies. American journal of obstetrics and gynecology. 1996; 174(5):1575–83. [PubMed: 9065132]
42. Cox BD, Calame DP. Changes in plasma amino acid levels during the human menstrual cycle and in early pregnancy. A preliminary report. Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme. 1978; 10(5):428–33. [PubMed: 711138]

43. Holden DP, Fickling SA, Whitley GS, Nussey SS. Plasma concentrations of asymmetric dimethylarginine, a natural inhibitor of nitric oxide synthase, in normal pregnancy and preeclampsia. American journal of obstetrics and gynecology. 1998; 178(3):551–6. [PubMed: 9539525]
44. Chesley LC. Blood pressure, edema and proteinuria in pregnancy. 9. Proposal for classification. Progress in clinical and biological research. 1976; 7:249–68. [PubMed: 1030794]
45. Gomez-Lopez N, Guilbert LJ, Olson DM. Invasion of the leukocytes into the fetal-maternal interface during pregnancy. J Leukoc Biol. 2010; 88(4):625–33. [PubMed: 20519637]
46. Widmer M, Villar J, Benigni A, Conde-Agudelo A, Karumanchi SA, Lindheimer M. Mapping the theories of preeclampsia and the role of angiogenic factors: a systematic review. Obstetrics and gynecology. 2007; 109(1):168–80. [PubMed: 17197602]
47. Chaiworapongsa T, Chaemsaithong P, Korzeniewski SJ, Yeo L, Romero R. Pre-eclampsia part 2: prediction, prevention and management. Nat Rev Nephrol. 2014; 10(9):531–40. [PubMed: 25003612]
48. Possomato-Vieira JS, Khalil RA. Mechanisms of Endothelial Dysfunction in Hypertensive Pregnancy and Preeclampsia. Adv Pharmacol. 2016; 77:361–431. [PubMed: 27451103]
49. Premawardhana LD, Parkes AB, Ammari F, John R, Darke C, Adams H, et al. Postpartum thyroiditis and long-term thyroid status: prognostic influence of thyroid peroxidase antibodies and ultrasound echogenicity. J Clin Endocrinol Metab. 2000; 85(1):71–5. [PubMed: 10634366]
50. Lonstein JS, Maguire J, Meinlschmidt G, Neumann ID. Emotion and mood adaptations in the peripartum female:complementary contributions of GABA and oxytocin. J Neuroendocrinol. 2014; 26(10):649–64. [PubMed: 25074620]
51. Hoekzema E, Barba-Muller E, Pozzobon C, Picado M, Lucco F, Garcia-Garcia D, et al. Pregnancy leads to long-lasting changes in human brain structure. Nat Neurosci. 2016
52. Wilkins MR, Pasquali C, Appel RD, Ou K, Golaz O, Sanchez JC, et al. From proteins to proteomes: large scale protein identification by two-dimensional electrophoresis and amino acid analysis. Biotechnology (N Y). 1996; 14(1):61–5. [PubMed: 9636313]
53. Romero R, Kuivaniemi H, Tromp G, Olson J. The design, execution, and interpretation of genetic association studies to decipher complex diseases. American journal of obstetrics and gynecology. 2002; 187(5):1299–312. [PubMed: 12439524]
54. Romero R, Tromp G. High-dimensional biology in obstetrics and gynecology: functional genomics in microarray studies. American journal of obstetrics and gynecology. 2006; 195(2):360–3. [PubMed: 16890547]
55. Romero R, Espinoza J, Gotsch F, Kusanovic JP, Friel LA, Erez O, et al. The use of high-dimensional biology (genomics, transcriptomics, proteomics, and metabolomics) to understand the preterm parturition syndrome. BJOG. 2006; 113(Suppl 3):118–35. [PubMed: 17206980]
56. Chard T. Pregnancy tests: a review. Human reproduction (Oxford, England). 1992; 7(5):701–10.
57. Hinney B, Bertagnoli C, Tobler-Sommer M, Osmers R, Wuttke W, Kuhn W. Diagnosis of early ectopic pregnancy by measurement of the maternal serum to cul-de-sac fluid beta-hCG ratio. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology. 1995; 5(4):260–6.
58. Nyberg DA, Filly RA, Mahony BS, Monroe S, Laing FC, Jeffrey RB Jr. Early gestation: correlation of HCG levels and sonographic identification. AJR American journal of roentgenology. 1985; 144(5):951–4. [PubMed: 3885694]
59. Enk L, Wikland M, Hammarberg K, Lindblom B. The value of endovaginal sonography and urinary human chorionic gonadotropin tests for differentiation between intrauterine and ectopic pregnancy. Journal of clinical ultrasound : JCU. 1990; 18(2):73–8. [PubMed: 2156909]
60. Feldkamp CS, Pfeffer WH. The measurement of human chorionic gonadotropin for pregnancy testing. Henry Ford Hospital medical journal. 1982; 30(4):207–13. [PubMed: 7169378]
61. Jouppila P, Tapanainen J, Huhtaniemi I. Plasma hCG and ultrasound in suspected ectopic pregnancy. European journal of obstetrics, gynecology, and reproductive biology. 1980; 10(1):3–12.

62. Landesman R, Saxena BB. Results of the first 1000 radioreceptorassays for the determination of human chorionic gonadotropin: a new, rapid, reliable, and sensitive pregnancy test. Fertility and sterility. 1976; 27(4):357–68. [PubMed: 944652]
63. Mishell DR Jr, Davajan V. Quantitative immunologic assay of human chorionic gonadotropin in normal and abnormal pregnancies. American journal of obstetrics and gynecology. 1966; 96(2): 231–9. [PubMed: 5921063]
64. Nyberg DA, Filly RA, Laing FC, Mack LA, Zarutskie PW. Ectopic pregnancy. Diagnosis by sonography correlated with quantitative HCG levels. Journal of ultrasound in medicine : official journal of the American Institute of Ultrasound in Medicine. 1987; 6(3):145–50. [PubMed: 3550138]
65. Pittaway DE, Wentz AC. Evaluation of early pregnancy by serial chorionic gonadotropin determinations: a comparison of methods by receiver operating characteristic curve analysis. Fertility and sterility. 1985; 43(4):529–33. [PubMed: 3987923]
66. Saxena BB, Landesman R. The use of a radioreceptorassay of human chorionic gonadotropin for the diagnosis and management of ectopic pregnancy. Fertility and sterility. 1975; 26(5):397–404. [PubMed: 1168588]
67. Steier JA, Bergsjo P, Myking OL. Human chorionic gonadotropin in maternal plasma after induced abortion, spontaneous abortion, and removed ectopic pregnancy. Obstetrics and gynecology. 1984; 64(3):391–4. [PubMed: 6462569]
68. Kadar N, DeCherney AH, Romero R. Receiver operating characteristic (ROC) curve analysis of the relative efficacy of single and serial chorionic gonadotropin determinations in the early diagnosis of ectopic pregnancy. Fertility and sterility. 1982; 37(4):542–7. [PubMed: 7067849]
69. Kadar N, DeVore G, Romero R. Discriminatory hCG zone: its use in the sonographic evaluation for ectopic pregnancy. Obstetrics and gynecology. 1981; 58(2):156–61. [PubMed: 7254727]
70. Kadar N, Taylor KJ, Rosenfield AT, Romero R. Combined use of serum HCG and sonography in the diagnosis of ectopic pregnancy. AJR American journal of roentgenology. 1983; 141(3):609–15. [PubMed: 6603780]
71. Redline RW, Boyd T, Campbell V, Hyde S, Kaplan C, Khong TY, et al. Maternal vascular underperfusion: nosology and reproducibility of placental reaction patterns. Pediatr Dev Pathol. 2004; 7(3):237–49. [PubMed: 15022063]
72. Redline RW, Faye-Petersen O, Heller D, Qureshi F, Savell V, Vogler C. Amniotic infection syndrome: nosology and reproducibility of placental reaction patterns. Pediatr Dev Pathol. 2003; 6(5):435–48. [PubMed: 14708737]
73. Redline RW, Heller D, Keating S, Kingdom J. Placental diagnostic criteria and clinical correlation-a workshop report. Placenta. 2005; 26(Suppl A):S114–7. [PubMed: 15837060]
74. Kusanovic JP, Romero R, Espinoza J, Nien JK, Kim CJ, Mittal P, et al. Twin-to-twin transfusion syndrome: an antiangiogenic state? American journal of obstetrics and gynecology. 2008; 198(4): 382.e1–8. [PubMed: 18395032]
75. Romero R, Chaiworapongsa T. Preeclampsia: a link between trophoblast dysregulation and an antiangiogenic state. J Clin Invest. 2013; 123(7):2775–7. [PubMed: 23934119]
76. Espinoza J, Romero R, Nien JK, Gomez R, Kusanovic JP, Goncalves LF, et al. Identification of patients at risk for early onset and/or severe preeclampsia with the use of uterine artery Doppler velocimetry and placental growth factor. American journal of obstetrics and gynecology. 2007; 196(4):326.e1–13. [PubMed: 17403407]
77. Seeber BE, Barnhart KT. Suspected ectopic pregnancy. Obstetrics and gynecology. 2006; 107(2 Pt 1):399–413. [PubMed: 16449130]
78. Cox J, Mann M. Quantitative, high-resolution proteomics for data-driven systems biology. Annu Rev Biochem. 2011; 80:273–99. [PubMed: 21548781]
79. Wilhelm M, Schlegl J, Hahne H, Gholami AM, Lieberenz M, Savitski MM, et al. Mass-spectrometry-based draft of the human proteome. Nature. 2014; 509(7502):582–7. [PubMed: 24870543]
80. Levitt M. Nature of the protein universe. Proc Natl Acad Sci U S A. 2009; 106(27):11079–84. [PubMed: 19541617]

81. Han X, Aslanian A, Yates JR 3rd. Mass spectrometry for proteomics. Curr Opin Chem Biol. 2008; 12(5):483–90. [PubMed: 18718552]

82. Amniotic-fluid alpha-fetoprotein measurement in antenatal diagnosis of anencephaly and open spina bifida in early pregnancy. Second report of the U.K. Collaborative Study on Alpha-fetoprotein in Relation to Neural-tube Defects. Lancet (London, England). 1979; 2(8144):651–62.

83. Bond EB, Thompson W, Elwood JH, Cran GW. Evaluation of measurement of maternal plasma alpha-fetoprotein levels as a screening test for fetal neural tube defects. British journal of obstetrics and gynaecology. 1977; 84(8):574–7. [PubMed: 70214]

84. Merkatz IR, Nitowsky HM, Macri JN, Johnson WE. An association between low maternal serum alpha-fetoprotein and fetal chromosomal abnormalities. American journal of obstetrics and gynecology. 1984; 148(7):886–94. [PubMed: 6201071]

85. Wald N, Barker S, Peto R, Brock DJ, Bonnar J. Maternal serum alpha-fetoprotein and previous neural tube defects. British journal of obstetrics and gynaecology. 1976; 83(3):213–6. [PubMed: 56196]

86. Weiss RR, Macri JN, Elligers K, Princler GL, McIntire R, Waldman TA. Amniotic fluid alpha-fetoprotein as a marker in prenatal diagnosis of neural tube defects. Obstetrics and gynecology. 1976; 47(2):148–51. [PubMed: 55990]

87. Cuckle HS, Wald NJ, Lindenbaum RH. Maternal serum alpha-fetoprotein measurement: a screening test for Down syndrome. Lancet (London, England). 1984; 1(8383):926–9.

88. Heyl PS, Miller W, Canick JA. Maternal serum screening for aneuploid pregnancy by alpha-fetoprotein, hCG, and unconjugated estriol. Obstetrics and gynecology. 1990; 76(6):1025–31. [PubMed: 1700348]

89. Crossley JA, Aitken DA, Connor JM. Prenatal screening for chromosome abnormalities using maternal serum chorionic gonadotrophin, alpha-fetoprotein, and age. Prenat Diagn. 1991; 11(2): 83–101. [PubMed: 1712100]

90. Wald NJ, Rodeck C, Hackshaw AK, Walters J, Chitty L, Mackinson AM. First and second trimester antenatal screening for Down's syndrome: the results of the Serum, Urine and Ultrasound Screening Study (SURUSS). Health Technol Assess. 2003; 7(11):1–77.

91. Malone FD, Canick JA, Ball RH, Nyberg DA, Comstock CH, Bukowski R, et al. First-trimester or second-trimester screening, or both, for Down's syndrome. N Engl J Med. 2005; 353(19):2001–11. [PubMed: 16282175]

92. Bredaki FE, Wright D, Matos P, Syngelaki A, Nicolaides KH. First-trimester screening for trisomy 21 using alpha-fetoprotein. Fetal Diagn Ther. 2011; 30(3):215–8. [PubMed: 21952001]

93. Wright D, Syngelaki A, Bradbury I, Akolekar R, Nicolaides KH. First-trimester screening for trisomies 21, 18 and 13 by ultrasound and biochemical testing. Fetal Diagn Ther. 2014; 35(2):118–26. [PubMed: 24356462]

94. Bogart MH, Pandian MR, Jones OW. Abnormal maternal serum chorionic gonadotropin levels in pregnancies with fetal chromosome abnormalities. Prenat Diagn. 1987; 7(9):623–30. [PubMed: 2447576]

95. MacDonald ML, Wagner RM, Slotnick RN. Sensitivity and specificity of screening for Down syndrome with alpha-fetoprotein, hCG, unconjugated estriol, and maternal age. Obstetrics and gynecology. 1991; 77(1):63–8. [PubMed: 1701526]

96. Spencer K, Macri JN, Aitken DA, Connor JM. Free beta-hCG as first-trimester marker for fetal trisomy. Lancet (London, England). 1992; 339(8807):1480.

