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Molecular and Vascular Targets in the Pathogenesis and Management of the Hypertension Associated with Preeclampsia

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Abstract

Normal pregnancy is associated with significant hemodynamic changes and vasodilation of the uterine and systemic circulation in order to meet the metabolic demands of the mother and developing fetus. Preeclampsia (PE) is one of the foremost complications of pregnancy and a major cause of maternal and fetal mortality. The pathophysiological mechanisms of PE have been elusive, but some parts of the puzzle have begun to unravel. Genetic factors such as leptin gene polymorphism, environmental and dietary factors such as Ca^{2+} and vitamin D deficiency, and co-morbidities such as obesity and diabetes may increase the susceptibility of pregnant women to develop PE. An altered maternal immune response may also play a role in the development of PE. Although the pathophysiology of PE is unclear, most studies have implicated inadequate invasion of cytotrophoblasts into the uterine artery, leading to reduced uteroplacental perfusion pressure (RUPP) and placental ischemia/hypoxia. Placental ischemia induces the release of biologically active factors such as growth factor inhibitors, anti-angiogenic factors, inflammatory cytokines, reactive oxygen species, hypoxia-inducible factors, and antibodies to vascular angiotensin II (AngII) receptor. These bioactive factors could cause vascular endotheliosis and consequent increase in vascular resistance and blood pressure, as well as glomerular endotheliosis with consequent proteinuria. The PE-associated vascular endotheliosis could be manifested as decreased vasodilator mediators such as nitric oxide, prostacyclin and hyperpolarizing factor and increased vasoconstrictor mediators such as endothelin-1, AngII and thromboxane A_2 . PE could also involve enhanced mechanisms of vascular smooth muscle contraction including intracellular Ca^{2+} , and Ca^{2+} sensitization pathways such as protein kinase C and Rho-kinase. PE-associated changes in the extracellular matrix composition and matrix metalloproteinases activity also promote vascular remodeling and further vasoconstriction in the uterine and systemic circulation. Some of these biologically active factors and vascular mediators have been proposed as biomarkers for early prediction or diagnosis of PE, and as potential targets for prevention or treatment of the disease.

Keywords

pregnancy; preeclampsia; blood pressure; endothelium; vascular smooth muscle

INTRODUCTION

Normal pregnancy (Norm-Preg) is associated with increased heart rate, plasma volume and cardiac output, as well as mid-gestational decrease in vascular resistance and blood pressure (BP). These hemodynamic changes ensure sufficient blood and nutrient supply to the growing fetus. In 5–10% of pregnancies women develop HTN-Preg in one of 4 forms: chronic HTN

that predates pregnancy, preeclampsia (PE)-eclampsia, chronic HTN with superimposed PE, and nonproteinuric gestational HTN [1]. PE is a multisystem disorder of unknown cause that is unique to human pregnancy and with potentially serious consequences to the mother and fetus. PE occurs in 3–8% of pregnancies [2,3] and accounts for 60,000 maternal deaths annually worldwide [4]. PE is clinically manifested as elevation of BP, proteinuria, and occasionally edema and enhanced platelet aggregation. PE may also feature hemolysis, low platelets count and elevated liver enzymes as part of HELLP syndrome. PE may also cause intrauterine fetal growth retardation (IUGR) and reduced birth weight. If untreated, PE can lead to eclampsia with life-threatening neurovascular complications including convulsions and severe HTN [5].

Several risk factors have been implicated in PE including genetic polymorphism, environmental and dietary factors, co-morbidities such as obesity and diabetes, and immunologic factors and immune maladaptation. These factors could cause abnormal placental development, induce an imbalance in circulating angiogenic and anti-angiogenic factors and lead to generalized endotheliosis [6]. Endotheliosis in systemic blood vessels may cause the increase in vascular resistance and HTN. Glomerular endotheliosis is likely the cause of proteinuria. Cerebrovascular endotheliosis could affect the blood-brain barrier and lead to severe headache, visual disturbance and seizures (Fig. 1). Endotheliosis in the hepatic vessels could lead to changes in liver enzymes and other manifestations of HELLP syndrome. Because of the difficulty to perform mechanistic studies in pregnant women, animal models of HTN-Preg with characteristics similar to those observed in human PE have been developed. Animal models of HTN-Preg have demonstrated significant changes in the renal control mechanisms of BP, and highlighted glomerular injury and altered kidney function as possible causes of the increased BP [7]. Studies have also highlighted prominent changes in the vasculature of animal models of HTN-Preg [5,8].

