Journal of
Proteomics & Bioinformatics

Wong and Cox, J Proteomics Bioinform 2014, S10
DOI: 10.4172/0974-276X.S10-001

Research Article Open Access

Proteomics Analysis of Preeclampsia, a Systematic Review of Maternal and Fetal Compartments

Frances Wong and Brian Cox*

Department of Physiology, University of Toronto, Toronto, Canada, M5S 1A8

Abstract

Preeclampsia is a relatively common hypertensive disorder of pregnancy that remains a high cause of maternal and fetal death due to the lack of early detection and treatment options. While the etiology is unclear, the placenta is the origin of preeclampsia and it releases factors into maternal circulation to induce systemic endothelial dysfunction. Preeclampsia is a heterogeneous condition and a single biomarker is likely incapable of identifying all women at risk of developing the condition. Proteomic efforts have been largely directed to analysis of maternal blood serum or the placental tissue. Altered protein expression in the placenta suggests a role in the development of preeclampsia while altered protein expression in maternal serum indicates factors secreted from the placenta or maternal responses to these factors.

In this systematic review, 12 studies comparing samples from preeclamptic and normotensive pregnancies using mass spectrometry based techniques were selected and 401 proteins with significantly altered expression in preeclampsia were observed across all studies. Inter-study comparison identified 52 proteins as significant in two or more studies. These 52 proteins were enriched for 22 pathways, including several previously implicated in preeclampsia such as hemostasis, immune response, and lipid metabolism which is a focus of this analysis. Significantly, the proteins complement component 4 and apolipoprotein E were observed with aberrant expression at week 12 before the clinical diagnosis of preeclampsia indicating promising roles as clinical biomarkers. Future studies of secretions from placenta villi explants can relate misrepresented proteins in the placenta to the altered protein profile in the maternal circulation resulting in systemic dysfunction.

Keywords: Proteomics; Preeclampsia; Mass spectrometry

Abbreviations: sFlt-1: Soluble fms-Like Tyrosine Kinase-1; PlGF: Placental Growth Factor; 2DE: 2-Dimensional Gel Electrophoresis; MALDI: Matrix-Assisted Laser Desorption/Ionization; TOF: Time-of-Flight; LC: Liquid Chromatography; ESI: Electrospray Ionization; C8: Complement Component 8; C9: Complement Component; MAC: Membrane Attack Complex; C3: Complement Component 3; C5a: Complement Component 5a; C4: Complement Component 4; LDL: low Density Lipoproteins

Introduction

Preeclampsia, affecting 2-7% of all pregnancies, is a vasculopathy that results from aberrant placenta development and it is the third highest cause of pregnancy related deaths following hemorrhage and embolism [1-3]. While the etiology of preeclampsia is poorly understood, a leading theory is that insufficient spiral artery remodeling in the placenta may result in a hypoxic environment with reperfusion injury and up-regulation of oxidative stress [4,5]. The preeclamptic placenta secretes factors such as inflammatory cytokines and reactive oxygen species into maternal circulation to induce hypertension through local production of vasoconstrictors, notably endothelin-1 [6]. As a result, the mother shows signs of preeclampsia after the 20th week of gestation by new onset hypertension and proteinuria.

Evidence for the placenta being the origin of preeclampsia comes from its occurrence in molar pregnancies, consisting of a placenta without a fetus, and symptoms subsiding when the placenta is expelled at delivery [7]. The placenta maintains essential communication between the mother and the developing fetus throughout pregnancy and complications such as preeclampsia can greatly impact fetal health and development [8]. The placenta mediates communication between the mother and fetus by transporting nutrients towards the developing fetus, removing metabolic products, and secreting hormones [8]. Currently, there are no preventative measures or treatment available for preeclampsia other than preterm delivery to minimize mortality of the mother, but frequently endangers infant health. Candidate based searches for altered protein expression in women with preeclampsia identified potential biomarkers such as endoglin, soluble fms-like tyrosine kinase-1 (sFlt-1), and placental growth factor (PlGF) [9]. While these biomarkers are correlated with the onset of preeclampsia, they lack sensitivity and as a consequence, biomarkers have yet to be incorporated into standard diagnostic procedures [10]. Preeclampsia is a complex disorder with many etiologies and this makes it unlikely that a single biomarker could robustly identify all preeclamptic patients. To identify new insights in the etiology of preeclampsia and novel predictive biomarkers, researchers are turning towards high throughput proteomics to enable robust comparisons between the protein content in preeclamptic and normotensive pregnancies. While there are multiple proteomic approaches available, mass spectrometry based techniques remain a popular choice and are the focus of this systematic review. Researchers have taken two approaches: proteomic analysis of maternal blood serum or the placental tissue. As the placenta is in direct contact with maternal circulation, it secretes factors in to

***Corresponding author:** Brian Cox, Department of Physiology, Faculty of Medicine, Medical Sciences Building, 3360, University of Toronto, King's College Circle, Toronto, ON Canada M5S 1A8, Tel: 416-978-3241; E-mail: b.cox@utoronto.ca

Received May 04, 2014; **Accepted** May 27, 2014; **Published** May 30, 2014

Citation: Wong F, Cox B (2014) Proteomics Analysis of Preeclampsia, a Systematic Review of Maternal and Fetal Compartments. J Proteomics Bioinform S10: 001. doi:10.4172/0974-276X.S10-001

the serum that that may serve as potential biomarkers. Additionally, pregnancy induces adaptive changes to maternal physiology that can alter serum protein profiles from a variety of maternal sources. In the case of preeclampsia, aberrant levels of select serum proteins, regardless of their origin, may be responsible for the systemic pathology of this disease. A comparison of proteins in the serum of preeclamptic and normal pregnancies is hoped to identify causative or diagnostic markers.