97. Kagan KO, Wright D, Valencia C, Maiz N, Nicolaides KH. Screening for trisomies 21, 18 and 13 by maternal age, fetal nuchal translucency, fetal heart rate, free beta-hCG and pregnancy-associated plasma protein-A. Human reproduction (Oxford, England). 2008; 23(9):1968–75.

98. Kagan KO, Anderson JM, Anwandter G, Neksasova K, Nicolaides KH. Screening for triploidy by the risk algorithms for trisomies 21, 18 and 13 at 11 weeks to 13 weeks and 6 days of gestation. Prenat Diagn. 2008; 28(13):1209–13. [PubMed: 19039823]

99. Kagan KO, Wright D, Spencer K, Molina FS, Nicolaides KH. First-trimester screening for trisomy 21 by free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-A: impact of maternal and pregnancy characteristics. Ultrasound in obstetrics & gynecology : the official

journal of the International Society of Ultrasound in Obstetrics and Gynecology. 2008; 31(5):493–502.

100. Wright D, Spencer K, Kagan KK, Torring N, Petersen OB, Christou A, et al. First-trimester combined screening for trisomy 21 at 7–14 weeks' gestation. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology. 2010; 36(4):404–11.
101. Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, Yu KF, et al. Circulating angiogenic factors and the risk of preeclampsia. N Engl J Med. 2004; 350(7):672–83. [PubMed: 14764923]
102. Chaiworapongsa T, Romero R, Kim YM, Kim GJ, Kim MR, Espinoza J, et al. Plasma soluble vascular endothelial growth factor receptor-1 concentration is elevated prior to the clinical diagnosis of pre-eclampsia. J Matern Fetal Neonatal Med. 2005; 17(1):3–18. [PubMed: 15804781]
103. Levine RJ, Lam C, Qian C, Yu KF, Maynard SE, Sachs BP, et al. Soluble endoglin and other circulating antiangiogenic factors in preeclampsia. N Engl J Med. 2006; 355(10):992–1005. [PubMed: 16957146]
104. Erez O, Romero R, Espinoza J, Fu W, Todem D, Kusanovic JP, et al. The change in concentrations of angiogenic and anti-angiogenic factors in maternal plasma between the first and second trimesters in risk assessment for the subsequent development of preeclampsia and small-for-gestational age. J Matern Fetal Neonatal Med. 2008; 21(5):279–87. [PubMed: 18446652]
105. Romero R, Nien JK, Espinoza J, Todem D, Fu W, Chung H, et al. A longitudinal study of angiogenic (placental growth factor) and anti-angiogenic (soluble endoglin and soluble vascular endothelial growth factor receptor-1) factors in normal pregnancy and patients destined to develop preeclampsia and deliver a small for gestational age neonate. J Matern Fetal Neonatal Med. 2008; 21(1):9–23. [PubMed: 18175241]
106. Kusanovic JP, Romero R, Chaiworapongsa T, Erez O, Mittal P, Vaisbuch E, et al. A prospective cohort study of the value of maternal plasma concentrations of angiogenic and anti-angiogenic factors in early pregnancy and midtrimester in the identification of patients destined to develop preeclampsia. J Matern Fetal Neonatal Med. 2009; 22(11):1021–38. [PubMed: 19900040]
107. Chaiworapongsa T, Romero R, Savasan ZA, Kusanovic JP, Ogge G, Soto E, et al. Maternal plasma concentrations of angiogenic/anti-angiogenic factors are of prognostic value in patients presenting to the obstetrical triage area with the suspicion of preeclampsia. J Matern Fetal Neonatal Med. 2011; 24(10):1187–207. [PubMed: 21827221]
108. Chaiworapongsa T, Romero R, Korzeniewski SJ, Kusanovic JP, Soto E, Lam J, et al. Maternal plasma concentrations of angiogenic/antiangiogenic factors in the third trimester of pregnancy to identify the patient at risk for stillbirth at or near term and severe late preeclampsia. American journal of obstetrics and gynecology. 2013; 208(4):287.e1–e15. [PubMed: 23333542]
109. Chaiworapongsa T, Chaemsaithong P, Yeo L, Romero R. Pre-eclampsia part 1: current understanding of its pathophysiology. Nat Rev Nephrol. 2014; 10(8):466–80. [PubMed: 25003615]
110. Chaiworapongsa T, Romero R, Korzeniewski SJ, Cortez JM, Pappas A, Tarca AL, et al. Plasma concentrations of angiogenic/anti-angiogenic factors have prognostic value in women presenting with suspected preeclampsia to the obstetrical triage area: a prospective study. J Matern Fetal Neonatal Med. 2014; 27(2):132–44. [PubMed: 23687930]
111. Espinoza J, Chaiworapongsa T, Romero R, Kim YM, Kim GJ, Nien JK, et al. Unexplained fetal death: another anti-angiogenic state. J Matern Fetal Neonatal Med. 2007; 20(7):495–507. [PubMed: 17674262]
112. Romero R, Chaiworapongsa T, Erez O, Tarca AL, Gervasi MT, Kusanovic JP, et al. An imbalance between angiogenic and anti-angiogenic factors precedes fetal death in a subset of patients: results of a longitudinal study. J Matern Fetal Neonatal Med. 2010; 23(12):1384–99. [PubMed: 20459337]
113. Chaiworapongsa T, Romero R, Whitten AE, Korzeniewski SJ, Chaemsaithong P, Hernandez-Andrade E, et al. The use of angiogenic biomarkers in maternal blood to identify which SGA fetuses will require a preterm delivery and mothers who will develop pre-eclampsia. J Matern Fetal Neonatal Med. 2016; 29(8):1214–28. [PubMed: 26303962]

114. Stepan H, Faber R. Cytomegalovirus-induced mirror syndrome associated with elevated levels of angiogenic factors. Obstetrics and gynecology. 2007; 109(5):1205–6. author reply 6. [PubMed: 17470610]
115. Akolekar R, Zaragoza E, Poon LC, Pepes S, Nicolaides KH. Maternal serum placental growth factor at 11 + 0 to 13 + 6 weeks of gestation in the prediction of pre-eclampsia. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology. 2008; 32(6):732–9.
116. Sacks G, Sargent I, Redman C. Innate immunity in pregnancy. Immunol Today. 2000; 21(4):200–1.
117. Sacks GP, Clover LM, Bainbridge DR, Redman CW, Sargent IL. Flow cytometric measurement of intracellular Th1 and Th2 cytokine production by human villous and extravillous cytotrophoblast. Placenta. 2001; 22(6):550–9. [PubMed: 11440543]
118. Southcombe J, Tannetta D, Redman C, Sargent I. The immunomodulatory role of syncytiotrophoblast microvesicles. PLoS One. 2011; 6(5):e20245. [PubMed: 21633494]
119. Karagiannis G, Akolekar R, Sarquis R, Wright D, Nicolaides KH. Prediction of small-for-gestation neonates from biophysical and biochemical markers at 11–13 weeks. Fetal Diagn Ther. 2011; 29(2):148–54. [PubMed: 21079385]
120. Nucci M, Poon LC, Demirdjian G, Darbouret B, Nicolaides KH. Maternal serum placental growth factor (PlGF) isoforms 1 and 2 at 11–13 weeks' gestation in normal and pathological pregnancies. Fetal Diagn Ther. 2014; 36(2):106–16. [PubMed: 24457972]
121. Dragan I, Wright D, Fiolna M, Leipold G, Nicolaides KH. Development of preeclampsia within four weeks of sFLT to PLGF ratio >38: Comparison of performance at 31–34 versus 35–37 weeks' gestation. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology. 2016
122. Khalil A, Maiz N, Garcia-Mandujano R, Penco JM, Nicolaides KH. Longitudinal changes in maternal serum placental growth factor and soluble fms-like tyrosine kinase-1 in women at increased risk of pre-eclampsia. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology. 2016; 47(3):324–31.
123. Espinoza J, Romero R, Nien JK, Kusanovic JP, Richani K, Gomez R, et al. A role of the anti-angiogenic factor sVEGFR-1 in the 'mirror syndrome' (Ballantyne's syndrome). J Matern Fetal Neonatal Med. 2006; 19(10):607–13. [PubMed: 17118734]
124. Varkonyi T, Nagy B, Fule T, Tarca AL, Karaszi K, Schonleber J, et al. Microarray profiling reveals that placental transcriptomes of early-onset HELLP syndrome and preeclampsia are similar. Placenta. 2011; 32(Suppl):S21–9. [PubMed: 20541258]
125. Romero R, Kuivaniemi H, Tromp G. Functional genomics and proteomics in term and preterm parturition. J Clin Endocrinol Metab. 2002; 87(6):2431–4. [PubMed: 12050194]
126. Finishing the euchromatic sequence of the human genome. Nature. 2004; 431(7011):931–45. [PubMed: 15496913]
127. Lee J, Romero R, Chaiworapongsa T, Dong Z, Tarca AL, Xu Y, et al. Characterization of the fetal blood transcriptome and proteome in maternal anti-fetal rejection: evidence of a distinct and novel type of human fetal systemic inflammatory response. Am J Reprod Immunol. 2013; 70(4): 265–84. [PubMed: 23905683]
128. Reshetova P, Smilde AK, van Kampen AH, Westerhuis JA. Use of prior knowledge for the analysis of high-throughput transcriptomics and metabolomics data. BMC Syst Biol. 2014; 8(Suppl 2):S2.
129. Cox B, Leavey K, Nosi U, Wong F, Kingdom J. Placental transcriptome in development and pathology: expression, function, and methods of analysis. American journal of obstetrics and gynecology. 2015; 213(4 Suppl):S138–51. [PubMed: 26428493]
130. Edlow AG, Guedj F, Pennings JL, Sverdlov D, Neri C, Bianchi DW. Males are from Mars, and females are from Venus: sex-specific fetal brain gene expression signatures in a mouse model of maternal diet-induced obesity. American journal of obstetrics and gynecology. 2016; 214(5): 623.e1–e10. [PubMed: 26945603]

131. Edlow AG, Slonim DK, Wick HC, Hui L, Bianchi DW. The pathway not taken: understanding 'omics data in the perinatal context. American journal of obstetrics and gynecology. 2015; 213(1):59.e1–172. [PubMed: 25772209]

132. Lassance L, Haghiac M, Leahy P, Basu S, Minium J, Zhou J, et al. Identification of early transcriptome signatures in placenta exposed to insulin and obesity. American journal of obstetrics and gynecology. 2015; 212(5):647.e1–11. [PubMed: 25731694]

133. Monk D. Genomic imprinting in the human placenta. American journal of obstetrics and gynecology. 2015; 213(4 Suppl):S152–62. [PubMed: 26428495]

134. Pew BK, Harris RA, Sbrana E, Guaman MC, Shope C, Chen R, et al. Structural and transcriptomic response to antenatal corticosteroids in an Erk3-null mouse model of respiratory distress. American journal of obstetrics and gynecology. 2016; 215(3):384.e1–e89. [PubMed: 27143398]

135. Rosa-e-Silva JC, Virginio LA Jr, Meola J, Dentillo DB, Ferriani RA, Giuliatti S. A system for storing, retrieving, and comparing gene expression information of patients with endometriosis. American journal of obstetrics and gynecology. 2015; 212(3):407. [PubMed: 25434836]

136. Tain YL, Lee CT, Chan JY, Hsu CN. Maternal melatonin or N-acetylcysteine therapy regulates hydrogen sulfide-generating pathway and renal transcriptome to prevent prenatal NG-Nitro-L-arginine-methyl ester (L-NAME)-induced fetal programming of hypertension in adult male offspring. American journal of obstetrics and gynecology. 2016; 215(5):636.e1–e72. [PubMed: 27457113]

137. Whitehead CL, McNamara H, Walker SP, Alexiadis M, Fuller PJ, Vickers DK, et al. Identifying late-onset fetal growth restriction by measuring circulating placental RNA in the maternal blood at 28 weeks' gestation. American journal of obstetrics and gynecology. 2016; 214(4):521.e1–8. [PubMed: 26880734]

138. Bujold E, Romero R, Kusanovic JP, Erez O, Gotsch F, Chaiworapongsa T, et al. Proteomic profiling of amniotic fluid in preterm labor using two-dimensional liquid separation and mass spectrometry. J Matern Fetal Neonatal Med. 2008; 21(10):697–713. [PubMed: 19012186]

139. Romero R, Espinoza J, Rogers WT, Moser A, Nien JK, Kusanovic JP, et al. Proteomic analysis of amniotic fluid to identify women with preterm labor and intra-amniotic inflammation/infection: the use of a novel computational method to analyze mass spectrometric profiling. J Matern Fetal Neonatal Med. 2008; 21(6):367–88. [PubMed: 18570116]

140. Romero R, Kusanovic JP, Gotsch F, Erez O, Vaisbuch E, Mazaki-Tovi S, et al. Isobaric labeling and tandem mass spectrometry: a novel approach for profiling and quantifying proteins differentially expressed in amniotic fluid in preterm labor with and without intra-amniotic infection/inflammation. J Matern Fetal Neonatal Med. 2010; 23(4):261–80. [PubMed: 19670042]

141. Lee DC, Hassan SS, Romero R, Tarca AL, Bhatti G, Gervasi MT, et al. Protein profiling underscores immunological functions of uterine cervical mucus plug in human pregnancy. J Proteomics. 2011; 74(6):817–28. [PubMed: 21362502]

142. Zhang AH, Sun H, Yan GL, Han Y, Wang XJ. Serum proteomics in biomedical research: a systematic review. Appl Biochem Biotechnol. 2013; 170(4):774–86. [PubMed: 23609910]

143. Rolland T, Tasan M, Charloteaux B, Pevzner SJ, Zhong Q, Sahni N, et al. A proteome-scale map of the human interactome network. Cell. 2014; 159(5):1212–26. [PubMed: 25416956]