Currently, the only definitive treatment for PE is delivery of the infant and the placenta, accounting for 15% of preterm births in the United States [2]. Prevention and specific treatment of PE are hindered by the fact that the etiology remains unclear [9]. In this review, we will present current knowledge of the genetic, environmental, and immunologic factors implicated in PE. We will discuss how impaired placentation could cause an imbalance in angiogenic and anti-angiogenic factors and result in the generalized endotheliosis and the changes in the vascular endothelial cells (ECs), vascular smooth muscle (VSM) and extracellular matrix (ECM). We will also discuss potential biomarkers for early prediction and diagnosis, and explore future directions for prevention and treatment of PE.

Genes and Genetic Imprinting in PE

According to the genetic-conflict theory, fetal genes are selected to increase the transfer of nutrients to the fetus, and maternal genes are selected to restrict transfer that exceeds maternal optimum [10]. With genetic imprinting, a similar conflict occurs within fetal cells between maternally- and paternally-derived genes. The conflict theory predicts that placental factors and fetal genes act to raise maternal BP, whereas maternal factors act to reduce BP. Thus, the EC dysfunction associated with PE may represent a fetal-rescue strategy to enhance non-placental resistance when the uteroplacental blood supply is inadequate [11]. Several genes have been implicated in PE including maternal, fetal and paternal genes.

Maternal Genes

Female offspring of a pregnancy complicated by PE may have a higher risk of developing PE in their own pregnancies, possibly due to inheritance of PE susceptibility genes [12]. Studies have estimated a 31% heritability for PE and 20% for gestational HTN [13]. A population-based Swedish cohort study has suggested that genetic factors may account for >50% of an individual's susceptibility to PE [14]. Genome-wide linkage studies have identified more than

one gene locus on chromosomes 1, 2p13, 2q, 3p, 4q, 9, 10q, 11q23–24, 12q, 15q, 18 and 22q, suggesting that PE is a multigene disorder [15] (Table 1). Also, regions on chromosomes 2p25 and 9p13 may harbor susceptibility genes for PE [16]. A recent study compared the global gene expression in the first trimester placentas from PE and Norm-Preg women and identified 36 major genes in PE placentas, 31 of which were downregulated [17]. Some of the genes implicated in PE include those of VEGF, hypoxia-inducible transcription factor (HIF), Fas, and leptin.

Subjects carrying the T allele VEGF 936C/T genotype have lower VEGF plasma levels than subjects carrying the VEGF 936C/C genotype, and changes in VEGF gene and plasma levels could play a role in the vascular endotheliosis observed in PE [18].

HIF-1 α and -2 α proteins and regulated genes are increased in PE placenta. Upregulation of placental HIF-1 α may induce the expression of the various factors that are secreted into the maternal circulation e.g. soluble fms-like tyrosine kinase 1 (sFLT-1) and soluble endoglin (sEng), which lead to disruption of the endothelium and other manifestations of PE. Accumulation of HIF-1 α proteins in PE placenta may be due to both increased formation secondary to ischemia/hypoxia and reduced degradation after reperfusion/oxygenation as a result of proteasomal dysfunction [19].

The Fas ligand-Fas is a known pathway of apoptosis. The binding of Fas ligand to Fas triggers the activation of caspases 3, 7 and 9 and leads to apoptotic event. Polymorphisms of Fas genes and Fas ligand genes may play a role in PE. In Norm-Preg, activated T lymphocytes, which recognize paternal antigens, express Fas and interact with the cytotrophoblasts expressing the Fas ligand, leading to apoptosis of T cells and reduction in their ability to recognize and destroy the cytotrophoblasts invading the myometrium and spiral arteries. In PE, the expression of Fas 670 G variant is associated with reduced Fas production in activated T lymphocytes, leading to reduced T cell apoptosis, increased destruction of cytotrophoblasts, and inadequate invasion of spiral arteries [20].

An increase in circulating levels of leptin and *leptin* gene polymorphism may be associated with PE [21]. Recent data have demonstrated increased leptin expression in the basal plate of PE placenta compared with Norm-Preg control tissue. Also, genes encoding sialic acid binding Ig-like lectin 6 (Siglec-6), a potential leptin receptor, and pappalysin-2 (PAPP-A2), a protease that cleaves insulin-like growth factor (IGF) binding proteins, are over-expressed in PE [2], supporting a link between increased leptin expression and PE.