Special Consideration of Human Biomaterial and Pathology

There are a few considerations to keep in mind when consolidating data across studies of human subjects. Firstly, diagnostic criteria for preeclampsia may differ between studies. Generally, preeclampsia is defined by new onset hypertension and proteinuria after 20 weeks gestation. While hypertension is typically defined as a systolic/diastolic blood pressure greater than 140/90 mmHg, many studies selected for severe hypertension defined by a blood pressure of 160/110 mmHg or greater. Proteinuria was diagnosed with a minimum of 300 mg of protein in urine within 24 hours but some studies set a more severe cutoff up to 5 g. The process of labor and delivery can alter protein expression compared to a placenta during pregnancy and thus, many groups selected for placentas from elective term cesarean sections. In addition, consideration is given to maternal and pregnancy characteristics such as age, gestational age, and parity (numbers of pregnancies and live births) to keep the subjects comparable and minimize the influence from other factors.

The placenta is a large organ and like all organs, is structurally heterogeneous. Replicate sampling is an important consideration to control for regional biases, typically more pronounced in an organ suffering from a developmental pathology [11-13]. By pooling multiple samples from different regions of the placenta, it is hoped that an average sample representing the placenta can be developed.

Proteomic Methods

A variety of techniques can be used to separate and identify proteins, with 2-dimensional gel electrophoresis (2DE) coupled with and matrix-assisted laser desorption/ionization (MALDI) time-of-flight (TOF) being one of the older but more common techniques. Two dimensional liquid chromatography (LC) separation of peptides coupled with electrospray ionization (ESI) into an ion trap mass spectrometer is a more modern approach that typically has a higher yield of identification rate but can be more complex to establish as a robust method. Mass spectrometry analysis and identification methods have previously been reviewed in detail [14].

Review Criteria and Methods

For our systematic review, we selected studies that evaluated preeclamptic and normotensive pregnancies sampling either placental tissue or maternal serum using any mass spectrometry based technique. In addition, we required the availability of the data set or complete table of identified proteins. All supplied protein identifiers were mapped to UniProt IDs and manually curated to ensure maximal and accurate conversion.

Tables of significantly up or down regulated proteins in each study were aggregated using R to determine if the proteins appeared in more than one study. Ontological analysis of the gene sets was performed using Metabolic Mine (www.metabolicmine.org). A network map was generated using Cytoscape to represent proteins identified in multiple studies and the pathway memberships.

Proteomics in Preeclampsia

Our review identified 12 studies that concentrate on three different proteomes affected by preeclampsia: placental tissue, blood serum, and the secretome of the placental trophoblast cells (Table 1-3). Eight studies quantified the protein complement of the placenta by analyzing the total protein extracted from tissue homogenates.

First Author, Year (PMID)	Number of			Inclusion Criteria for		Additional Criteria
	Sample	Control	PE	Hypertension	Proteinuria	
Epiney, 2012 [4] (22234358)	Secreted	6	4	SBP ≥ 160 mmHg or DBP ≥ 110 mmHg	≥ 5g/24 hr	N/A
Feng, 2012 [39] (22414876)	Tissue	10	10	SBP ≥ 140 mm Hg or DBP ≥ 90 mm Hg	≥ 0.3 g/24 hr or ≥ 2 by dipstick measurement	C-section, epidural anesthesia, age and gestational age matched
Gharesi-Fard, 2010 [40] (19954843)	Tissue	5	5	SBP ≥ 160 mmHg or DBP ≥ 110 mmHg on two occasions 6 hrs apart	≥ 500 mg/24 hrs	C-section, age and gestational Age
Jin, 2008 [41] (18940332)	Tissue	N/A	8	BP ≥ 140/90	≥ 300 mg/24 hrs	C-section, age, time of pregnancy
Kolla, 2012 [42] (22570525)	Serum	6	6	N/A	N/A	Age and gestational age Matched
Lui, 2011 [43] (21145106)	Serum	5	5	SBP ≥ 160 mmHg or DBP ≥ 110 mmHg on 2 occasions at least 6hrs apart	≥ 5 g/24 h or ≥ 2 by dipstick measurement	C-section, third trimester, gestational age-matched
Ma, 2013 [44] (24343450)	Tissue	1	1	N/A	N/A	C-section, age, gestational Age, and parity matched
Park, 2011 [45] (21646846)	Serum	5	5	SBP ≥ 160 mmHg or DBP ≥ 110 mmHg	≥ 2 by dipstick measurement at least 6 hrs apart	C-section, age matched
Shi, 2013 [30] (23671712)	Tissue	4	4	BP ≥ 160/110 mmHG on two occasions w/in 4 hrs	≥ 2g /24 hrs	C-section, age, gestational age, and parity matched
Shin, 2011 [46] (21335933)	Tissue	10	10	SBP ≥ 160 mmHg or DBP ≥ 90 mmHg	≥ 300 mg/24 hr	C-section,
Wang, 2013 [28] (24260401)	Tissue	20	20	SBP ≥ 150 mmHg or DBP ≥ 110 mmHg on 2 occasions in 6 hrs	N/A	N/A
Wang, 2013 [29] (24205073)	Tissue	20	20	SBP ≥ 150 mmHg or DBP ≥ 110 mmHg on 2 occasions in 6 hrs	N/A	N/A

Table 1: Inclusion characteristics for each sample and number of participants. BP: Blood Pressure; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; C-Section: Cesarean-Section.