144. Menni C, Kiddle SJ, Mangino M, Vinuela A, Psatha M, Steves C, et al. Circulating Proteomic Signatures of Chronological Age. J Gerontol A Biol Sci Med Sci. 2015; 70(7):809–16. [PubMed: 25123647]

145. Lynch AM, Wagner BD, Deterding RR, Giclas PC, Gibbs RS, Janoff EN, et al. The relationship of circulating proteins in early pregnancy with preterm birth. American journal of obstetrics and gynecology. 2016; 214(4):517.e1–8. [PubMed: 26576488]

146. Manuck TA, Esplin MS, Biggio J, Bukowski R, Parry S, Zhang H, et al. The phenotype of spontaneous preterm birth: application of a clinical phenotyping tool. American journal of obstetrics and gynecology. 2015; 212(4):487.e1–e11. [PubMed: 25687564]

147. Manuck TA, Esplin MS, Biggio J, Bukowski R, Parry S, Zhang H, et al. Predictors of response to 17-alpha hydroxyprogesterone caproate for prevention of recurrent spontaneous preterm birth. American journal of obstetrics and gynecology. 2016; 214(3):376.e1–8. [PubMed: 26692181]

148. Saade GR, Boggess KA, Sullivan SA, Markenson GR, Iams JD, Coonrod DV, et al. Development and validation of a spontaneous preterm delivery predictor in asymptomatic women. American journal of obstetrics and gynecology. 2016; 214(5):633.e1–e24. [PubMed: 26874297]

149. Gervasi MT, Chaiworapongsa T, Naccasha N, Blackwell S, Yoon BH, Maymon E, et al. Phenotypic and metabolic characteristics of maternal monocytes and granulocytes in preterm labor with intact membranes. American journal of obstetrics and gynecology. 2001; 185(5): 1124–9. [PubMed: 11717645]

150. Racicot K, Kwon JY, Aldo P, Silasi M, Mor G. Understanding the complexity of the immune system during pregnancy. Am J Reprod Immunol. 2014; 72(2):107–16. [PubMed: 24995526]

151. Chaiworapongsa T, Gervasi MT, Refuerzo J, Espinoza J, Yoshimatsu J, Berman S, et al. Maternal lymphocyte subpopulations (CD45RA+ and CD45RO+) in preeclampsia. American journal of obstetrics and gynecology. 2002; 187(4):889–93. [PubMed: 12388971]

152. Koga K, Izumi G, Mor G, Fujii T, Osuga Y. Toll-like receptors at the maternal-fetal interface in normal pregnancy and pregnancy complications. Am J Reprod Immunol. 2014; 72(2):192–205. [PubMed: 24754320]

153. Mor G, Cardenas I. The immune system in pregnancy: a unique complexity. Am J Reprod Immunol. 2010; 63(6):425–33. [PubMed: 20367629]

154. Mor G, Cardenas I, Abrahams V, Guller S. Inflammation and pregnancy: the role of the immune system at the implantation site. Ann N Y Acad Sci. 2011; 1221:80–7. [PubMed: 21401634]

155. Bubeck RW. Acute pyelonephritis during pregnancy with anuria, septicemia and thrombocytopenia. Del Med J. 1968; 40(5):143–7. [PubMed: 5655841]

156. Cunningham FG, Morris GB, Mickal A. Acute pyelonephritis of pregnancy: A clinical review. Obstetrics and gynecology. 1973; 42(1):112–7. [PubMed: 4720190]

157. Dilworth EE, Ward JV. Bacteremic shock in pyelonephritis and criminal abortion. Obstetrics and gynecology. 1961; 17:160–7. [PubMed: 13722912]

158. Romero R, Mazaki-Tovi S, Vaisbuch E, Kusanovic JP, Chaiworapongsa T, Gomez R, et al. Metabolomics in premature labor: a novel approach to identify patients at risk for preterm delivery. J Matern Fetal Neonatal Med. 2010; 23(12):1344–59. [PubMed: 20504069]

159. Wishart DS, Mandal R, Stanislaus A, Ramirez-Gaona M. Cancer Metabolomics and the Human Metabolome Database. Metabolites. 2016; 6(1)

160. Bahado-Singh RO, Syngelaki A, Akolekar R, Mandal R, Bjondahl TC, Han B, et al. Validation of metabolomic models for prediction of early-onset preeclampsia. American journal of obstetrics and gynecology. 2015; 213(4):530.e1–e10. [PubMed: 26116099]

161. Ghartey J, Bastek JA, Brown AG, Anglim L, Elovitz MA. Women with preterm birth have a distinct cervicovaginal metabolome. American journal of obstetrics and gynecology. 2015; 212(6):776.e1–e12. [PubMed: 25827503]

162. Jelliffe-Pawlowski LL, Norton ME, Baer RJ, Santos N, Rutherford GW. Gestational dating by metabolic profile at birth: a California cohort study. American journal of obstetrics and gynecology. 2016; 214(4):511.e1–13. [PubMed: 26688490]

163. Pinto J, Almeida LM, Martins AS, Duarte D, Domingues MR, Barros AS, et al. Impact of fetal chromosomal disorders on maternal blood metabolome: toward new biomarkers? American journal of obstetrics and gynecology. 2015; 213(6):841.e1–e15. [PubMed: 26220113]

164. Ventura JE, Villa M, Mizraji R, Ferreiros R. Acute renal failure in pregnancy. Ren Fail. 1997; 19(2):217–20. [PubMed: 9101594]

165. Mabie WC, Barton JR, Sibai B. Septic shock in pregnancy. Obstetrics and gynecology. 1997; 90(4 Pt 1):553–61. [PubMed: 9380315]

166. Pruett K, Faro S. Pyelonephritis associated with respiratory distress. Obstetrics and gynecology. 1987; 69(3 Pt 2):444–6. [PubMed: 3543771]

167. Cunningham FG, Lucas MJ, Hankins GD. Pulmonary injury complicating antepartum pyelonephritis. American journal of obstetrics and gynecology. 1987; 156(4):797–807. [PubMed: 3578394]

168. Cunningham FG, Lucas MJ. Urinary tract infections complicating pregnancy. Baillieres Clin Obstet Gynaecol. 1994; 8(2):353–73. [PubMed: 7924012]

169. Catanzarite VA, Willms D. Adult respiratory distress syndrome in pregnancy: report of three cases and review of the literature. Obstet Gynecol Surv. 1997; 52(6):381–92. [PubMed: 9178312]

170. Bianchi DW. From prenatal genomic diagnosis to fetal personalized medicine: progress and challenges. Nat Med. 2012; 18(7):1041–51. [PubMed: 22772565]

171. Butte AJ. It takes a genome to understand a village: Population scale precision medicine. Proc Natl Acad Sci U S A. 2016; 113(44):12344–6. [PubMed: 27791179]

172. Edlow AG, Slonim DK, Wick HC, Hui L, Bianchi DW. The pathway not taken: understanding 'omics data in the perinatal context. American journal of obstetrics and gynecology. 2015; 213(1):59.e1–172. [PubMed: 25772209]

173. Gligorijevic V, Malod-Dognin N, Przulj N. Integrative methods for analyzing big data in precision medicine. Proteomics. 2016; 16(5):741–58. [PubMed: 26677817]

174. Fletcher DA, Mullins RD. Cell mechanics and the cytoskeleton. Nature. 2010; 463(7280):485–92. [PubMed: 20110992]

175. Green D. Coagulation cascade. Hemodial Int. 2006; 10(Suppl 2):S2–4.

176. Cimmino G, Ciccarelli G, Golino P. Role of Tissue Factor in the Coagulation Network. Semin Thromb Hemost. 2015; 41(7):708–17. [PubMed: 26408920]

177. Blaser H, Dostert C, Mak TW, Brenner D. TNF and ROS Crosstalk in Inflammation. Trends Cell Biol. 2016; 26(4):249–61. [PubMed: 26791157]

178. Tarca AL, Romero R, Draghici S. Analysis of microarray experiments of gene expression profiling. American journal of obstetrics and gynecology. 2006; 195(2):373–88. [PubMed: 16890548]

179. Wei L, Sun S, Zhang J, Zhu H, Xu Y, Ma Q, et al. Endocrine cells of the adenohypophysis in severe acute respiratory syndrome (SARS). Biochem Cell Biol. 2010; 88(4):723–30. [PubMed: 20651845]

180. Phillips KP, O'Sullivan TL, Dow D, Amaratunga CA. Infectious respiratory disease outbreaks and pregnancy: occupational health and safety concerns of Canadian nurses. Prehosp Disaster Med. 2011; 26(2):114–21. [PubMed: 21888731]

181. Wang G, Zhou Y, Gong S, Dong H, Wu G, Xiang X, et al. A PREGNANT WOMAN WITH AVIAN INFLUENZA A (H7N9) VIRUS PNEUMONIA AND ARDS MANAGED WITH EXTRACORPOREAL MEMBRANE OXYGENATION. Southeast Asian J Trop Med Public Health. 2015; 46(3):444–8. [PubMed: 26521517]

182. Maxwell C, McGeer A, Tai KF, Seimer A. SOGC Clinical Practice Guideline. Management guidelines for obstetric patients and neonates born to mothers with suspected or probable severe acute respiratory syndrome (SARS). No. 225, April 2009. Int J Gynaecol Obstet. 2009; 107(1): 82–6. [PubMed: 19780222]

183. Heron M. Deaths: Leading Causes for 2012. Natl Vital Stat Rep. 2015; 64(10):1–93.

184. Stockman LJ, Lowther SA, Coy K, Saw J, Parashar UD. SARS during pregnancy, United States. Emerg Infect Dis. 2004; 10(9):1689–90. [PubMed: 15503406]

185. D'Mello T, Brammer L, Blanton L, Kniss K, Smith S, Mustaquim D, et al. Update: Influenza activity-United States, September 28, 2014-February 21, 2015. MMWR Morb Mortal Wkly Rep. 2015; 64(8):206–12. [PubMed: 25742380]

186. Gresh L, Kuan G, Sanchez N, Azziz-Baumgartner E, Ojeda S, Melendez M, et al. Burden of Influenza and Influenza-associated Pneumonia in the First Year of Life in a Prospective Cohort Study in Managua, Nicaragua. Pediatr Infect Dis J. 2016; 35(2):152–6. [PubMed: 26421805]

187. Svinarich DM, Wolf NA, Gomez R, Gonik B, Romero R. Detection of human defensin 5 in reproductive tissues. American journal of obstetrics and gynecology. 1997; 176(2):470–5. [PubMed: 9065200]

188. Chu HY, Katz J, Tielsch J, Khatry SK, Shrestha L, LeClerq SC, et al. Clinical Presentation and Birth Outcomes Associated with Respiratory Syncytial Virus Infection in Pregnancy. PLoS One. 2016; 11(3):e0152015. [PubMed: 27031702]

189. Acosta CD, Harrison DA, Rowan K, Lucas DN, Kurinczuk JJ, Knight M. Maternal morbidity and mortality from severe sepsis: a national cohort study. BMJ Open. 2016; 6(8):e012323.

190. Svinarich DM, Gomez R, Romero R. Detection of human defensins in the placenta. Am J Reprod Immunol. 1997; 38(4):252–5. [PubMed: 9352011]

191. Us E, Cengiz SA. Prevalence and phenotypic evaluation of Candida dubliniensis in pregnant women with vulvovaginal candidosis in a university hospital in Ankara. Mycoses. 2007; 50(1): 13–20. [PubMed: 17302742]

192. Audisio T, Penacino M, Cannistraci R, Bertolotto P. Detection of bacterial vaginosis, Trichomonas vaginalis infection, and vaginal Candida infection: a comparative study of methods of extracting exudates, with and without a speculum, during pregnancy. J Low Genit Tract Dis. 2005; 9(4):213–5. [PubMed: 16205190]

193. Kim SK, Romero R, Kusanovic JP, Erez O, Vaisbuch E, Mazaki-Tovi S, et al. The prognosis of pregnancy conceived despite the presence of an intrauterine device (IUD). Journal of perinatal medicine. 2010; 38(1):45–53. [PubMed: 19650756]

194. Chaim W, Mazor M, Meril T, Peleg R, Maor E. Late miscarriage and intraamniotic candidiasis in a woman with a retained intrauterine contraceptive device. Arch Gynecol Obstet. 1993; 253(3): 157–60. [PubMed: 8250605]

195. Horn LC, Nenoff P, Ziegert M, Hockel M. Missed abortion complicated by Candida infection in a woman with rested IUD. Arch Gynecol Obstet. 2001; 264(4):215–7. [PubMed: 11205713]

196. Marelli G, Mariani A, Frigerio L, Leone E, Ferrari A. Fetal Candida infection associated with an intrauterine contraceptive device. European journal of obstetrics, gynecology, and reproductive biology. 1996; 68(1–2):209–12.