Fetal Genes

Another factor in the development of PE is fetal genes. PE is more common in women homozygous for the inhibitory killer immunoglobulin-like receptor (KIR) haplotypes (AA) than in women homozygous for the stimulator KIR haplotypes (BB), and the effect is strongest if the fetus is homozygous for the human leukocyte antigen class II (HLA-C2) haplotype [29]. Also, gene-to-gene interaction between fetal HLA-G and maternal KIR2DL4 is associated with PE risk in multigravid pregnancy [30]. In mice, mutations in the cyclin-dependent kinase inhibitor *cdkn1c* (*p57^{Kip2}*), a regulator of embryonic growth, induces changes in the placenta architecture indicative of the RUPP found in HTN-Preg. Also, pregnant mice expressing wild-type levels of *p57^{Kip2}* develop a PE-like condition when carrying *p57^{Kip2}*-deficient pups, supporting a role for fetal genes in the development of PE [15].

Paternal Genes

Paternal genes could play a role in the development of PE. Studies compared men whose mothers had PE to men whose mothers did not have PE and found that a paternal family history

of PE was associated with more than 2-fold increase in risk of PE [31,32]. PE is also common in hydatid-form mole-pregnancies in which all the fetal chromosomes originate from the father [33].

Ethnic Background and PE

Maternal ethnicity may be a factor in the development of PE. African-American women have the highest incidence rate of PE (5.2%), while Asian women have the lowest rate (3.5%) [34]. Paternal ethnicity may also play a role in the incidence of PE. Among African-American women the incidence of PE is greater if paternal ethnicity is different from maternal ethnicity (5.8%) than if the paternal and maternal ethnicities are the same (5.2%). Among Asian women the incidence of PE is greater if the paternal ethnicity is different (4.6%) than if it is the same as maternal ethnicity (3.2%). In contrast, among Native-American women the incidence of PE is greater if the paternal ethnicity is the same (9.7%) than if it is different from maternal ethnicity (3.3%) [35].

Environmental and Dietary Risk Factors of PE

Environmental factors may contribute to the development of PE. The high incidence of PE in under-developed countries suggests that inadequate diet may be a risk factor. Deficiencies in dietary calcium, magnesium, potassium, and vitamins may be associated with PE.

Calcium, Magnesium, Potassium

Ca^{2+} concentration in the extracellular fluid is strictly maintained at a normal serum level of 2.2–2.6 mmol/L. 40% of serum calcium is bound to albumin, 10% in complex with citrate and the rest is ionized Ca^{2+} – the most important fraction [36]. During pregnancy, Ca^{2+} is transferred to the fetus through the placenta. The higher Ca^{2+} requirement during pregnancy requires physiologic adaptation of the maternal Ca^{2+} homeostatic mechanisms including intestinal absorption, urinary excretion, and Ca^{2+} turnover in the bones. Ca^{2+} deficiency may increase the risk of PE particularly among teenagers, and Ca^{2+} -supplementation may reduce the incidence of PE in this age group. The beneficial effects of Ca^{2+} -supplementation may depend not only on the mother's age but also on the socioeconomic status, particularly in geographical locations where Ca^{2+} intake is low [36]. Some studies have suggested that Ca^{2+} -supplementation in the range of 375–2000mg/day in a population with low Ca^{2+} intake is a safe, effective and inexpensive measure to reduce the risk of HTN-Preg and PE [37]. However, in one study, although women who ingested Ca^{2+} tablets 2000 mg/day had a 66.7% lower risk of PE, among women who developed PE there was no significant reduction in the severity of the disease [38]. Also, the Ca^{2+} for PE Prevention (CPEP) clinical trial has shown no effect of 2000 mg/day Ca^{2+} -supplementation on BP or incidence of PE [39]. Experimental studies suggest beneficial effects of dietary Ca^{2+} during pregnancy. In pregnant ewes, restricted Ca^{2+} intake is associated with decreased plasma Ca^{2+} levels and uterine blood flow, and increased BP and urinary protein; all symptoms similar to those of PE [40]. Also, low Ca^{2+} intake in pregnant rats is associated with increased pressor effects of AngII [41]. In both Norm-Preg and nonpregnant rats, a high Ca^{2+} diet (1.7%–2.1%) is associated with reduction in BP, and attenuated VSM reactivity [42]. Thus, Ca^{2+} -supplementation may have the strongest influence on PE when dietary Ca^{2+} is low. If Ca^{2+} intake is adequate, no Ca^{2+} -supplementation may be needed [36].