First Author, Year (PMID)	Samples	Sample Details	Separation	MS Machine Model	Protein Identification Database
Epiney,2012 [4] (22234358)	Secreted	Media from primary cultured cytotrophoblasts	LC-ESI-MS/MS	linear trap quadrupole (LTQ) Orbitrap Velos	SwissProt/ trEMBL database
Feng, 2012 [39] (22414876)	Tissue	1 x 5 g decidual tissue	2DE, MALDI-TOF-MS	Voyager-DE STR 4307	SwissProt database via Mascot-Wizard Engine
Gharesi-Fard, 2010 [40] (19954843)	Tissue	5 x 500 mg samples from each placenta	2DE, MALDI-TOF-TOF	N/A	NCBI via Mascot Engine
Jin, 2008 [41] (18940332)	Tissue	1 x 1 cm squared sample from the center of each placenta; LCM	LC- 2DE, TOF-TOFMS/MS	Linear trap quadrupole (LTQ) Orbitrap	IPI human v3.25 database via Mascot Engine
Kolla, 2012 [42] (22570525)	Serum	Week 12 blood sample, 9ml	iTRAQ labeling, Nano-LC-MALDI-TOF/TOFMS/MS	4800 Plus MALDITOF/ TOF Analyzer (Applied Biosystems)	PANTHER database
Lui, 2011 [43] (21145106)	Serum	Blood Samples	Tris-Glycine SDS-gel, LC-MS/MS	Thermo LTQ-Orbitrap mass spectrometer	Human IPI database via Mascot engine
Ma, 2013 [44] (24343450)	Tissue	1 x 1 cm squared sample from the center of maternal face of the placenta; LCM	ICAT, 2DE, LC-MS/MS	Esquire 3000 ion trap mass spectrometer	Geneontology.org
Park, 2011 [45] (21646846)	Serum	10 ul venous blood before delivery	LC-MS/MS	LTQ ion-trap mass spectrometer	NCBInr database via SEQUEST algorithms
Shi, 2013 [30] (23671712)	Tissue	1 x 1 cm squared sample from the center of maternal face of the placenta; isolate for mitochondria	iTRAQ labeling, nano-LC-MS/MS	LTQ-Orbitrap MS	Mascot engine
Shin, 2011 [46] (21335933)	Tissue	5 x 5 core samples of feto-maternal interface of the placenta	2DE, MALDI-TOF-MS	Voyager TM-delayed extraction STR biospectrometry workstation	SwissProt or NCBI
Wang, 2013 [28] (24260401)	Tissue	1 x 0.5 g sample from the maternal face of the placenta, isolate for plasma memb.	ICAT, UPLC-MS/MS	LTQ-Orbitrap instrument	UniProt via Maxquant
Wang, 2013 [29] (24205073)	Tissue	1 x 0.5 g sample from the maternal face of the placenta	ICAT, UPLC-MS/MS	LTQ-Orbitrap instrument	UniProt via Maxquant

Table 2: Details of samples collected and processing workflow. LCM: Laser Capture Microdissection; LC: Liquid Chromatography; UPLC: Ultra Performance Liquid Chromatography; ESI: Electrospray Ionization; MS: Mass Spectrometry; MALDI: Matrix-Assisted Laser Desorption/Ionization; TOF: Time-of-Flight; iTraq: Isobaric Tag for Relative and Absolute Quantitation

First Author, Year (PMID)	Sample	Total Proteins Detected	Total Proteins Altered in preeclampsia	Upregulated	Downregulated	Dataset Available?
Epiney, 2012 [4] (22234358)	Secreted	273	23	9	14	Table in paper
Feng, 2012 [39] (22414876)	Tissue	~ 420	9	9	0	Table in paper
Gharesi-Fard, 2010 [40](19954843)	Tissue	520	11	4	7	Table in paper
Jin, 2008 [41] (18940332)	Tissue	963	13	10	3	Table in paper
Kolla, 2012 [42] (22570525)	Serum	N/A	16	8	8	Supplementary Table
Lui, 2011 [43] (21145106)	Serum	~ 1160	51	31	20	Table in paper
Ma, 2013 [44] (24343450)	Tissue	831	70	39	31	Table in paper
Park, 2011 [45] (21646846)	Serum	237	62	27	35	Table in paper
Shi, 2013 [30] (23671712)	Tissue	N/A	26	4	22	Table in paper
Shin, 2011 [46] (21335933)	Tissue	>300	12	4	8	Table in paper
Wang, 2013 [28] (24260401)	Tissue	3121	38	15	23	Supplementary Table
Wang, 2013 [29] (24205073)	Tissue	2636	171	147	24	Supplementary Table

Table 3: Summary of proteins detected in each study.

Proteins contributing to the development of preeclampsia are more likely to be detected in placenta tissue rather than serum. Importantly, the hypoxic placenta secretes factors into the maternal circulation to induce systemic vascular dysfunction, therefore analysis of the protein complement in blood serum may identify these factors. Maternal blood serum was examined in three studies. To causally link the correlation of changes in serum proteins to the placenta tissue, one study assessed changes in the profile of secreted proteins from cultures of placental cells from normal and preeclamptic tissues.