197. Smith CV, Horenstein J, Platt LD. Intraamniotic infection with Candida albicans associated with a retained intrauterine contraceptive device: a case report. American journal of obstetrics and gynecology. 1988; 159(1):123–4. [PubMed: 3394730]

198. Bider D, Ben-Rafael Z, Barkai G, Mashiach S. Intrauterine fetal death apparently due to Candida chorioamnionitis. Arch Gynecol Obstet. 1989; 244(3):175–7. [PubMed: 2735775]

199. Potasman I, Leibovitz Z, Sharf M. Candida sepsis in pregnancy and the postpartum period. Rev Infect Dis. 1991; 13(1):146–9. [PubMed: 2017614]

200. Chaim W, Mazor M, Wiznitzer A. The prevalence and clinical significance of intraamniotic infection with Candida species in women with preterm labor. Arch Gynecol Obstet. 1992; 251(1):9–15. [PubMed: 1550392]

201. Spaun E, Klunder K. Candida chorioamnionitis and intra-uterine contraceptive device. Acta obstetricia et gynecologica Scandinavica. 1986; 65(2):183–4. [PubMed: 3727943]

202. Delprado WJ, Baird PJ, Russell P. Placental candidiasis: report of three cases with a review of the literature. Pathology. 1982; 14(2):191–5. [PubMed: 7099725]

203. Klein J, Buffin-Meyer B, Mullen W, Carty DM, Delles C, Vlahou A, et al. Clinical proteomics in obstetrics and neonatology. Expert Rev Proteomics. 2014; 11(1):75–89. [PubMed: 24404900]

204. Kim MS, Pinto SM, Getnet D, Nirujogi RS, Manda SS, Chaerkady R, et al. A draft map of the human proteome. Nature. 2014; 509(7502):575–81. [PubMed: 24870542]

205. Kusebauch U, Campbell DS, Deutsch EW, Chu CS, Spicer DA, Brusniak MY, et al. Human SRMAtlas: A Resource of Targeted Assays to Quantify the Complete Human Proteome. Cell. 2016; 166(3):766–78. [PubMed: 27453469]

206. Omenn GS, Lane L, Lundberg EK, Beavis RC, Overall CM, Deutsch EW. Metrics for the Human Proteome Project 2016: Progress on Identifying and Characterizing the Human Proteome, Including Post-Translational Modifications. J Proteome Res. 2016; 15(11):3951–60. [PubMed: 27487407]

207. Schweitzer B, Roberts S, Grimwade B, Shao W, Wang M, Fu Q, et al. Multiplexed protein profiling on microarrays by rolling-circle amplification. Nat Biotechnol. 2002; 20(4):359–65. [PubMed: 11923841]

208. Fredriksson S, Dixon W, Ji H, Koong AC, Mindrinos M, Davis RW. Multiplexed protein detection by proximity ligation for cancer biomarker validation. Nat Methods. 2007; 4(4):327–9. [PubMed: 17369836]

209. Zichi D, Eaton B, Singer B, Gold L. Proteomics and diagnostics: Let's Get Specific, again. Curr Opin Chem Biol. 2008; 12(1):78–85. [PubMed: 18275862]

210. Gold L, Ayers D, Bertino J, Bock C, Bock A, Brody EN, et al. Aptamer-based multiplexed proteomic technology for biomarker discovery. PLoS One. 2010; 5(12):e15004. [PubMed: 21165148]

211. Loffredo FS, Steinhauser ML, Jay SM, Gannon J, Pancoast JR, Yalamanchi P, et al. Growth differentiation factor 11 is a circulating factor that reverses age-related cardiac hypertrophy. Cell. 2013; 153(4):828–39. [PubMed: 23663781]
212. Ostroff RM, Bigbee WL, Franklin W, Gold L, Mehan M, Miller YE, et al. Unlocking biomarker discovery: large scale application of aptamer proteomic technology for early detection of lung cancer. PLoS One. 2010; 5(12):e15003. [PubMed: 21170350]
213. Ostroff RM, Mehan MR, Stewart A, Ayers D, Brody EN, Williams SA, et al. Early detection of malignant pleural mesothelioma in asbestos-exposed individuals with a noninvasive proteomics-based surveillance tool. PLoS One. 2012; 7(10):e46091. [PubMed: 23056237]
214. Mehan MR, Ayers D, Thirstrup D, Xiong W, Ostroff RM, Brody EN, et al. Protein signature of lung cancer tissues. PLoS One. 2012; 7(4):e35157. [PubMed: 22509397]
215. Mehan MR, Williams SA, Siegfried JM, Bigbee WL, Weissfeld JL, Wilson DO, et al. Validation of a blood protein signature for non-small cell lung cancer. Clin Proteomics. 2014; 11(1):32. [PubMed: 25114662]
216. Webber J, Stone TC, Katilius E, Smith BC, Gordon B, Mason MD, et al. Proteomics analysis of cancer exosomes using a novel modified aptamer-based array (SOMAscan) platform. Mol Cell Proteomics. 2014; 13(4):1050–64. [PubMed: 24505114]
217. Hathout Y, Brody E, Clemens PR, Cripe L, DeLisle RK, Furlong P, et al. Large-scale serum protein biomarker discovery in Duchenne muscular dystrophy. Proc Natl Acad Sci U S A. 2015; 112(23):7153–8. [PubMed: 26039989]
218. Drolet DW, Green LS, Gold L, Janjic N. Fit for the Eye: Aptamers in Ocular Disorders. Nucleic Acid Ther. 2016
219. Davies DR, Gelinas AD, Zhang C, Rohloff JC, Carter JD, O'Connell D, et al. Unique motifs and hydrophobic interactions shape the binding of modified DNA ligands to protein targets. Proc Natl Acad Sci U S A. 2012; 109(49):19971–6. [PubMed: 23139410]
220. SomaLogic. SOMAmer Technical notes. http://www.somalogic.com/somalogic/media/Assets/PDFs/SSM-017-Rev-3-SOMAmer-Technical-Note-3-7-15.pdf
221. Bates, D., Maechler, M., Bolker, B., Walker, S. lme4: Linear mixed-effects models using Eigen and S4. 2014. http://arxivorg/abs/14065823
222. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J Royal Stat Soc B. 1995; 57(1):289–300.
223. Allison DB, Cui X, Page GP, Sabripour M. Microarray data analysis: from disarray to consolidation and consensus. Nat Rev Genet. 2006; 7(1):55–65. [PubMed: 16369572]
224. Mittal P, Romero R, Tarca AL, Gonzalez J, Draghici S, Xu Y, et al. Characterization of the myometrial transcriptome and biological pathways of spontaneous human labor at term. Journal of perinatal medicine. 2010; 38(6):617–43. [PubMed: 20629487]
225. Pappas A, Chaiworapongsa T, Romero R, Korzeniewski SJ, Cortez JC, Bhatti G, et al. Transcriptomics of maternal and fetal membranes can discriminate between gestational-age matched preterm neonates with and without cognitive impairment diagnosed at 18–24 months. PLoS One. 2015; 10(3):e0118573. [PubMed: 25822971]
226. Tarca AL, Lauria M, Unger M, Bilal E, Boue S, Kumar Dey K, et al. Strengths and limitations of microarray-based phenotype prediction: lessons learned from the IMPROVER Diagnostic Signature Challenge. Bioinformatics. 2013; 29(22):2892–9. [PubMed: 23966112]
227. Koenker R. quantreg: Quantile Regression. R package 5.05 ed.
228. Koenker, RW. Quantile Regression. Cambridge U. Press; 2005.
229. Tibshirani R, Guenther W, Hastie T. Estimating the number of clusters in a data set via the gap statistic. J Royal Stat Soc B. 2001; 63(2):411–23.
230. Maglott D, Ostell J, Pruitt KD, Tatusova T. Entrez Gene: gene-centered information at NCBI. Nucleic Acids Res. 2005; 33(Database issue):D54–8. [PubMed: 15608257]
231. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, et al. Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. Nat Genet. 2000; 25(1):25–9. [PubMed: 10802651]

232. Peri S, Navarro JD, Kristiansen TZ, Amanchy R, Surendranath V, Muthusamy B, et al. Human protein reference database as a discovery resource for proteomics. Nucleic Acids Res. 2004; 32(Database issue):D497–501. [PubMed: 14681466]

233. Alexander GR, Kogan MD, Himes JH. 1994–1996 U.S. singleton birth weight percentiles for gestational age by race, Hispanic origin, and gender. Matern Child Health J. 1999; 3(4):225–31. [PubMed: 10791363]

234. Baltajian K, Bajracharya S, Salahuddin S, Berg AH, Geahchan C, Wenger JB, et al. Sequential plasma angiogenic factors levels in women with suspected preeclampsia. American journal of obstetrics and gynecology. 2016; 215(1):89.e1–e10. [PubMed: 26827880]

235. Holme AM, Roland MC, Henriksen T, Michelsen TM. In vivo uteroplacental release of placental growth factor and soluble Fms-like tyrosine kinase-1 in normal and preeclamptic pregnancies. American journal of obstetrics and gynecology. 2016; 215(6):782.e1–e9. [PubMed: 27503620]

236. Kim MY, Buyon JP, Guerra MM, Rana S, Zhang D, Laskin CA, et al. Angiogenic factor imbalance early in pregnancy predicts adverse outcomes in patients with lupus and antiphospholipid antibodies: results of the PROMISSE study. American journal of obstetrics and gynecology. 2016; 214(1):108.e1–e14. [PubMed: 26432463]

237. Yang J, Pearl M, DeLorenze GN, Romero R, Dong Z, Jelliffe-Pawlowski L, et al. Racial-ethnic differences in midtrimester maternal serum levels of angiogenic and antiangiogenic factors. American journal of obstetrics and gynecology. 2016; 215(3):359.e1–9. [PubMed: 27073062]

238. Chaiworapongsa T, Romero R, Korzeniewski SJ, Chaemsaithong P, Hernandez-Andrade E, Segars JH, et al. Pravastatin to prevent recurrent fetal death in massive perivillous fibrin deposition of the placenta (MPFD). J Matern Fetal Neonatal Med. 2016; 29(6):855–62. [PubMed: 25893545]

239. Whitten AE, Romero R, Korzeniewski SJ, Tarca AL, Schwartz AG, Yeo L, et al. Evidence of an imbalance of angiogenic/antiangiogenic factors in massive perivillous fibrin deposition (maternal floor infarction): a placental lesion associated with recurrent miscarriage and fetal death. American journal of obstetrics and gynecology. 2013; 208(4):310.e1–e11. [PubMed: 23333548]

240. Bixel K, Silasi M, Zelop CM, Lim KH, Zsengeller Z, Stillman IE, et al. Placental origins of angiogenic dysfunction in mirror syndrome. Hypertens Pregnancy. 2012; 31(2):211–7. [PubMed: 22150085]

241. Fisher SJ. Why is placentation abnormal in preeclampsia? American journal of obstetrics and gynecology. 2015; 213(4 Suppl):S115–22. [PubMed: 26428489]

242. Graham N, Garrod A, Bullen P, Heazell AE. Placental expression of anti-angiogenic proteins in mirror syndrome: a case report. Placenta. 2012; 33(6):528–31. [PubMed: 22401877]

243. Llurba E, Marsal G, Sanchez O, Dominguez C, Alijotas-Reig J, Carreras E, et al. Angiogenic and antiangiogenic factors before and after resolution of maternal mirror syndrome. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology. 2012; 40(3):367–9.

244. Rana S, Venkatesha S, DePaepe M, Chien EK, Paglia M, Karumanchi SA. Cytomegalovirus-induced mirror syndrome associated with elevated levels of circulating antiangiogenic factors. Obstetrics and gynecology. 2007; 109(2 Pt2):549–52. [PubMed: 17267891]

245. Spencer K, Tul N, Nicolaides KH. Maternal serum free beta-hCG and PAPP-A in fetal sex chromosome defects in the first trimester. Prenat Diagn. 2000; 20(5):390–4. [PubMed: 10820406]

246. Colosi E, D'Ambrosio V, Periti E. FIRST TRIMESTER CONTINGENT SCREENING FOR TRISOMIES 21,18,13: IS THIS MODEL COST EFFICIENT AND FEASIBLE IN PUBBLIC HEALTH SYSTEM? J Matern Fetal Neonatal Med. 2016:1–13.

247. Gupta S, Fox NS, Rebarber A, Saltzman DH, Klauser CK, Roman AS. Biochemical screening for aneuploidy in patients with donor oocyte pregnancies compared with autologous pregnancies. J Matern Fetal Neonatal Med. 2014; 27(14):1418–21. [PubMed: 24228730]

248. Wiechec M, Knafel A, Nocun A, Ludwin A, Ludwin I, Maczka M, et al. Screening for trisomy 18 using traditional combined screening vs. ultrasound-based protocol in tertiary center environment. J Matern Fetal Neonatal Med. 2016:1–6.

249. Poon LC, Maiz N, Valencia C, Plasencia W, Nicolaides KH. First-trimester maternal serum pregnancy-associated plasma protein-A and pre-eclampsia. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology. 2009; 33(1):23–33.

250. Poon LC, Syngelaki A, Akolekar R, Lai J, Nicolaides KH. Combined screening for preeclampsia and small for gestational age at 11–13 weeks. Fetal Diagn Ther. 2013; 33(1):16–27. [PubMed: 22986844]

251. Akolekar R, Syngelaki A, Poon L, Wright D, Nicolaides KH. Competing risks model in early screening for preeclampsia by biophysical and biochemical markers. Fetal Diagn Ther. 2013; 33(1):8–15. [PubMed: 22906914]

252. Conde-Agudelo A, Romero R. Prediction of preterm birth in twin gestations using biophysical and biochemical tests. American journal of obstetrics and gynecology. 2014; 211(6):583–95. [PubMed: 25072736]

253. Han CS, Platt LD. Noninvasive prenatal testing: need for informed enthusiasm. American journal of obstetrics and gynecology. 2014; 211(6):577–80. [PubMed: 25459560]

254. Odibo AO. Pregnancy associated-plasma protein-A (PAPP-A) and alfa-fetoprotein (AFP) associated with placental abruption. American journal of obstetrics and gynecology. 2014; 211(2):89–90. [PubMed: 24837457]

255. O'Gorman N, Wright D, Syngelaki A, Akolekar R, Wright A, Poon LC, et al. Competing risks model in screening for preeclampsia by maternal factors and biomarkers at 11–13 weeks gestation. American journal of obstetrics and gynecology. 2016; 214(1):103.e1–e12. [PubMed: 26297382]

256. Abdel Moety GA, Almohamady M, Sherif NA, Raslana AN, Mohamed TF, El Moneam HM, et al. Could first-trimester assessment of placental functions predict preeclampsia and intrauterine growth restriction? A prospective cohort study. J Matern Fetal Neonatal Med. 2016; 29(3):413–7. [PubMed: 25594239]

257. Dukhovny S, Zera C, Little SE, McElrath T, Wilkins-Haug L. Eliminating first trimester markers: will replacing PAPP-A and betahCG miss women at risk for small for gestational age? J Matern Fetal Neonatal Med. 2014; 27(17):1761–4. [PubMed: 24460472]

258. Duran M, Kosus A, Kosus N, Turhan NO. Relation between serum PAPP-A level and umbilical cord thickness during first trimester of pregnancy. J Matern Fetal Neonatal Med. 2014; 27(4): 385–7. [PubMed: 23795813]

259. Krauskopf AL, Knippel AJ, Verde PE, Kozlowski P. Predicting SGA neonates using first-trimester screening: influence of previous pregnancy's birthweight and PAPP-A MoM. J Matern Fetal Neonatal Med. 2016; 29(18):2962–7. [PubMed: 26551433]

260. Kumar M, Singh S, Sharma K, Singh R, Ravi V, Bhattacharya J. Adverse fetal outcome: is first trimester ultrasound and Doppler better predictor than biomarkers? J Matern Fetal Neonatal Med. 2016:1–7.