Magnesium (Mg^{2+}) is an essential dietary mineral and a cofactor for many enzymes that regulate temperature and protein synthesis. Mg^{2+} also maintains the membrane potential in nerves and muscle [43]. Observational studies of medical records have suggested that Mg^{2+} -supplementation during pregnancy reduces the risk of IUGR and PE. Maternal Mg^{2+} intake is also positively associated with birth weight. Diets high in potassium and fiber are also

associated with reduced risk of HTN [44]. Thus, maternal intake of recommended amounts of Ca^{2+} , Mg^{2+} , potassium and fiber may reduce the risk of PE.

Vitamins

Vitamin D (cholecalciferol) is a major cofactor in Ca^{2+} absorption and metabolism. Cholecalciferol is formed in the skin during exposure to sunlight and UV irradiation and absorbed from ingested food, then converted in the liver to 25(OH) cholecalciferol or 25(OH) D, and further hydroxylated by 1 α -hydroxylase in the kidney to 1,25 dihydroxycholecalciferol or 1,25(OH)₂D₃, the most active form of vitamin D. Adequate levels of 25(OH)D (>80 nmol/L) are needed for optimal health of the skeletal system, and may influence the cardiovascular system and BP. 1,25(OH)₂D₃ levels are elevated during pregnancy due to increased activity of renal 1 α -hydroxylase and placental production. Also, vitamin D receptors are found in the placenta [36]. Vitamin D deficiency and altered Ca^{2+} metabolism have been implicated in abnormal placentation and PE [45]. Circulating 1,25-(OH)₂D₃ levels in both maternal and umbilical cord compartments are lower in PE compared with Norm-Preg [46]. Vitamin D receptor null mice show an increase in renin expression, increased plasma AngII, and HTN. Also, in wild-type mice, inhibition of 1,25(OH)₂D₃ synthesis leads to increased renin expression, and 1,25(OH)₂D₃ injection causes renin suppression, supporting a role of 1,25 (OH)₂D₃ in the regulation of the renin-angiotensin system (RAS) and BP [47].

Vitamins may influence the oxidants and antioxidants balance and the risk of PE. Serum levels of vitamins E and C are decreased in women with PE, and vitamin supplementation may decrease the incidence of PE in women at high risk [48]. Folic acid or folate is a coenzyme in the production of nucleic acids and cell growth. Folic acid may play a role in the implantation and development of placenta and could reduce the risk of PE by improving EC function in the placenta and systemic vessels and by lowering plasma homocysteine level [49]. In a recent study, women who received multivitamins containing folic acid demonstrated increased serum folate, lower plasma homocysteine, and reduced risk of PE by 63% compared to women with no supplementation [50].

Co-morbidities and Risk of PE

Co-morbidities such as obesity and diabetes can adversely affect the outcome of pregnancy and increase the risk of PE and preterm delivery.

Obesity

The prevalence of obesity is rapidly increasing worldwide. In the United States, 28% of women aged 20–39 years are obese and 54% are either obese or overweight [51]. The risk of PE rises strikingly with the increase in pre-pregnancy body mass index (BMI). Compared with women with a BMI of 21, the risk of PE doubles at a BMI of 26, triples at a BMI of 30 and increases further with severe obesity [52]. In a study assessing the risk factors of severe PE, other than a history of PE, severe obesity (BMI >32.2) was the major maternal risk factor [53]. Obesity is associated with low-grade inflammation and increased circulating inflammatory markers [54]. Plasma levels of C-reactive protein, TNF- α , IL-6 and IL-8 are elevated in obese subjects, and body fat is a possible source of these inflammatory markers. Markers of inflammation are also increased in the vasculature of obese women and this may contribute to Vascular Targets in the vascular changes associated with PE. Obesity is also associated with increased circulating levels of leptin, and a leptin gene polymorphism has been linked to increased risk of developing PE [21].