Identified Proteins

Across the 12 identified studies, 401 proteins were observed with differential expression in preeclamptic placentas (Table 3). Only 20 proteins could not be mapped to UniProt IDs because the original protein/gene identifier was no longer valid or the original identifier description was ambiguous. Of these, 53 proteins were identified in at least two studies as differentially regulated (Supplementary Table 1) and were further analyzed for significant enrichment of affected pathways and Gene Ontology (GO) terms. These 53 proteins were enriched for 22 pathways (FDR corrected p-value<0.05) and are graphically represented as a network in Figure 1. We will focus on the pathways of hemostasis, immune response, and lipid metabolism as these are known to be linked to preeclampsia.

Hemostasis

Many proteins involved in hemostasis and blood coagulation pathways were observed as consistently upregulated through different studies. Proteins were also enriched in pathways for the clotting cascade and O_2 and CO_2 exchange in erythrocytes. These proteins are cross annotated to GO terms for blood coagulation, platelet activation, platelet degranulation, and wound healing. There is a delicate balance between opposing coagulation factors and we see a greater upregulation of pro-coagulation factors promoting a hypercoagulative state. Pregnancy is normally associated with a hypercoagulative state induced by increased pro-coagulation factors as well as reduced anti-coagulation factors [15]. This is believed to be an adaptive measure to prevent blood loss during delivery when the placenta is separated from the uterus. The hypercoagulative state is enhanced in preeclampsia and this is observed by multiple studies through the upregulation of proteins including fibrinogen beta chain (2/2 studies), fibrinogen gamma chain (2/2 studies), and kininogen (2/2 studies), all involved in fibrin clot formation during the coagulation cascade. These results support previous targeted studies that detected elevated fibrinogen levels in serum of women with preeclampsia [16,17]. Fibronectin is detected as upregulated by four studies and is only expressed by activated platelets as a feedback to limit excessive response. Coagulation is initiated by

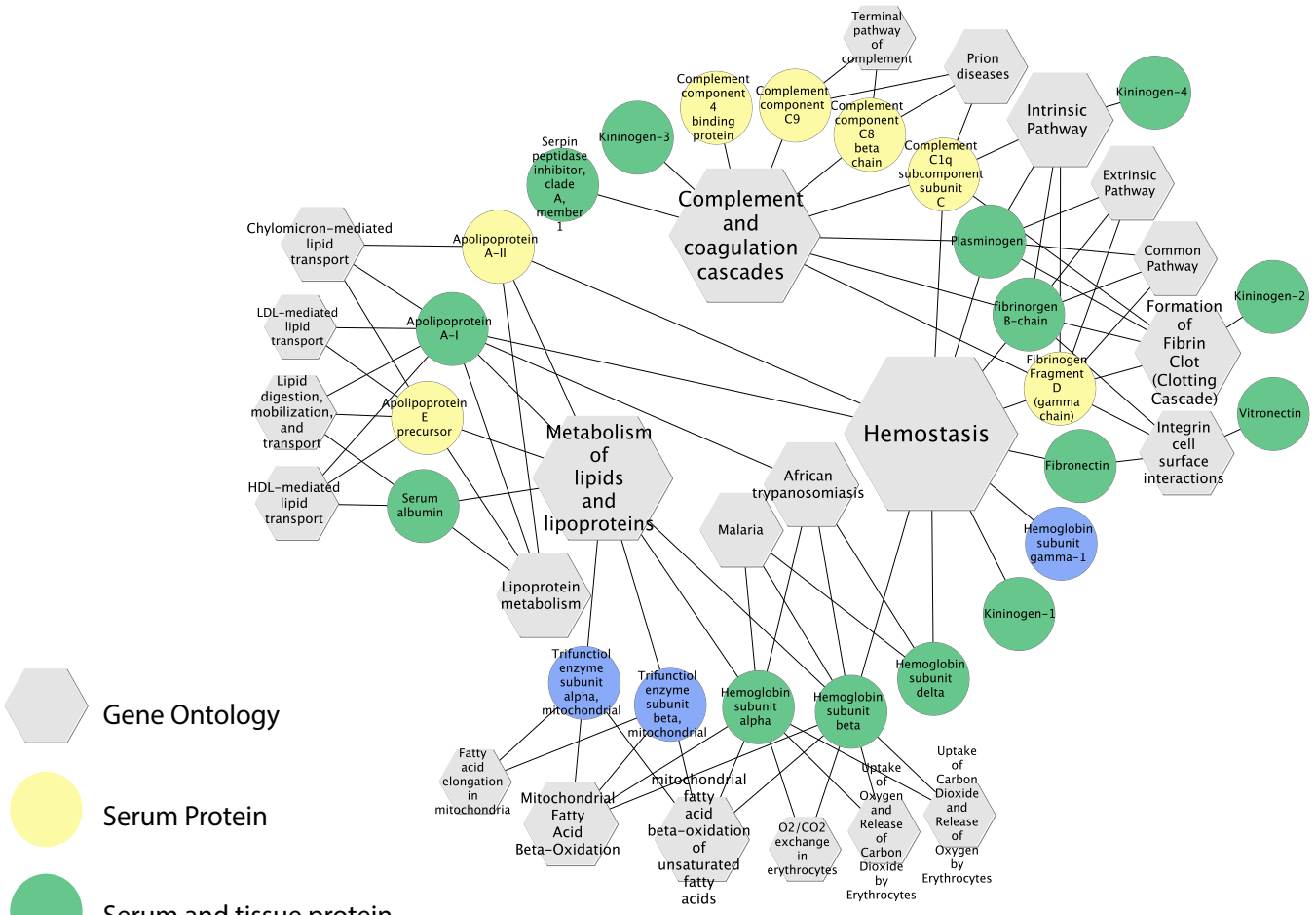

Figure 1: Network of proteins identified in two or more studies and the pathways they are involved in. Circles represent proteins while hexagons represent pathways. Proteins identified exclusively in serum or secretome are colored yellow, proteins identified exclusively in tissue are colored blue, and proteins identified in both serum and tissue are colored green. The size of the pathways nodes are representative of the number of connections.