261. Moon M, Odibo A. First-trimester screening for preeclampsia: impact of maternal parity on modeling and screening effectiveness. J Matern Fetal Neonatal Med. 2015; 28(17):2028–33. [PubMed: 25330843]

262. Lynch AM, Wagner BD, Deterding RR, Giclas PC, Gibbs RS, Janoff EN, et al. The relationship of circulating proteins in early pregnancy with preterm birth. American journal of obstetrics and gynecology. 2015

263. Wright D, Silva M, Papadopoulos S, Wright A, Nicolaides KH. Serum pregnancy-associated plasma protein-A in the three trimesters of pregnancy: effects of maternal characteristics and medical history. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology. 2015; 46(1):42–50.

264. Crocker PR, Varki A. Siglecs, sialic acids and innate immunity. Trends Immunol. 2001; 22(6): 337–42. [PubMed: 11377294]

265. von Gunten S, Simon HU. Sialic acid binding immunoglobulin-like lectins may regulate innate immune responses by modulating the life span of granulocytes. FASEB J. 2006; 20(6):601–5. [PubMed: 16581967]

266. Crocker PR, Paulson JC, Varki A. Siglecs and their roles in the immune system. Nat Rev Immunol. 2007; 7(4):255–66. [PubMed: 17380156]

267. Macauley MS, Crocker PR, Paulson JC. Siglec-mediated regulation of immune cell function in disease. Nat Rev Immunol. 2014; 14(10):653–66. [PubMed: 25234143]

268. Lam KK, Chiu PC, Lee CL, Pang RT, Leung CO, Koistinen H, et al. Glycodelin-A protein interacts with Siglec-6 protein to suppress trophoblast invasiveness by down-regulating extracellular signal-regulated kinase (ERK)/c-Jun signaling pathway. J Biol Chem. 2011; 286(43):37118–27. [PubMed: 21880722]

269. Rumer KK, Post MD, Larivee RS, Zink M, Uyenishi J, Kramer A, et al. Siglec-6 is expressed in gestational trophoblastic disease and affects proliferation, apoptosis and invasion. Endocr Relat Cancer. 2012; 19(6):827–40. [PubMed: 23089140]

270. Brinkman-Van der Linden EC, Hurtado-Ziola N, Hayakawa T, Wiggleton L, Benirschke K, Varki A, et al. Human-specific expression of Siglec-6 in the placenta. Glycobiology. 2007; 17(9):922–31. [PubMed: 17580316]

271. Patel N, Brinkman-Van der Linden EC, Altmann SW, Gish K, Balasubramanian S, Timans JC, et al. OB-BP1/Siglec-6. a leptin- and sialic acid-binding protein of the immunoglobulin superfamily. J Biol Chem. 1999; 274(32):22729–38. [PubMed: 10428856]

272. Rumer KK, Uyenishi J, Hoffman MC, Fisher BM, Winn VD. Siglec-6 expression is increased in placentas from pregnancies complicated by preterm preeclampsia. Reprod Sci. 2013; 20(6):646–53. [PubMed: 23171684]

273. Winn VD, Gormley M, Paquet AC, Kjaer-Sorensen K, Kramer A, Rumer KK, et al. Severe preeclampsia-related changes in gene expression at the maternal-fetal interface include sialic acid-binding immunoglobulin-like lectin-6 and pappalysin-2. Endocrinology. 2009; 150(1):452–62. [PubMed: 18818296]

274. Tsai S, Hardison NE, James AH, Motsinger-Reif AA, Bischoff SR, Thames BH, et al. Transcriptional profiling of human placentas from pregnancies complicated by preeclampsia reveals disregulation of sialic acid acetylesterase and immune signalling pathways. Placenta. 2011; 32(2):175–82. [PubMed: 21183218]

275. Yayon A, Klagsbrun M, Esko JD, Leder P, Ornitz DM. Cell surface, heparin-like molecules are required for binding of basic fibroblast growth factor to its high affinity receptor. Cell. 1991; 64(4):841–8. [PubMed: 1847668]

276. De Cat B, David G. Developmental roles of the glypicans. Semin Cell Dev Biol. 2001; 12(2):117–25. [PubMed: 11292377]

277. Song HH, Filmus J. The role of glypicans in mammalian development. Biochim Biophys Acta. 2002; 1573(3):241–6. [PubMed: 12417406]

278. Fransson LA, Belting M, Cheng F, Jonsson M, Mani K, Sandgren S. Novel aspects of glypican glycobiology. Cell Mol Life Sci. 2004; 61(9):1016–24. [PubMed: 15112050]

279. Jakubovic BD, Jothy S. Glypican-3: from the mutations of Simpson-Golabi-Behmel genetic syndrome to a tumor marker for hepatocellular carcinoma. Exp Mol Pathol. 2007; 82(2):184–9. [PubMed: 17258707]

280. Fico A, Maina F, Dono R. Fine-tuning of cell signaling by glypicans. Cell Mol Life Sci. 2011; 68(6):923–9. [PubMed: 18087675]

281. Filmus J, Capurro M. Glypican-3: a marker and a therapeutic target in hepatocellular carcinoma. FEBS J. 2013; 280(10):2471–6. [PubMed: 23305321]

282. Ogawa M, Yanoma S, Nagashima Y, Okamoto N, Ishikawa H, Haruki A, et al. Paradoxical discrepancy between the serum level and the placental intensity of PP5/TFPI-2 in preeclampsia and/or intrauterine growth restriction: possible interaction and correlation with glypican-3 hold the key. Placenta. 2007; 28(2–3):224–32. [PubMed: 16580726]

283. Chui A, Murthi P, Brennecke SP, Ignjatovic V, Monagle PT, Said JM. The expression of placental proteoglycans in pre-eclampsia. Gynecologic and obstetric investigation. 2012; 73(4):277–84. [PubMed: 22516801]

284. Coolman M, de Groot CJ, Steegers EA, Geurts-Moespot A, Thomas CM, Steegers-Theunissen RP, et al. Concentrations of plasminogen activators and their inhibitors in blood

preconceptionally, during and after pregnancy. European journal of obstetrics, gynecology, and reproductive biology. 2006; 128(1–2):22–8.

285. Hills FA, English J, Chard T. Circulating levels of IGF-I and IGF-binding protein-1 throughout pregnancy: relation to birthweight and maternal weight. J Endocrinol. 1996; 148(2):303–9. [PubMed: 8699144]
286. Skjaerbaek C, Frystyk J, Orskov H, Flyvbjerg A. Free IGF-I, IGFBP-1, and the binary complex of IGFBP-1 and IGF-I are increased during human pregnancy. Horm Res. 2004; 62(5):215–20. [PubMed: 15467298]
287. Olausson H, Lof M, Brismar K, Lewitt M, Forsum E, Sohlstrom A. Longitudinal study of the maternal insulin-like growth factor system before, during and after pregnancy in relation to fetal and infant weight. Horm Res. 2008; 69(2):99–106. [PubMed: 18059090]
288. Monaghan JM, Godber IM, Lawson N, Kaur M, Wark G, Teale D, et al. Longitudinal changes of insulin-like growth factors and their binding proteins throughout normal pregnancy. Ann Clin Biochem. 2004; 41(Pt 3):220–6. [PubMed: 15117436]
289. Hills FA, Iles RK, Sullivan MH. Differential proteolysis of insulin-like growth factor binding protein-1 (IGFBP-1) in pregnancy. Journal of perinatal medicine. 2013; 41(3):241–9. [PubMed: 23183724]
290. Lee SE, Han BD, Park IS, Romero R, Yoon BH. Evidence supporting proteolytic cleavage of insulin-like growth factor binding protein-1 (IGFBP-1) protein in amniotic fluid. Journal of perinatal medicine. 2008; 36(4):316–23. [PubMed: 18598121]
291. van Santen S, Kroot JJ, Zijderveld G, Wiegerinck ET, Spaanderman ME, Swinkels DW. The iron regulatory hormone hepcidin is decreased in pregnancy: a prospective longitudinal study. Clin Chem Lab Med. 2013; 51(7):1395–401. [PubMed: 23241678]
292. Dashe JS, Casey BM, Wells CE, McIntire DD, Byrd EW, Leveno KJ, et al. Thyroid-stimulating hormone in singleton and twin pregnancy: importance of gestational age-specific reference ranges. Obstetrics and gynecology. 2005; 106(4):753–7. [PubMed: 16199632]
293. Janeway CA Jr. Approaching the asymptote? Evolution and revolution in immunology. Cold Spring Harb Symp Quant Biol. 1989; (54 Pt 1):1–13.
294. Janeway C. Immunogenicity signals 1,2,3 … and 0. Immunol Today. 1989; 10(9):283–6. [PubMed: 2590379]
295. Janeway CA Jr, Medzhitov R. Innate immune recognition. Annu Rev Immunol. 2002; 20:197–216. [PubMed: 11861602]
296. Medzhitov R, Janeway CA Jr. Decoding the patterns of self and nonself by the innate immune system. Science. 2002; 296(5566):298–300. [PubMed: 11951031]
297. Matzinger P. The danger model: a renewed sense of self. Science. 2002; 296(5566):301–5. [PubMed: 11951032]
298. Oppenheim JJ, Tewary P, de la Rosa G, Yang D. Alarmins initiate host defense. Adv Exp Med Biol. 2007; 601:185–94. [PubMed: 17713005]
299. Bianchi ME. DAMPs, PAMPs and alarmins: all we need to know about danger. J Leukoc Biol. 2007; 81(1):1–5.
300. Bianchi ME, Manfredi AA. Immunology. Dangers in and out. Science. 2009; 323(5922):1683–4. [PubMed: 19325105]
301. Redman CW, Sargent IL. Immunological disorders of human pregnancy. Oxf Rev Reprod Biol. 1986; 8:223–65. [PubMed: 3540804]
302. Sacks GP, Studena K, Sargent K, Redman CW. Normal pregnancy and preeclampsia both produce inflammatory changes in peripheral blood leukocytes akin to those of sepsis. American journal of obstetrics and gynecology. 1998; 179(1):80–6. [PubMed: 9704769]
303. Zasloff M. Antimicrobial peptides in health and disease. N Engl J Med. 2002; 347(15):1199–200. [PubMed: 12374882]
304. Ganz T. The role of antimicrobial peptides in innate immunity. Integr Comp Biol. 2003; 43(2): 300–4. [PubMed: 21680437]
305. Ganz T. Defensins: antimicrobial peptides of innate immunity. Nat Rev Immunol. 2003; 3(9): 710–20. [PubMed: 12949495]

306. Yeaman MR, Yount NY. Unifying themes in host defence effector polypeptides. Nat Rev Microbiol. 2007; 5(9):727–40. [PubMed: 17703227]

307. Frew L, Stock SJ. Antimicrobial peptides and pregnancy. Reproduction. 2011; 141(6):725–35. [PubMed: 21474606]

308. Yarbrough VL, Winkle S, Herbst-Kralovetz MM. Antimicrobial peptides in the female reproductive tract: a critical component of the mucosal immune barrier with physiological and clinical implications. Hum Reprod Update. 2015; 21(3):353–77. [PubMed: 25547201]

309. Espinoza J, Chaiworapongsa T, Romero R, Edwin S, Rathnasabapathy C, Gomez R, et al. Antimicrobial peptides in amniotic fluid: defensins, calprotectin and bacterial/permeability-increasing protein in patients with microbial invasion of the amniotic cavity, intra-amniotic inflammation, preterm labor and premature rupture of membranes. J Matern Fetal Neonatal Med. 2003; 13(1):2–21. [PubMed: 12710851]

310. Soto E, Espinoza J, Nien JK, Kusanovic JP, Erez O, Richani K, et al. Human beta-defensin-2: a natural antimicrobial peptide present in amniotic fluid participates in the host response to microbial invasion of the amniotic cavity. J Matern Fetal Neonatal Med. 2007; 20(1):15–22. [PubMed: 17437194]

311. Galask RP, Snyder IS. Antimicrobial factors in amniotic fluid. American journal of obstetrics and gynecology. 1970; 106(1):59–65. [PubMed: 4983127]

312. Thadepalli H, Appleman MD, Maidman JE, Arce JJ, Davidson EC Jr. Antimicrobial effect of amniotic fluid against anaerobic bacteria. American journal of obstetrics and gynecology. 1977; 127(3):250–4. [PubMed: 835621]

313. Tafari N, Ross SM, Naeye RL, Galask RP, Zaar B. Failure of bacterial growth inhibition by amniotic fluid. American journal of obstetrics and gynecology. 1977; 128(2):187–9. [PubMed: 324281]