Adiponectin, a hormone produced abundantly by adipose tissue, plays a role in obesity-related inflammation. In women with normal body weight (BMI <25 kg/m²), an increase in adiponectin

levels may suppress the expression of adhesion molecules in vascular ECs and cytokine production from macrophages in order to minimize the inflammatory process associated with PE. However, in obese women ($\text{BMI} \geq 25 \text{ kg/m}^2$) with PE, this inflammation-minimizing mechanism may fail and the adiponectin concentration is reduced [55].

Ethnic differences in obesity may influence the incidence of PE. African-American women are more likely to be overweight prior to pregnancy and to gain the required weight during pregnancy, while Asian women gain less than the required weight [56]. Thus, ethnicities most likely to gain weight during pregnancy correlate with those most at risk of developing PE.

Diabetes

During Norm-Preg, women experience brief insulin resistance and glycemia after a meal. Increased sugar consumption in pregnant women may lead to hyperglycemia, which in turn inhibits endothelium-dependent vasorelaxation. The risk of PE is increased in women with family history of type 2 diabetes or pre-pregnancy hyperinsulinemia and insulin resistance [57]. Also, the prevalence of metabolic syndrome (characterized by visceral obesity, insulin resistance, hyperglycemia, type 2 diabetes, dyslipidemia, and high BP) is 3-fold higher in PE women than in women with gestational diabetes [58]. Women with metabolic syndrome have endothelial dysfunction likely because their blood vessels are more sensitive to the circulating factors released during PE. The rate of PE is greater in women with pre-pregnancy diabetes (~12%) than in women without diabetes (~3%). Also, the incidence rate of PE with abruption or infarction of placenta in women with pre-pregnancy diabetes (2.2%) is greater than that in women without diabetes (1.8%) [59]. Gestational diabetes is also associated with high BMI and increased risk of PE [60].

Immune Theory of PE

Altered immune response may play a role in the development of PE. Epidemiological studies have implicated maternal immune maladaptation to fetal or paternal factors in PE [61]. Nulliparous women have a higher risk of PE than multiparous women, and the protective effect of multiparity is lost with a change of partner. Among nulliparous women, a previous spontaneous or induced abortion has a protective effect against PE, but nulliparous women who had an abortion with a different partner are at the same risk as primigravidas. These observations suggest that paternal antigen-specific tolerance develop during the first pregnancy, and that memory T cells, which induce paternal antigen-specific tolerance, quickly increase in the next pregnancy and lower the risk of maladaptation during pregnancy [62]. This is supported by report that maternal T cells acquire a transient state of tolerance to specific paternal alloantigens during pregnancy in mice [63].

A second piece of evidence of altered immune response is that prolonged exposure of the female reproductive system to seminal fluid may decrease the risk of PE. The seminal fluid contains soluble MHC-class I antigens which are taken up by vaginal or uterine antigen presenting cells (APC), and lead to specific tolerance to paternal MHC class I [64]. Soluble MHC class I antigens may also induce apoptosis of T cells and reduce alloreactive maternal T cells. Seminal fluid also contains abundant amounts of transforming growth factor (TGF)- β , which initiates inflammatory response and increases pro-inflammatory cytokines and chemokines in uterine tissues, leading to deviation of the immune activity of T-cells towards the inflammatory reaction and thereby decreased activity against paternal sperm alloantigens [62]. This is supported by experiments in rodents demonstrating that exposure of the female reproductive tract to seminal TGF- β initiates an influx of APC that sample ejaculate antigens and subsequently activate the lymphocytes in lymph nodes draining the uterus and as a consequence induce systemic tolerance to paternal MHC class I antigens [65]. Thus prolonged exposure to soluble MHC-class I antigens may be important for the induction of tolerance, while limited

exposure or low levels of soluble MHC-class I antigens in sperms may be a contributing factor in PE [64].

Natural killer (NK) cells may play a role in induction of MHC-class I tolerance. Uterine NK cells produce angiogenic factors such as vascular endothelial growth factor (VEGF), placenta growth factor (PlGF) and TGF- β [66]. During Norm-Preg, activation of decidual uterine NK cells by paternal MHC class I antigens causes an increase in VEGF which reacts with Flt-1 receptors on human intermediate and extravillous trophoblasts (EVT) and thereby causes efficient EVT invasion. The failure of interplay between the immunologically driven expression of growth factors by decidual uterine NK cells and trophoblast receptors may be responsible for the decreased EVT invasion in PE [67].