cleaving fibrinogen to fibrin and activating platelets [18]. Fibrinogen beta chain and kininogen are observed as upregulated in both serum and tissue studies while fibrinogen gamma chain was only observed as upregulated in serum studies.

Despite the consistency observed in coagulation factors, of the proteins annotated to the GO term negative regulation of blood coagulation, annexin A2, apolipoprotein E, and plasminogen there was ambiguous regulation in relation to the preeclamptic state since they were identified as upregulated or downregulated different studies. We speculate that this could be due to heterogeneity in sample sources (Table 1) or maternal response the pathology. Plasminogen is primarily synthesized in the liver and is cleaved into plasmin at the site of insult by tissue or urokinase plasminogen activators in the early steps of fibrinolysis to the active protease plasmin [18,19]. Annexin A2 also acts in the fibrinolysis pathway. Plasminogen was observed as upregulated in three studies, both tissue and serum, while one study of serum observed it as down regulated. While plasmin was not detected as differentially regulated in preeclampsia by any of the 12 studies, it is likely upregulated as well in this thrombolytic disease. Detection methods may not have aimed to discriminate between cleavage products resulting in plasmin not detected as differentially regulated in preeclamptic women. Further studies are required to confirm the expression of proteins in the fibrinolysis pathway and how it affects the placenta during preeclampsia.

Pathways for blood disorders malaria and African trypanosomiasis were enriched, suggesting preeclampsia may share downstream targets resulting in the systemic hypertensive state. Malaria is a known risk factor for developing preeclampsia in tropical countries, further supporting the notion of preeclampsia as a blood disorder, and suggesting a possible causal molecular link between idiopathic and malarial induced preeclampsia [20]. The results from the 12 studies indicate there is deregulation in the hemostasis pathway during preeclampsia. The markers are found in both tissue and serum indicating it may be relevant in the etiology of preeclampsia and secreted into maternal circulation to induce systemic vascular function.

Immune Response

Women with autoimmune diseases are at a greater risk of developing preeclampsia indicating these pregnancy complications may be a result of altered immune regulation [21]. Our analysis of affected pathways identified dysregulation of intrinsic, extrinsic, and compliment activation pathways. The complement cascade involves opsonization, chemotaxis, and lysis of foreign material as part of the innate immune system and is potentiated in normotensive pregnancy [22]. Most of the complement proteins are synthesized by the liver but they can also be produced locally by specialized cells such as macrophages and blood monocytes [23]. Complement proteins were previously shown to be up-regulated in preeclamptic placentas but these proteins were only observed as differentially regulated in studies of serum [21]. Circulating complement products indicate the complement system is activated but surface receptors are saturated causing the factors to accumulate in plasma [22]. Complement activation by the complement component 3 (C3) and complement component 5a (C5a) fragments are associated with pregnancy loss showing complement activation can adversely affect pregnancy. C8 and C9 are activated in the final steps to form pores on target cells and induce cell death by contributing to the membrane attack complex (MAC) [24]. Complement component 8 (C8) and complement component (C9) were observed as ambiguously regulated in two studies, one serum source reported C8 and C9 as down regulated at delivery while the other reported up- regulated but the gestational age of the samples was not reported (Table2 and Supplementary Table 1) also ambiguous was Complement factor H-1 related protein, which was previously identified as a potential biomarker for preeclampsia [4]. Additionally, Complement component 4 (C4) was ambiguous, but interestingly, C4 binding protein was observed as upregulated in Kolla's study of blood serum during week 12 of pregnancy while the same protein was observed as downregulated in Park's study of blood serum immediately before delivery. This suggests the regulation of C4 is a potential biomarker of preeclampsia because its expression is altered and can be detected early on in gestation. C4 is associated with elevated risk of lupus but its role in pregnancy has yet to be identified [25,26]. Also of interest is our observation of Complement C1q subcomponent subunit C as up-regulated in 2/2 studies of maternal PE serum (Supplementary Table 1), yet the knockout mouse model of this factor has PE like symptoms [27]. Differential regulation of complement proteins could be an important area to pursue in future studies using larger patient cohorts and more targeted quantitative approaches.

Lipid Metabolism

We observed significant enrichment of proteins involved in lipid and lipoprotein metabolism pathways as altered in women with preeclampsia. Annexin A2 is found to be elevated in tissue but down regulated in cytotrophoblast media. Mitochondria trifunctional enzyme subunit alpha and beta are only significantly altered in placenta tissue although with conflicting results. This conflict may result from differences in sampling as Wang et al. [28,29] (Table 1-3) did not specify the control group use nor did they include proteinuria as a diagnostic for PE, while Shi et al. [30] (Table 1-3) defined both. Apolipoprotein A-II was consistently down-regulated in 2/2 studies and apolipoprotein E (apoE) was ambiguous.