314. Thadepalli H, Bach VT, Davidson EC Jr. Antimicrobial effect of amniotic fluid. Obstetrics and gynecology. 1978; 52(2):198–204. [PubMed: 355963]

315. Appelbaum PC, Shulman G, Chambers NL, Simon NV, Granados JL, Fairbrother PF, et al. Studies on the growth-inhibiting property of amniotic fluids from two United States population groups. American journal of obstetrics and gynecology. 1980; 137(5):579–82. [PubMed: 6992582]

316. Thadepalli H, Gangopadhyay PK, Maidman JE. Amniotic fluid analysis for antimicrobial factors. Int J Gynaecol Obstet. 1982; 20(1):65–72. [PubMed: 6126410]

317. Cherry SH, Filler M, Harvey H. Lysozyme content of amniotic fluid. American journal of obstetrics and gynecology. 1973; 116(5):639–42. [PubMed: 4713126]

318. Ford LC, DeLange RJ, Lebherz TB. Identification of a bactericidal factor (B-lysin) in amnionic fluid at 14 and 40 weeks' gestation. American journal of obstetrics and gynecology. 1977; 127(7):788–92. [PubMed: 403764]

319. Yoshio H, Tollin M, Gudmundsson GH, Lagercrantz H, Jornvall H, Marchini G, et al. Antimicrobial polypeptides of human vernix caseosa and amniotic fluid: implications for newborn innate defense. Pediatr Res. 2003; 53(2):211–6. [PubMed: 12538777]

320. Akinbi HT, Narendran V, Pass AK, Markart P, Hoath SB. Host defense proteins in vernix caseosa and amniotic fluid. American journal of obstetrics and gynecology. 2004; 191(6):2090–6. [PubMed: 15592296]

321. Otsuki K, Yoda A, Saito H, Mitsuhashi Y, Toma Y, Shimizu Y, et al. Amniotic fluid lactoferrin in intrauterine infection. Placenta. 1999; 20(2–3):175–9. [PubMed: 10195738]

322. Pacora P, Maymon E, Gervasi MT, Gomez R, Edwin SS, Yoon BH, et al. Lactoferrin in intrauterine infection, human parturition, and rupture of fetal membranes. American journal of obstetrics and gynecology. 2000; 183(4):904–10. [PubMed: 11035335]

323. Heine RP, Wiesenfeld H, Mortimer L, Greig PC. Amniotic fluid defensins: potential markers of subclinical intrauterine infection. Clin Infect Dis. 1998; 27(3):513–8. [PubMed: 9770150]

324. Marinoni E, Di Iorio R, Letizia C, Villaccio B, Alberini A, Cosmi EV. Amniotic fluid concentrations of adrenomedullin in preterm labor. Obstetrics and gynecology. 1999; 93(6):964–7. [PubMed: 10362163]

325. Akinbiyi AA, Watson R, Feyi-Waboso P. Prevalence of Candida albicans and bacterial vaginosis in asymptomatic pregnant women in South Yorkshire, United Kingdom. Outcome of a prospective study. Arch Gynecol Obstet. 2008; 278(5):463–6. [PubMed: 18299865]

326. Purtilo DT. Opportunistic mycotic infections in pregnant women. American journal of obstetrics and gynecology. 1975; 122(5):607–10. [PubMed: 1146924]

327. Robinson SC, Nicholas WC, Lee DT, Wanklin JM, Zwicker B. The relationship of pregnancy, vaginal candidiasis and glucose metabolism. Can Med Assoc J. 1967; 96(10):583–4. [PubMed: 6020207]

328. Brandsma MA, Braaksma JT, van der Harten JJ. Immature delivery after intrauterine Candida albicans infection. European journal of obstetrics, gynecology, and reproductive biology. 1975; 5(6):331–5.

329. Walhout AJ, Vidal M. Protein interaction maps for model organisms. Nat Rev Mol Cell Biol. 2001; 2(1):55–62. [PubMed: 11413466]

330. Peri S, Navarro JD, Amanchy R, Kristiansen TZ, Jonnalagadda CK, Surendranath V, et al. Development of human protein reference database as an initial platform for approaching systems biology in humans. Genome Res. 2003; 13(10):2363–71. [PubMed: 14525934]

331. Knox R, Jiang X. Fyn in Neurodevelopment and Ischemic Brain Injury. Dev Neurosci. 2015; 37(4–5):311–20. [PubMed: 25720756]

332. Schenone S, Brullo C, Musumeci F, Biava M, Falchi F, Botta M. Fyn kinase in brain diseases and cancer: the search for inhibitors. Curr Med Chem. 2011; 18(19):2921–42. [PubMed: 21651487]

Summary

This is the first comprehensive study to characterize the longitudinal maternal plasma proteome in pregnancy with the use of novel technology. Since the proteomics technique used in this study provided protein abundance measurements expressed in relative fluorescence units, we could not derive references ranges of protein concentrations so that one can compare future data directly against these references. However, the patterns of change with gestation that we describe can still be useful in discovering disease markers in future studies provided that they deviate from the expected trajectory, regardless of the baseline concentration, which may be subject to experimental batch effects.

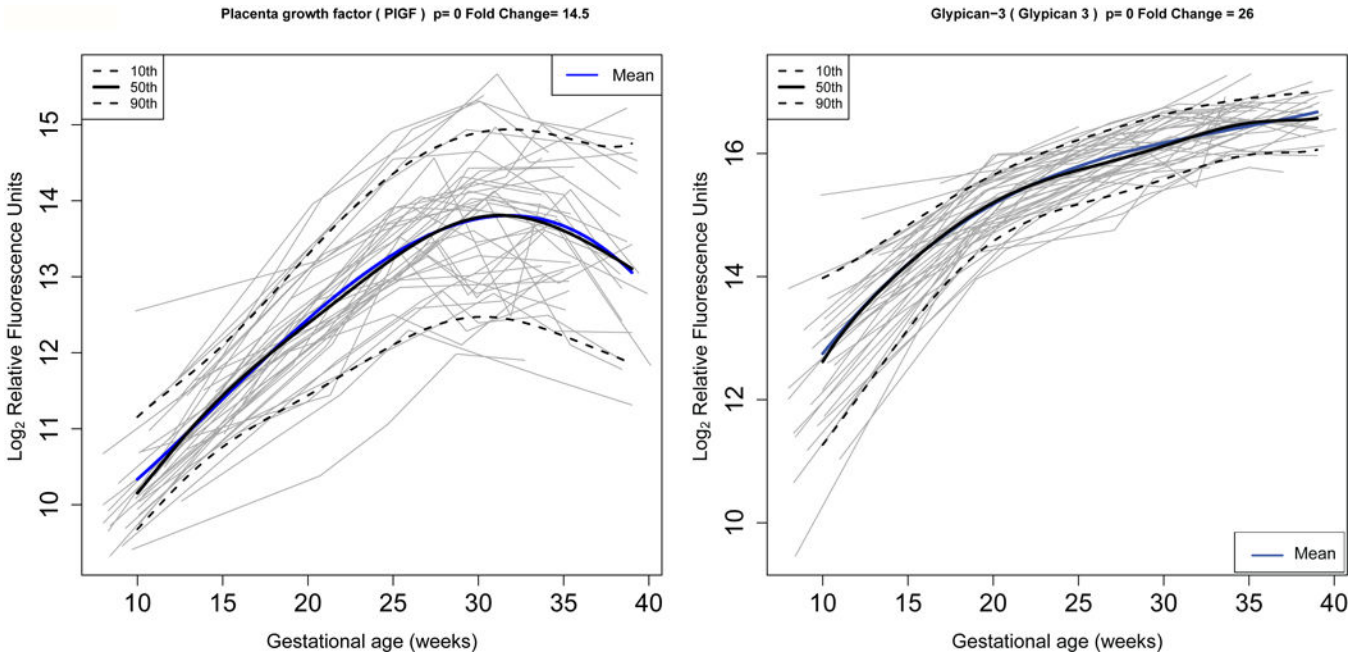

Figure 1.

Longitudinal profiles of placenta growth factor (left) and glypican-3 (right) in normal pregnancy. Protein abundance in (log, base 2, of) relative fluorescence units is shown for each of the 43 patients (grey lines). Mean protein abundance estimated by linear mixed-effects models with cubic splines (thick blue line) as well as median level (thick black line) and 10^{th} / 90^{th} centiles computed by quantile regression are also shown. Fold change (FC) is computed as the ratio in abundance between the highest and lowest value of the mean abundance over gestation.

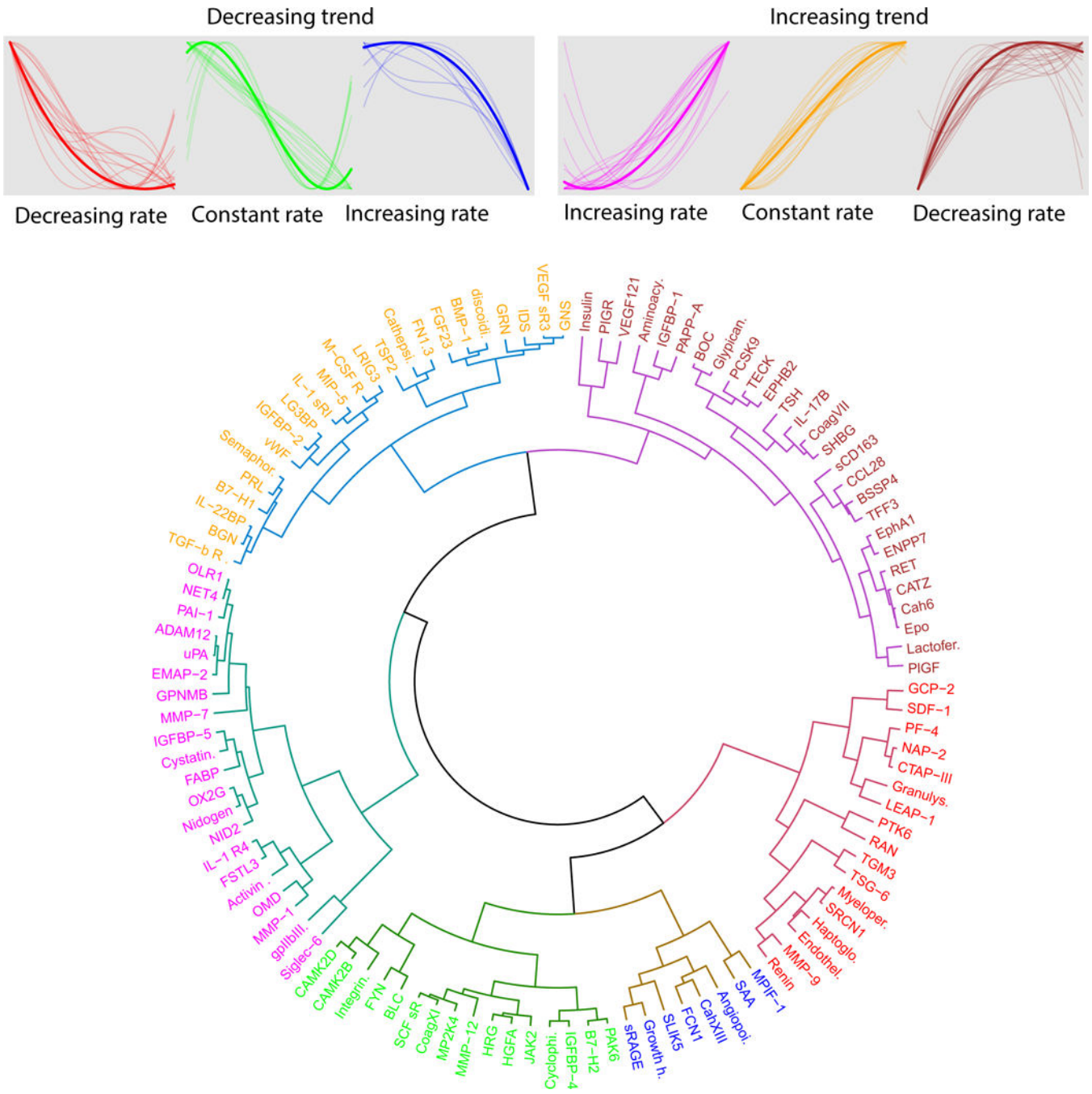

Figure 2.

Clustering of maternal plasma average protein profiles. The figure shows three clusters of proteins with increasing overall trends (increasing rate: n=21, constant rate: n=23, and decreasing rate: n=27) and three clusters with decreasing overall trends (increasing rate: n=8, constant rate: n=16, and increasing rate: n=17).