During Norm-Preg, T cells which react with autoantigens are removed by Fas ligand/Fas-mediated apoptosis, a process known as 'clonal deletion', leading to tolerance. HLA-G and the Fas ligand expressed on trophoblasts may play a role in clonal deletion [62]. Soluble HLA-G1 is produced by syncytiotrophoblasts and probably EVT, and may exert immunotolerance to fetal or paternal antigens by inducing apoptosis of activated maternal CD8⁺ T cells via the Fas ligand/Fas system [68]. The lack of, or decreased soluble HLA-G1 expression in trophoblast may cause inadequate tolerance at the feto-maternal interface resulting in PE [62].

There may be a relation between inflammation or infection and PE. Anti-cytomegalovirus (CMV) and anti-Chlamydia pneumonia antibodies are higher in early than late onset PE or Norm-Preg [69]. Also, women sero-negative for antibodies against viral agents such as CMV, herpes-simplex virus type2, and Epstein-Barr virus are at higher risk of PE than sero-positive women because sero-negative women are more susceptible to primary infection during pregnancy [70].

Cytokine-secreting cells could also be involved in the immunologic response associated with PE. The CD4-positive T helper cells are classified into Th1 and Th2 cells. Th1 cells produce IL-2, IFN γ - and TGF- β , which acts on effector cells to enhance cell-mediated immunity, delayed type hypersensitivity and rejection response. Th2 cells secrete IL-4, IL-5, IL-6 and IL-13, and are involved in antibody production and suppression of cell-mediated immunity. In Norm-preg, the predominance of Th2 over Th1 cells causes downregulation of the allogeneic immune responses to the fetus and reduces inflammation. In PE, the balance between Th1 and Th2 cells may shift toward Th1 cells [62]. Also, the serum level of soluble CD30, a member of the TNF receptor superfamily expressed by Th2 cells, is lower in PE than Norm-Preg women, suggesting that PE is associated with a polarized Th1 immune response due to a decrease in Th2 response [71].

Abnormal Placentation as an Initiating Pathology of PE

Although the pathophysiology of PE remains undefined, placental ischemia/hypoxia is considered an important factor. The vascular development of the placenta involves first, vasculogenesis and formation of a primitive vascular network from endothelial progenitor cells; second, branching angiogenesis; and third, non-branching angiogenesis [72]. During early pregnancy, cytotrophoblasts invade the uterine spiral arteries, replacing the endothelial layer and destroying the medial elastic, muscular, and neural tissue. Cytotrophoblasts initially express adhesion molecules characteristic of epithelial cells such as integrins $\alpha 6/\beta 1$, $\alpha \omega/\beta 5$. As cytotrophoblasts take the invasive pathway, epithelial cell-like adhesion molecules cease to be expressed and expression of EC adhesion markers such as integrins $\alpha 1/\beta 1$, $\alpha v/\beta 3$ and E-cadherin is observed and referred to as vascular mimicry or pseduovasculogenesis [73]. By the end of the second trimester, the uterine spiral arteries are lined exclusively by cytotrophoblast, and ECs are no longer present in the endometrial or superficial myometrial regions. The

remodeling of the spiral arteries results in a low resistance arteriolar system with a dramatic increase in blood supply to the growing fetus. In PE, abnormal expression of epithelial cell-like adhesion molecules and apoptosis of cytotrophoblasts lead to limited invasion of the spiral arteries to only the superficial layers of the decidua, with 30% to 50% of the spiral arteries escaping endovascular trophoblast remodeling [73,74]. The failure of trophoblast invasion results in RUPP and ischemic placenta, leading to PE and fetal IUGR [75]. The ischemic placenta may induce the release of bioactive circulating factors that cause the EC dysfunction observed in PE. Animal models of RUPP in late pregnant sheep, dogs, rabbits and rats exhibit a HTN state that resembles PE (Table 2). Studies in the RUPP model have suggested that the ischemic placenta contributes to maternal EC dysfunction by altering the circulating levels of angiogenic and antiangiogenic factors such as soluble fms-like tyrosine kinase-1 (sFlt-1) and soluble endoglin (sEng), and other vasoactive factors such as cytokines and AngII type 1 receptor autoantibody (AT1-AA) [76,77].