Of these proteins, apoE is the most extensively studied in the context of preeclampsia. ApoE is detected exclusively in studies of serum because it is predominantly synthesized by the liver and macrophages before being secreted into circulation with minimal local production in the placenta [31]. Previous findings are conflicted because apoE levels have been detected as unchanged or increasing in preeclamptic patients [31-33]. ApoE was observed as downregulated in the study of week 12 serum while it was detected as upregulated in the study of serum at term. This interesting pattern of expression suggests apoE is a candidate biomarker for early preeclampsia detection. ApoE participates in various physiological systems because it has three different isoforms: ApoE2, ApoE3, and ApoE4 [34]. ApoE4 is associated with pathological conditions including atherosclerosis and Alzheimer's disease. During pregnancy, ApoE4 promotes atherosclerosis by elevating low density lipoproteins (LDL) levels as well as promoting thrombosis, as discussed earlier. The majority of the population expresses ApoE3 so it is inefficient marker of disease but high ApoE2 and ApoE4 levels is correlated with elevated risk of recurrent spontaneous abortion [34]. Recent studies suggest ApoE levels are unaffected by preeclampsia but polymorphisms result in misrepresentation of glycosylation isoforms in women with preeclampsia [31]. The basic deglycosylated form is increased and the acidic isoform is decreased in preeclamptic women [31]. This is a possible explanation for the conflicted reports of ApoE levels in women with preeclampsia because studies could be detecting different isoforms of ApoE due to different post-translational modifications.

Discussion: Biomarkers for Preeclampsia

Currently, the diagnosis of preeclampsia relies on maternal symptoms of hypertension and proteinuria but these symptoms

are variable between women and unspecific to the disorder [35]. Pathological mechanisms have already manifested by the time of diagnosis causing irreversible damage to maternal and fetal systems. There is a need then to identify early molecular markers that identify mothers at risk before the onset of clinical symptoms allowing health care professionals to take preventative measures and prevent the placenta from becoming hypoxic. Preeclampsia is a complex multisystem disorder that involves the miscommunication between mother and fetus; it has been suggested that there are three potential origins to this pathology: fetal, maternal or a contribution from both. As a result, a single biomarker may not be capable of identifying all women at risk. An accurate and early biomarker is essential to determining the most appropriate treatment plan [9,36].

Proteomics studies are costly and require a great deal of resources, limiting the number of replicates that can be assessed. Pooling datasets from multiple studies allows the analysis of many more samples and studies with similar conclusion can be validated. Through a systematic review of mass spectrometry based proteomics literature centered around preeclampsia, 53 proteins were detected in at least two of the 12 studies as differentially expressed in preeclamptic patients. Additionally, these were enriched to pathways previously implicated in the pathology of preeclampsia: hemostasis, immune response, and lipid metabolism.

Proteomics offers a promising alternative to classical candidate based approaches for identifying biomarkers of preeclampsia because of its ability to detect the whole protein complement. Additionally, the ability to investigate changes in placental tissue, its secreted fraction and maternal serum enable the elucidation of placental dysfunction and maternal responses underlying the origins and mechanisms behind this pathology. Altered plasma proteins may be a result of factors directly secreted from the placenta or a maternal response triggered against the aberrant placenta. Comparing proteins found in the tissue and serum will elude to how the placenta causes systemic maternal vascular dysfunction.

Ambiguity and conflicting results are endemic in large scale data sets. This arises though multiple mechanisms. While some causes are purely statistical, such as false positive discovery, or methodological, such as instrumentation and sample processing, others may arise from patient heterogeneity. Different patient populations and selection criteria can confound inter-study comparisons (Tables 1-3). After review of these published studies there is sufficient promise in the technique of proteomics to warrent expansion of these analyses to larger patient cohorts, but with careful consideration to the many pitfalls of such research, which some studies considered in this review are victims. There needs to be a better effort by journals and authors to make tables of raw data and mass spectrometry files available, to enable more robust comparisons between data sets, similar to frame works that govern microarray and next generation sequencing data sets.

Biomarkers in serum are more translatable to clinical applications because blood samples are routinely analyzed throughout pregnancy (e.g., glucose tolerance tests). Altered biomarker levels may precede clinical symptoms and these are important for early disease detection. Two proteins are observed with time dependent changes in expression. C4 was detected as upregulated at week 12 while it was detected as downregulated immediately before delivery in a separate study. Alternatively, apoE expression was observed as downregulated at week 12 and upregulated at term. C4 and apoE expression are altered in the serum long before diagnosis at week 20 suggesting they may have critical roles as biomarker of preeclampsia. While ApoE has been pursued as a marker C4 requires further study. Altered protein expression in tissue may elude to maternal pathways misregulated in preeclampsia except they unlikely contribute to the clinical symptoms of hypertension and proteinuria unless it is secreted into maternal circulation. Early detection will facilitate early intervention to prevent maternal and fetal damage. Preeclampsia remains a leading cause of maternal death because we lack early detection and effective treatment plans.

Proteomics enables detection of the protein complement and this systematic review is powerful because tissue and serum proteomes are compared to show how the preeclamptic placenta interacts with the maternal circulation. For example the proteoglycan Lumican and the glycoprotein Vitronectin are both detected as up regulated in placenta and serum studies (Supplementary Table 1), indicating a possible link between production by the placenta and deposition into serum. Additionally, we note strong supporting evidence for other recently identified markers of fetal growth restriction and PE, such as Clic3 [37], which was detected as up regulated in 4 or 4 studies.