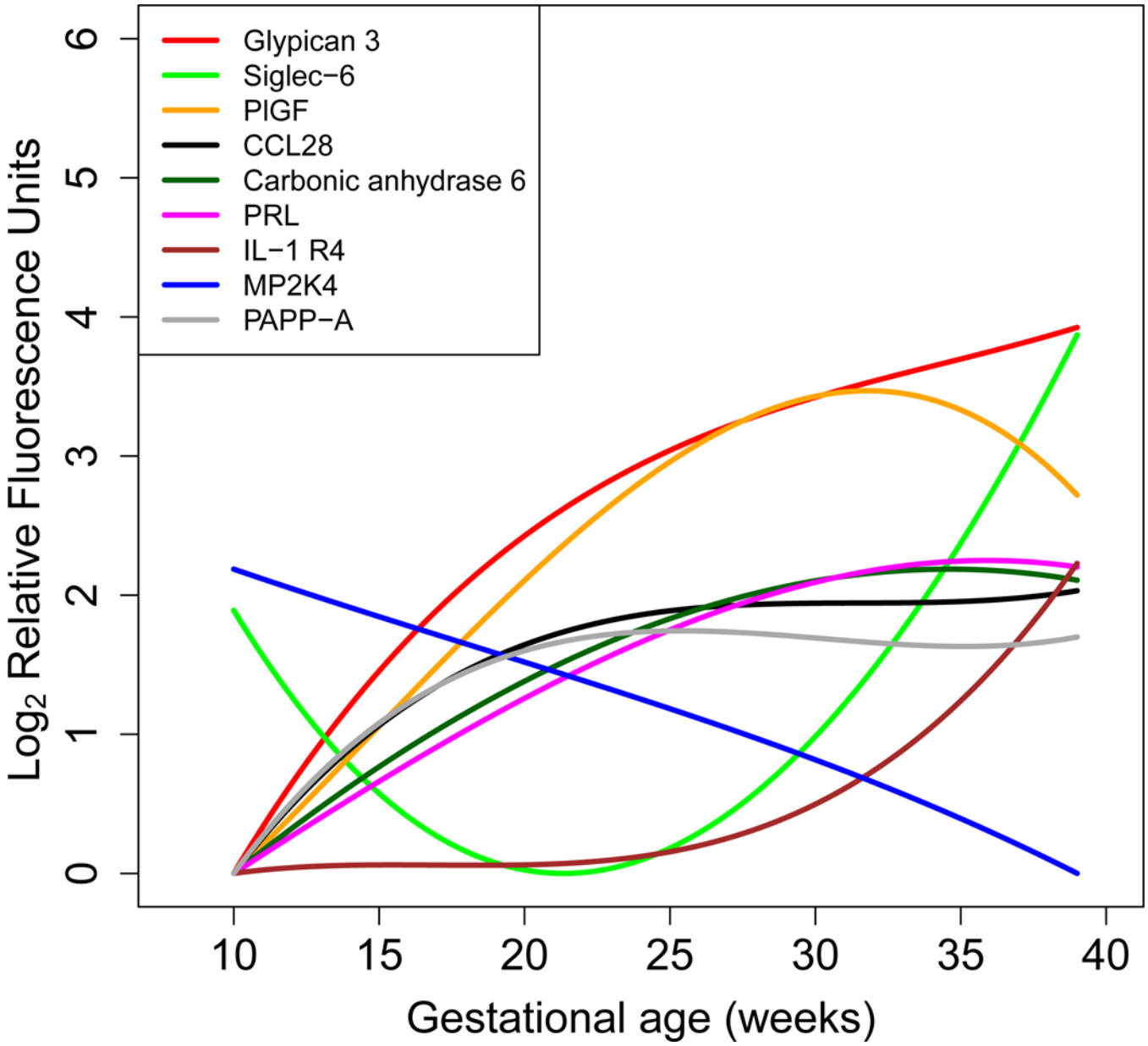

Figure 3.

Maternal plasma average protein abundance for nine highest modulated proteins. The figure shows protein abundance in log (base 2) relative fluorescence units computed from linear mixed-effects models with cubic splines. Average profiles were shifted so that minimum expression corresponds to 0 for all preteins. Most changing nine proteins were (decreasing fold change order): Glypican-3, Siglec-6: Sialic acid-binding immunoglobulin-type lectins 6, PlGF: placental growth factor, CCL28: C-C motif 28, Carbonic anhydrase 6, PRL: Prolactin, IL-1 R4: Interleukin-1 receptor 4, MP2K4: Dual specificity mitogen-activated protein kinase kinase 4, PAPP-A: pregnancy-associated plasma protein A.

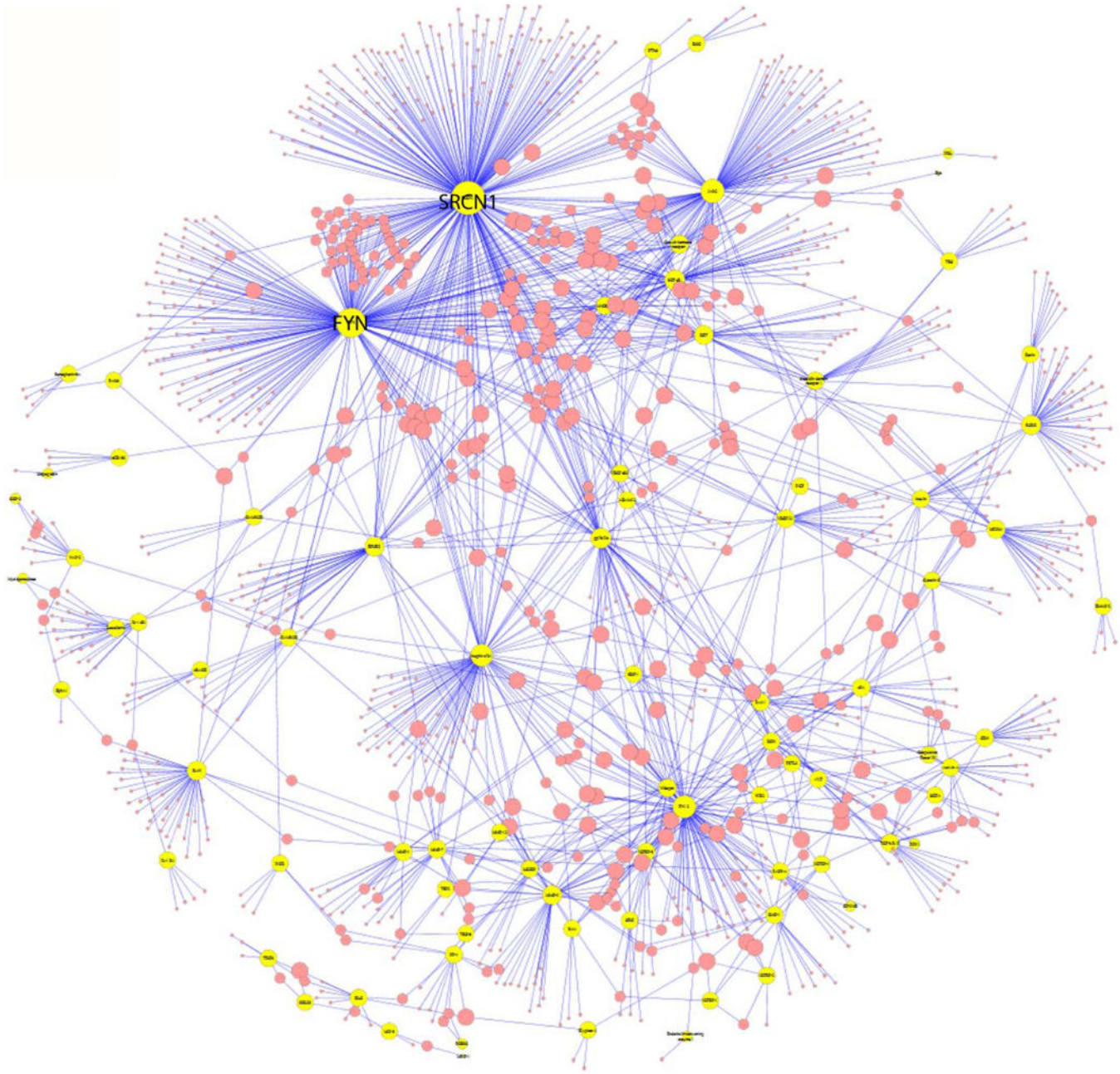

Figure 4.

Protein-protein interaction network in normal pregnancy. Yellow circles represent proteins profiled that change with gestation while red circles represent proteins from the Human Protein Reference Database known to interact with these. The size of the circles is proportional with the number of interactions. Proto-oncogene tyrosine-protein kinase Src (SRCN1) and Tyrosine-protein kinase Fyn (FYN) had 260 and 196 interactions, respectively.

Table 1

Clinical characteristics

Demographic characteristics of the study population. Data is presented as number (%percentage) for categorical variables or median/Inter quartile Range (IQR) for continuous variables.

Characteristics of the study population (n=43)	Median (IQR) or % (n)
Age (years)	25 (21–28)
Prepregnant BMI (kg/m^2)	25.2 (21.3–30.6)
Nulliparity (%)	27.9% (12)
Race (%) • African American • White • Other	88.4% (38) 4.7% (2) 7.0% (3)
Gestational age at delivery (weeks)	39.4 (39.0–40.1)
Route of delivery • Vaginal delivery • Cesarean delivery	67.4% (29) 32.6% (14)
Birthweight (grams)	3330 (3120–3545)

Table 2

List of proteins that change with gestational age in normal pregnancy. Somalogic identifiers and protein symbols and names are provided together with corresponding UniProt database identifiers and gene symbols. Fold change represents the ratio in protein abundance between the maximum and minimum average value across gestation. The protein trend types correspond to the clusters defined in Figure 1.

Target	Target Full Name	UniProt	Gene Symbol	FC	p	q	Direction	Trend Type
B7-H2	ICOS ligand	O75144	ICOSLG	1.84	0.0000	0.000	Decreasing	Constant rate
BLC	C-X-C motif chemokine 13	O43927	CXCL13	1.70	0.0000	0.000	Decreasing	Constant rate
CAMK2B	Calcium/calmodulin-dependent protein kinase type II subunit beta	Q13554	CAMK2B	1.53	0.0172	0.024	Decreasing	Constant rate
CAMK2D	Calcium/calmodulin-dependent protein kinase type II subunit delta	Q13557	CAMK2D	1.56	0.0183	0.025	Decreasing	Constant rate
Coagulation Factor XI	Coagulation Factor XI	P03951	F11	1.72	0.0000	0.000	Decreasing	Constant rate
Cyclophilin F	Peptidyl-prolyl cis-trans isomerase F, mitochondrial	P30405	PPIF	1.56	0.0471	0.059	Decreasing	Constant rate
FYN	Tyrosine-protein kinase Fyn	P06241	FYN	1.70	0.0029	0.005	Decreasing	Constant rate
HGFA	Hepatocyte growth factor activator	Q04756	HGFAC	1.91	0.0000	0.000	Decreasing	Constant rate
HRG	Histidine-rich glycoprotein	P04196	HRG	3.26	0.0000	0.000	Decreasing	Constant rate
IGFBP-4	Insulin-like growth factor-binding protein 4	P22692	IGFBP4	1.61	0.0000	0.000	Decreasing	Constant rate
Integrin a1b1	Integrin alpha-I: beta-1 complex	P56199, P05556	ITGA1 ITGB1	1.60	0.0012	0.002	Decreasing	Constant rate
JAK2	Tyrosine-protein kinase JAK2	O60674	JAK2	1.55	0.0004	0.001	Decreasing	Constant rate
MMP-12	Macrophage metalloelastase	P39900	MMP12	3.38	0.0000	0.000	Decreasing	Constant rate
MP2K4	Dual specificity mitogen-activated protein kinase kinase 4	P45985	MAP2K4	5.23	0.0000	0.000	Decreasing	Constant rate
PAK6	Serine/threonine-protein kinase PAK 6	Q9NQU5	PAK6	1.54	0.0000	0.000	Decreasing	Constant rate
SCF sR	Mast/stem cell growth factor receptor Kit	P10721	KIT	2.09	0.0000	0.000	Decreasing	Constant rate
CTAP-III	Connective tissue-activating peptide III	P02775	PPBP	1.52	0.0000	0.000	Decreasing	Decreasing rate
Endothelin-converting enzyme 1	Endothelin-converting enzyme 1	P42892	ECE1	2.21	0.0000	0.000	Decreasing	Decreasing rate
GCP-2	C-X-C motif chemokine 6	P80162	CXCL6	1.55	0.0000	0.000	Decreasing	Decreasing rate
Granulysin	Granulysin	P22749	GNLY	1.53	0.0000	0.000	Decreasing	Decreasing rate
Haptoglobin, Mixed Type	Haptoglobin	P00738	HP	2.11	0.0004	0.001	Decreasing	Decreasing rate
LEAP-1	Hepcidin	P81172	HAMP	4.32	0.0000	0.000	Decreasing	Decreasing rate
MMP-9	Matrix metalloproteinase-9	P14780	MMP9	1.56	0.0652	0.079	Decreasing	Decreasing rate
Myeloperoxidase	Myeloperoxidase	P05164	MPO	1.99	0.0000	0.000	Decreasing	Decreasing rate
NAP-2	Neutrophil-activating peptide 2	P02775	PPBP	1.59	0.0000	0.000	Decreasing	Decreasing rate