Biologically Active Factors Involved in PE

Placental ischemia/hypoxia is thought to induce the release of various bioactive factors. Increased plasma and vascular tissue levels of these bioactive factors could cause EC and VSM dysfunction, severe vasoconstriction and PE in humans (Table 3) and HTN-Preg in rats (Table 4). The EC dysfunction in PE may result from low circulating levels of the proangiogenic factors VEGF and PlGF and high circulating levels of the anti-angiogenic factors sFlt1 and sEng [6]. Other biologically active factors in PE include cytokines, oxidative stress, HIFs, matrix metalloproteinases (MMPs), sex hormone metabolites and antibodies to vascular AT₁R (Fig. 2).

Vascular Endothelial Growth Factor (VEGF)

VEGF is a major angiogenic factor and a prime regulator of EC proliferation, vasculogenesis and vascular permeability [103]. VEGF may also have vasodilator effects by stimulating the NO-cGMP vascular relaxation pathway. VEGF increases EC intracellular free calcium concentration ($[Ca^{2+}]_i$), which promotes calmodulin binding to eNOS and thereby stimulates NO production [104]. The gene encoding VEGF is located on chromosome 6 band p21 and comprises a 14 kb coding region with 8 exons and 7 introns [105]. VEGF binds to Flt-1 (VEGFR1) and flk1 (KDR VEGFR2), both are tyrosine kinase receptors present on EC membrane [103]. VEGF (or VEGF-A) belongs to a gene family that includes placental growth factor (PLGF), VEGF-B, VEGF-C and VEGF-D. While the circulating total VEGF levels are elevated in PE, the free (unbound) VEGF concentration is reduced possibly due to the elevated levels of sFlt1 receptor, which captures free VEGF [95,106]. Patients receiving VEGF antagonists such as bevacizumab for renal cell carcinoma develop HTN, proteinuria and endothelial activation resembling PE [107]. Also, mice lacking one VEGF allele in renal podocytes develop the typical renal pathology found in PE [107]. In non-pregnant mice, infusion of antibodies against VEGF to cause a 50% reduction of VEGF leads to glomerular endotheliosis and proteinuria similar to what is seen in PE [107,108]. Also, *in vitro* angiogenesis studies have shown that exogenous VEGF or an antibody against sFlt1 can reverse the anti-angiogenic effects of PE plasma [109]. These data support the contention that the VEGF levels and signaling pathways may be altered during PE.

Soluble fms-like tyrosine Kinase-1 (sFlt-1)

sFlt-1 is a VEGF and PlGF antagonist protein that binds VEGF and prevents its interaction with its endogenous receptors. sFlt1, which lacks the transmembrane and cytoplasmic domains, is made in large amounts by the PE placenta and is released into the maternal circulation [110]. Circulating sFlt1 levels are increased in women with established PE and may begin to rise before the onset of clinical symptoms [76]. Consistent with the antagonistic effect of sFlt1,

free (or unbound) VEGF and PlGF concentrations are decreased in PE women at disease presentation and even before the onset of clinical symptoms [76,95]. Increases in maternal sFlt1 are observed in several conditions that are considered risk factors for PE. For example, in twin pregnancy, which is associated with a 2 to 3-fold increase in the incidence of PE [111], circulating sFlt1 levels and sFlt1/PlGF ratios are twice as high as those in singleton pregnancy [112]. Also, the Flt-1/sFlt1 gene is located on chromosome 13q12, and in trisomy 13 an extra copy of this gene may lead to excess circulating sFlt1, reduced free PlGF level, and increased sFlt1/PlGF ratio and thereby contribute to the increased risk of PE [113]. In support of a role of sFlt-1 in PE, exogenous sFlt-1 given to pregnant rats results in HTN, proteinuria and glomerular endotheliosis, akin to the PE phenotype observed in human [95]. Also, in tissue culture, exposure of endothelial cells to conditioned medium containing PE plasma results in reduced angiogenesis, while removal of sFlt-1 by immunoprecipitation and adding the supernatant restores EC function and angiogenesis to normal levels [109].