Epiney et al. [4] study pioneers a promising direction for proteomics by quantifying protein in cytotrophoblast culture media. While Epiney et al. [4] study acknowledges that syncytial trophoblasts, not cytotrophoblast, directly exchange materials with the maternal circulation, their study begins to bridges the gap in placenta and serum proteome. This difference of cell type may explain why there is differential significance between serum studies and the cytotrophoblast sectromone. Future applications could include quantifying proteins in media of whole placenta villous explants because these structures are in contact with maternal circulation [38]. Combining systems biology approaches such as proteomics and genomics will give us more comprehensive understanding about preeclampsia and mechanistically relate the genome to the expressed phenotype.

References

1. MacKay AP, Berg CJ, Atrash HK (2001) Pregnancy-related mortality from preeclampsia and eclampsia. Obstet Gynecol 97: 533-538.
2. Masoura S, Kalogiannidis IA, Gitas G, Goutsioulis A, Koiou E, et al. (2012) Biomarkers in pre-eclampsia: a novel approach to early detection of the disease. J Obstet Gynaecol 32: 609-616.
3. Roberts JM, Escudero C (2012) The placenta in preeclampsia. Pregnancy Hypertens 2: 72-83.
4. Epiney M, Ribaux P, Arboit P, Irion O, Cohen M (2012) Comparative analysis of secreted proteins from normal and preeclamptic trophoblastic cells using proteomic approaches. J Proteomics 75: 1771-1777.
5. Lyall F, Robson SC, Bulmer JN (2013) Spiral artery remodeling and trophoblast invasion in preeclampsia and fetal growth restriction: relationship to clinical outcome. Hypertension 62: 1046-1054.
6. George EM, Granger JP (2011) Endothelin: key mediator of hypertension in preeclampsia. Am J Hypertens 24: 964-969.
7. Staff AC, Benton SJ, von Dadelszen P, Roberts JM, Taylor RN, et al. (2013) Redefining preeclampsia using placenta-derived biomarkers. Hypertension 61: 932-942.
8. Cross JC, Baczyk D, Dobric N, Hemberger M, Hughes M, et al. (2003) Genes, development and evolution of the placenta. Placenta 24: 123-130.
9. Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, et al. (2004) Circulating angiogenic factors and the risk of preeclampsia. N Engl J Med 350: 672-683.
10. Kleinrouweler CE, Wiegerinck MM, Ris-Stalpers C, Bossuyt PM, van der Post JA, et al. (2012) Accuracy of circulating placental growth factor, vascular endothelial growth factor, soluble fms-like tyrosine kinase 1 and soluble endoglin in the prediction of pre-eclampsia: a systematic review and meta-analysis. BJOG 119: 778-787.
11. Hemberger M, Udayashankar R, Tesar P, Moore H, Burton GJ (2010) ELF5-

enforced transcriptional networks define an epigenetically regulated trophoblast stem cell compartment in the human placenta. Hum Mol Genet 19: 2456-2467.