Target	Target Full Name	UniProt	Gene Symbol	FC	p	q	Direction	Trend Type
PF-4	Platelet factor 4	P02776	PF4	2.09	0.0000	0.000	Decreasing	Decreasing rate
PTK6	Protein-tyrosine kinase 6	Q13882	PTK6	1.55	0.0000	0.000	Decreasing	Decreasing rate
RAN	GTP-binding nuclear protein Ran	P62826	RAN	2.45	0.0002	0.000	Decreasing	Decreasing rate
Renin	Renin	P00797	REN	1.69	0.0000	0.000	Decreasing	Decreasing rate
SDF-1	Stromal cell-derived factor 1	P48061	CXCL12	1.54	0.0000	0.000	Decreasing	Decreasing rate
SRCN1	Proto-oncogene tyrosine-protein kinase Src	P12931	SRC	1.54	0.0000	0.000	Decreasing	Decreasing rate
TGM3	Protein-glutamine gamma-glutamyltransferase E	Q08188	TGM3	1.67	0.0000	0.000	Decreasing	Decreasing rate
TSG-6	Tumor necrosis factor-inducible gene 6 protein	P98066	TNFAIP6	1.61	0.0000	0.000	Decreasing	Decreasing rate
Angiopoietin-2	Angiopoietin-2	O15123	ANGPT2	2.27	0.0000	0.000	Decreasing	Increasing rate
Carbonic anhydrase XIII	Carbonic anhydrase 13	Q8N1Q1	CA13	1.83	0.0000	0.000	Decreasing	Increasing rate
FCN1	Ficolin-1	O00602	FCN1	1.71	0.0000	0.000	Decreasing	Increasing rate
Growth hormone receptor	Growth hormone receptor	P10912	GHR	1.89	0.0000	0.000	Decreasing	Increasing rate
MPIF-1	C-C motif chemokine 23	P55773	CCL23	1.57	0.0000	0.000	Decreasing	Increasing rate
SAA	Serum amyloid A-1 protein	P0DJI8	SAA1	2.04	0.0166	0.023	Decreasing	Increasing rate
SLIK5	SLIT and NTRK-like protein 5	O94991	SLITRK5	2.20	0.0000	0.000	Decreasing	Increasing rate
sRAGE	Advanced glycosylation end product-specific receptor, soluble	Q15109	AGER	1.54	0.0000	0.000	Decreasing	Increasing rate
B7-H1	Programmed cell death 1 ligand 1	Q9NZQ7	CD274	3.12	0.0000	0.000	Increasing	Constant rate
BGN	Biglycan	P21810	BGN	2.62	0.0000	0.000	Increasing	Constant rate
BMP-1	Bone morphogenetic protein 1	P13497	BMP1	3.78	0.0000	0.000	Increasing	Constant rate
Cathepsin A	Lysosomal protective protein	P10619	CTSA	1.66	0.0000	0.000	Increasing	Constant rate
discoidin domain receptor 1	Epithelial discoidin domain-containing receptor 1	Q08345	DDR1	3.48	0.0000	0.000	Increasing	Constant rate
FGF23	Fibroblast growth factor 23	Q9GZV9	FGF23	2.86	0.0000	0.000	Increasing	Constant rate
FN1.3	Fibronectin Fragment 3	P02751	FN1	1.66	0.0000	0.000	Increasing	Constant rate
GNS	N-acetylglucosamine-6-sulfatase	P15586	GNS	1.74	0.0000	0.000	Increasing	Constant rate
GRN	Granulins	P28799	GRN	2.69	0.0000	0.000	Increasing	Constant rate
IDS	Iduronate 2-sulfatase	P22304	IDS	2.31	0.0000	0.000	Increasing	Constant rate
IGFBP-2	Insulin-like growth factor-binding protein 2	P18065	IGFBP2	2.01	0.0000	0.000	Increasing	Constant rate
IL-1 sRI	Interleukin-1 receptor type 1	P14778	IL1R1	1.66	0.0000	0.000	Increasing	Constant rate
IL-22BP	Interleukin-22 receptor subunit alpha-2	Q969J5	IL22RA2	2.04	0.0000	0.000	Increasing	Constant rate
LG3BP	Galectin-3-binding protein	Q08380	LGALS3BP	1.61	0.0000	0.000	Increasing	Constant rate
LRIG3	Leucine-rich repeats and immunoglobulin-like domains protein 3	Q6UXM1	LRIG3	2.10	0.0000	0.000	Increasing	Constant rate

Target	Target Full Name	UniProt	Gene Symbol	FC	p	q	Direction	Trend Type
M-CSF R	Macrophage colony-stimulating factor 1 receptor	P07333	CSF1R	3.40	0.0000	0.000	Increasing	Constant rate
MIP-5	C-C motif chemokine 15	Q16663	CCL15	2.01	0.0000	0.000	Increasing	Constant rate
PRL	Prolactin	P01236	PRL	5.75	0.0000	0.000	Increasing	Constant rate
Semaphorin-6A	Semaphorin-6A	Q9H2E6	SEMA6A	2.52	0.0000	0.000	Increasing	Constant rate
TGF-b R III	Transforming growth factor beta receptor type 3	Q03167	TGFBR3	1.59	0.0000	0.000	Increasing	Constant rate
TSP2	Thrombospondin-2	P35442	THBS2	2.00	0.0000	0.000	Increasing	Constant rate
VEGF sR3	Vascular endothelial growth factor receptor 3	P35916	FLT4	1.75	0.0000	0.000	Increasing	Constant rate
vWF	von Willebrand factor	P04275	VWF	2.49	0.0000	0.000	Increasing	Constant rate
Aminoacylase-1	Aminoacylase-1	Q03154	ACY1	1.67	0.0001	0.000	Increasing	Decreasing rate
BOC	Brother of CDO	Q9BWV1	BOC	1.64	0.0000	0.000	Increasing	Decreasing rate
BSSP4	Brain-specific serine protease 4	Q9GZN4	PRSS22	2.18	0.0000	0.000	Increasing	Decreasing rate
Carbonic anhydrase 6	Carbonic anhydrase 6	P23280	CA6	5.79	0.0000	0.000	Increasing	Decreasing rate
CATZ	Cathepsin Z	Q9UBR2	CTSZ	1.60	0.0000	0.000	Increasing	Decreasing rate
CCL28	C-C motif chemokine 28	Q9NRJ3	CCL28	6.25	0.0000	0.000	Increasing	Decreasing rate
Coagulation Factor VII	Coagulation Factor VII	P08709	F7	1.59	0.0000	0.000	Increasing	Decreasing rate
ENPP7	Ectonucleotide pyrophosphatase/phosphodiesterase family member 7	Q6UWV6	ENPP7	1.84	0.0000	0.000	Increasing	Decreasing rate
EphA1	Ephrin type-A receptor 1	P21709	EPHA1	2.12	0.0000	0.000	Increasing	Decreasing rate
EPHB2	Ephrin type-B receptor 2	P29323	EPHB2	1.53	0.0000	0.000	Increasing	Decreasing rate
Epo	Erythropoietin	P01588	EPO	1.59	0.0000	0.000	Increasing	Decreasing rate
Glypican 3	Glypican-3	P51654	GPC3	26.04	0.0000	0.000	Increasing	Decreasing rate
IGFBP-1	Insulin-like growth factor-binding protein 1	P08833	IGFBP1	2.92	0.0000	0.000	Increasing	Decreasing rate
IL-17B	Interleukin-17B	Q9UHF5	IL17B	1.56	0.0000	0.000	Increasing	Decreasing rate
Insulin	Insulin	P01308	INS	1.54	0.0274	0.037	Increasing	Decreasing rate
Lactoferrin	Lactotransferrin	P02788	LTF	1.50	0.0000	0.000	Increasing	Decreasing rate
PAPP-A	Pappalysin-1	Q13219	PAPPA	5.18	0.0000	0.000	Increasing	Decreasing rate
PCSK9	Proprotein convertase subtilisin/kexin type 9	Q8NBP7	PCSK9	1.89	0.0000	0.000	Increasing	Decreasing rate
PIGR	Polymeric immunoglobulin receptor	P01833	PIGR	1.86	0.0000	0.000	Increasing	Decreasing rate
PlGF	Placenta growth factor	P49763	PGF	14.46	0.0000	0.000	Increasing	Decreasing rate
RET	Proto-oncogene tyrosine-protein kinase receptor Ret	P07949	RET	4.15	0.0000	0.000	Increasing	Decreasing rate
sCD163	Scavenger receptor cysteine-rich type 1 protein M130	Q86VB7	CD163	1.57	0.0000	0.000	Increasing	Decreasing rate
SHBG	Sex hormone-binding globulin	P04278	SHBG	2.38	0.0000	0.000	Increasing	Decreasing rate

Target	Target Full Name	UniProt	Gene Symbol	FC	p	q	Direction	Trend Type
TECK	C-C motif chemokine 25	O15444	CCL25	2.02	0.0000	0.000	Increasing	Decreasing rate
TFF3	Trefoil factor 3	Q07654	TFF3	4.31	0.0000	0.000	Increasing	Decreasing rate
TSH	Thyroid Stimulating Hormone	P01215 P01222	CGA TSHB	2.63	0.0000	0.000	Increasing	Decreasing rate
VEGF121	Vascular endothelial growth factor A, isoform 121	P15692	VEGFA	2.02	0.0000	0.000	Increasing	Decreasing rate
Activin A	Inhibin beta A chain	P08476	INHBA	3.21	0.0000	0.000	Increasing	Increasing rate
ADAM12	Disintegrin and metalloproteinase domain-containing protein 12	O43184	ADAM12	1.74	0.0000	0.000	Increasing	Increasing rate
Cystatin C	Cystatin-C	P01034	CST3	1.98	0.0000	0.000	Increasing	Increasing rate
EMAP-2	Endothelial monocyte-activating polypeptide 2	Q12904	AIMP1	1.85	0.0000	0.000	Increasing	Increasing rate
FABP	Fatty acid-binding protein, heart	P05413	FABP3	1.58	0.0000	0.000	Increasing	Increasing rate
FSTL3	Follistatin-related protein 3	O95633	FSTL3	1.56	0.0000	0.000	Increasing	Increasing rate
gpIIbIIIa	Integrin alpha-IIb: beta-3 complex	P08514 P05106	ITGA2B ITGB3	1.79	0.0696	0.084	Increasing	Increasing rate
GPNMB	Transmembrane glycoprotein NMB	Q14956	GPNMB	1.52	0.0000	0.000	Increasing	Increasing rate
IGFBP-5	Insulin-like growth factor-binding protein 5	P24593	IGFBP5	1.51	0.0000	0.000	Increasing	Increasing rate
IL-1 R4	Interleukin-1 receptor-like 1	Q01638	IL1RL1	5.49	0.0000	0.000	Increasing	Increasing rate
MMP-1	Interstitial collagenase	P03956	MMP1	1.58	0.0000	0.000	Increasing	Increasing rate
MMP-7	Matrilysin	P09237	MMP7	1.53	0.0000	0.000	Increasing	Increasing rate
NET4	Netrin-4	Q9HB63	NTN4	1.50	0.0000	0.000	Increasing	Increasing rate
NID2	Nidogen-2	Q14112	NID2	1.51	0.0000	0.000	Increasing	Increasing rate
Nidogen	Nidogen-1	P14543	NID1	1.79	0.0000	0.000	Increasing	Increasing rate
OLR1	Oxidized low-density lipoprotein receptor 1	P78380	OLR1	2.65	0.0000	0.000	Increasing	Increasing rate
OMD	Osteomodulin	Q99983	OMD	1.79	0.0000	0.000	Increasing	Increasing rate
OX2G	OX-2 membrane glycoprotein	P41217	CD200	2.60	0.0000	0.000	Increasing	Increasing rate
PAI-1	Plasminogen activator inhibitor 1	P05121	SERPINE1	2.00	0.0000	0.000	Increasing	Increasing rate
Siglec-6	Sialic acid-binding Ig-like lectin 6	O43699	SIGLEC6	16.92	0.0000	0.000	Increasing	Increasing rate
uPA	Urokinase-type plasminogen activator	P00749	PLAU	2.18	0.0000	0.000	Increasing	Increasing rate

Table 3

Biological processes enriched in proteins that change with gestational age. Odds ratios from a Fisher's exact test and p-values are provided for all biological processes that had 3 or more significant proteins.

Biological process	No. of Proteins	Corresponding Genes Symbols	Odds Ratio	p	Decreasing	Increasing
defense response to fungus	4	MPO;GNLY;HAMP;HRG	12.4	0.003	MPO;GNLY;HAMP;HRG	
defense response	6	MPO; CST3; INHBA; HP; TFF3; ICOSLG	5.7	0.003	MPO;HP;ICOSLG	CST3;INHBA;TFF3
germ cell migration	3	KIT;ITGA1 ITGB1;CXCL12	27.7	0.004	KIT;ITGA1 ITGB1;CXCL12	
smooth muscle cell migration	3	ITGA2B ITGB3;DDR1;PLAU	27.7	0.004		ITGA2B ITGB3;DDR1;PLAU
regulation of bone resorption	3	CSF1R;ITGA2B ITGB3;SRC	27.7	0.004	SRC	CSF1R;ITGA2B ITGB3
negative regulation of leukocyte tethering or rolling	3	CCL25;CCL28;CXCL12	27.7	0.004	CXCL12	CCL25;CCL28
proteolysis	20	F11;MMP9;LTF;MMP7;CTSA;F7; ACY1;BMP1;REN;ECE1;HGFAC;O LR1;PAPPA;PLAU;ADAM12;MMP 12;PRSS22;MMP1;CTSZ;PCSK9	2.1	0.006	F11;MMP9;REN;ECE1;HGF AC;MMP12	LTF;MMP7;CTSA;F7;ACY1;B MP1;OLR1;PAPPA;PLAU;A DAM12;PRSS22;MMP1;CTS Z;PCSK9
cell chemotaxis	8	KIT;CCL25;PPBP;CCL28;CXCL6;CC L15;CXCL12;PPBP	3.5	0.007	KIT;PPBP;CXCL6;CXCL12;PP BP	CCL25;CCL28;CCL15
leukocyte migration	11	MMP9;ANGPT2; CCL25;AIMP1;F N1;ITGA1 ITGB1;OLR1;ITGA2B ITGB3;FYN;MMP1;SRC	2.7	0.008	MMP9;ANGPT2;ITGA1 ITGB1;FYN;SRC	CCL25;AIMP1;FN1;OLR1;IT GA2B ITGB3;MMP1
cellular response to hormone stimulus	4	IGFBP2;GHR;PGF;CGA TSHB	7.4	0.008	GHR	IGFBP2;PGF;CGA TSHB
macrophage differentiation	3	MMP9;CSF1R;VEGFA	13.8	0.008	MMP9	CSF1R;VEGFA
cell-substrate adhesion	3	VWF;ITGA1 ITGB1;ITGA2B ITGB3	13.8	0.008	ITGA1 ITGB1	VWF;ITGA2B ITGB3
mesodermal cell differentiation	3	INHBA;ITGA1 ITGB1;ITGA2B ITGB3	13.8	0.008	ITGA1 ITGB1	INHBA;ITGA2B ITGB3
defense response to bacterium	7	PPBP;HP;GNLY;CXCL13;CXCL6;H AMP;PPBP	3.7	0.008	PPBP;HP;GNLY;CXCL13;CXC L6;HAMP;PPBP	

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