12. Mayhew TM (2008) Taking tissue samples from the placenta: an illustration of principles and strategies. Placenta 29: 1-14.
13. Takao T, Asanoma K, Kato K, Fukushima K, Tsunematsu R, et al. (2011) Isolation and characterization of human trophoblast side-population (SP) cells in primary villous cytotrophoblasts and HTR-8/SVneo cell line. PLoS One 6: e21990.
14. Aebersold R, Mann M (2003) Mass spectrometry-based proteomics. Nature 422: 198-207.
15. Pinheiro MB, Gomes KB, Dusse LM (2013) Fibrinolytic system in preeclampsia. Clin Chim Acta 416: 67-71.
16. Al-Ofi E, Coffelt SB, Anumba DO3 (2014) Fibrinogen, an endogenous ligand of Toll-like receptor 4, activates monocytes in pre-eclamptic patients. J Reprod Immunol 103: 23-28.
17. Comeglio P, Fedi S, Liotta AA, Cellai AP, Chiarantini E, et al. (1996) Blood clotting activation during normal pregnancy. Thromb Res 84: 199-202.
18. Cesarman-Maus G, Hajjar KA (2005) Molecular mechanisms of fibrinolysis. Br J Haematol 129: 307-321.
19. Gong Y, Zhao Y, Li Y, Fan Y, Hoover-Plow J (2014) Plasminogen regulates cardiac repair after myocardial infarction through its non-canonical function in stem cell homing to the infarcted heart. J Am Coll Cardiol .
20. Ndao CT, Dumont A, Fievet N, Doucoure S, Gaye A, et al. (2009) Placental malarial infection as a risk factor for hypertensive disorders during pregnancy in Africa: a case-control study in an urban area of Senegal, West Africa. Am J Epidemiol 170: 847-853.
21. Buurma A, Cohen D, Veraar K, Schonkeren D, Claas FH, et al. (2012) Preeclampsia is characterized by placental complement dysregulation. Hypertension 60: 1332-1337.
22. Richani K, Soto E, Romero R, Espinoza J, Chaiworapongsa T, et al. (2005) Normal pregnancy is characterized by systemic activation of the complement system. J Matern Fetal Neonatal Med 17: 239-245.
23. Perlmutter DH, Colten HR (1986) Molecular immunobiology of complement biosynthesis: a model of single-cell control of effector-inhibitor balance. Annu Rev Immunol 4: 231-251.
24. Weiland MH, Qian Y, Sodetz JM (2014) Membrane pore formation by human complement: functional importance of the transmembrane beta-hairpin (TMH) segments of C8alpha and C9. Mol Immunol 57: 310-316.
25. Puah SM, Lian LH, Chew CH, Chua KH, Tan SY (2007) A study of association of the complement C4 mutations with systemic lupus erythematosus in the Malaysian population. Lupus 16: 750-754.
26. Wu YL, Hauptmann G, Viguier M, Yu CY (2009) Molecular basis of complete complement C4 deficiency in two North-African families with systemic lupus erythematosus. Genes Immun 10: 433-445.
27. Singh J, Ahmed A, Girardi G (2011) Role of complement component C1q in the onset of preeclampsia in mice. Hypertension 58: 716-724.
28. Wang F, Wang L, Shi Z, Liang G (2013) Comparative N-glycoproteomic and phosphoproteomic profiling of human placental plasma membrane between normal and preeclampsia pregnancies with high-resolution mass spectrometry. PLoS One 8: e80480.
29. Wang F, Shi Z, Wang P, You W, Liang G (2013) Comparative proteome profile of human placenta from normal and preeclamptic pregnancies. PLoS One 8: e78025.
30. Shi Z, Long W, Zhao C, Guo X, Shen R, et al. (2013) Comparative proteomics analysis suggests that placental mitochondria are involved in the development of pre-eclampsia. PLoS One 8: e64351.
31. Atkinson KR, Blumenstein M, Black MA, Wu SH, Kasabov N, et al. (2009) An altered pattern of circulating apolipoprotein E3 isoforms is implicated in preeclampsia. J Lipid Res 50: 71-80.
32. Belo L, Gaffney D, Caslake M, Santos-Silva A, Pereira-Leite L, et al. (2004) Apolipoprotein E and cholesteryl ester transfer protein polymorphisms in normal and preeclamptic pregnancies. Eur J Obstet Gynecol Reprod Biol 112: 9-15.
33. Blumenstein M, McMaster MT, Black MA, Wu S, Prakash R, et al. (2009) A proteomic approach identifies early pregnancy biomarkers for preeclampsia: novel linkages between a predisposition to preeclampsia and cardiovascular disease. Proteomics 9: 2929-2945.
34. Li J, Chen Y, Wu H, Li L (2014) Apolipoprotein E (Apo E) gene polymorphisms and recurrent pregnancy loss: a meta-analysis. J Assist Reprod Genet 31: 139-148.
35. Alvarez-Fernández I, Prieto B, Rodríguez V, Ruano Y, Escudero AI, et al. (2014) New biomarkers in diagnosis of early onset preeclampsia and imminent delivery prognosis. Clin Chem Lab Med .
36. Cox B, Sharma P, Evangelou AI, Whiteley K, Ignatchenko V, et al. (2011) Translational analysis of mouse and human placental protein and mRNA reveals distinct molecular pathologies in human preeclampsia. Mol Cell Proteomics 10: M111.
37. Murthi P, Stevenson JL, Money TT, Borg AJ, Brennecke SP, et al. (2012) Placental CLIC3 is increased in fetal growth restriction and pre-eclampsia affected human pregnancies. Placenta 33: 741-744.
38. Cross JC (2006) Placental function in development and disease. Reprod Fertil Dev 18: 71-76.
39. Feng YL, Zhou CJ, Li XM, Liang XQ (2012) Alpha-1-antitrypsin acts as a preeclampsia-related protein: a proteomic study. Gynecol Obstet Invest 73: 252-259.
40. Gharesi-Fard B, Zolghadri J, Kamali-Sarvestani E (2010) Proteome differences of placenta between pre-eclampsia and normal pregnancy. Placenta 31: 121-125.
41. Jin H, Ma KD, Hu R, Chen Y, Yang F, et al. (2008) Analysis of expression and comparative profile of normal placental tissue proteins and those in preeclampsia patients using proteomic approaches. Anal Chim Acta 629: 158-164.
42. Kolla V, Jenö P, Moes S, Lapaire O, Hoesli I, et al. (2012) Quantitative proteomic (iTRAQ) analysis of 1st trimester maternal plasma samples in pregnancies at risk for preeclampsia. J Biomed Biotechnol 2012: 305964.
43. Liu C, Zhang N, Yu H, Chen Y, Liang Y, et al. (2011) Proteomic analysis of human serum for finding pathogenic factors and potential biomarkers in preeclampsia. Placenta 32: 168-174.
44. Ma K, Jin H, Hu R, Xiong Y, Zhou S, et al. (2014) A proteomic analysis of placental trophoblastic cells in preeclampsia-eclampsia. Cell Biochem Biophys 69: 247-258.
45. Park J, Cha DH, Lee SJ, Kim YN, Kim YH, et al. (2011) Discovery of the serum biomarker proteins in severe preeclampsia by proteomic analysis. Exp Mol Med 43: 427-435.
46. Shin JK, Baek JC, Kang MY, Park JK, Lee SA, et al. (2011) Proteomic analysis reveals an elevated expression of heat shock protein 27 in preeclamptic placentas. Gynecol Obstet Invest 71: 151-157.

This article was originally published in a special issue, **Applications of Proteomics in Pathology** handled by Editor. Dr. Alan Tackett, University of Arkansas for Medical Sciences, USA