Endoglin

Endoglin (Eng) or CD105, a co-receptor for TGF- β 1 and TGF- β 3, is highly expressed on cell membranes of vascular ECs and syncytiotrophoblasts [114]. Placental endoglin is upregulated in PE, releasing soluble endoglin (sEng) into the maternal circulation [115]. sEng is an anti-angiogenic protein that inhibits TGF- β 1 signaling in the vasculature [115]. Circulating sEng levels increase beginning 2 to 3 months before the onset of PE and more steeply in women who develop PE, and peak at the onset of clinical disease [76]. sEng may act in concert with sFlt-1 to cause EC dysfunction, resulting in severe PE and/or HELLP syndrome.

sEng disrupts formation of endothelial tubes in human umbilical vein endothelial cells (HUVECs) in culture and induces vascular permeability and HTN in pregnant rat *in vivo* [115]. Mutations in endoglin result in loss of capillaries, arteriovenous malformations, and hereditary hemorrhagic telangiectasia [6]. Pregnant rats with overexpression of both sEng and sFlt-1 demonstrate proteinuria, severe HTN, HELLP syndrome, and IUGR. Histological analysis of tissues from these pregnant rats reveals severe glomerular endotheliosis, infarction in the placenta, necrosis in the liver, and fragmented red blood cells (schistocytes) [115]. Also, placental ischemia in the RUPP rat model is associated with increased expression of sEng and HIF- α and shifts the balance of angiogenic factors in the maternal circulation toward an angiostatic state [77].

Placental Growth Factor (PlGF)

PlGF is a pro-angiogenic factor of the VEGF family that may play a role in EC growth and the control of vasculogenesis, angiogenesis, and placental development during pregnancy [85, 95,109]. During Norm-Preg, circulating PlGF levels are more than 40 times greater than VEGF, but PlGF has only 1/10th the affinity for the Flt-1 receptor compared to VEGF. During PE, circulating PlGF levels decrease while the levels of its antagonist protein sFlt-1 increase [116]. The sFlt-1/PlGF concentration ratio is much higher in PE than Norm-Preg women as early as the second trimester, and therefore can be used as an early indicator of the onset of PE [117]. However, the use of sFlt-1 and sEng as mediators or predictors of PE should be viewed with extreme caution as the levels of sFlt-1 and sEng may not be altered in some women or experimental animals with PE/HTN-Preg as compared to Norm-Preg.

Cytokines

During Norm-Preg, women may have elevated inflammatory response and increased plasma levels of plasminogen activator inhibitor-1 (PAI-1), TNF- α and C-reactive protein. In PE, the ischemic placenta may contribute to the maternal EC dysfunction by enhancing the synthesis of TNF- α , IL-6, and IL-8. TNF- α enhances the release of ET-1 from ECs and reduces acetylcholine-induced vasodilatation [118]. TNF- α induces oxidative damage as it destabilizes

electron flow in mitochondria, resulting in the release of oxidizing free radicals and formation of lipid peroxides that damage ECs [118]. The plasma level of TNF- α and IL-6 increases 2- to 3-fold in women with PE compared with third-trimester Norm-Preg and women with gestational HTN [118]. In contrast, IL-10, which may be involved in the maintenance of pregnancy by inducing corpus luteum maturation and progesterone production, is decreased in PE women [119]. RUPP during pregnancy and the ensuing placental ischemia are thought to increase the release of TNF- α and IL-6 into the maternal circulation, leading to generalized EC dysfunction and HTN. Also, chronic infusion of TNF- α or IL-6 in late pregnant rats causes significant elevation of BP, enhanced vascular contraction and reduced endothelium-dependent vascular relaxation possibly due to inhibition of the endothelial NO-cGMP pathway [80,120].

Reactive Oxygen Species (ROS)

Pregnancy is a state of oxidative stress characterized by placental production of ROS such as superoxide ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2). In Norm-Preg, the production of ROS is counterbalanced by abundant antioxidant defenses. However, in PE excessive ROS production overpowers antioxidant defenses, leading to increased oxidative stress and ROS including hydroxyl radicals ($\cdot OH$) and peroxynitrite ($ONOO^-$) [121]. Increased levels of thromboxane and lipid peroxides and loss of glutathione peroxidase (GPx) activity have been demonstrated in placentas from PE compared with Norm-Preg women [122]. Also, mRNA levels of ROS modulators such as hemoxygenase (HO)-1, (HO)-2, copper/zinc superoxide dismutase (SOD), GPx and catalase are decreased in blood cells of PE compared to Norm-Preg women and the reduction in the amount of HO-1 and HO-2 correlates with the severity of PE [123].

Neutrophils represent an important source of ROS and EC damage in PE. Stimulated neutrophils from women with PE produce more $O_2^{\bullet-}$ compared to those from Norm-Preg women. Neutrophils also produce NO, which can protect cells from $O_2^{\bullet-}$ -induced damage in Norm-Preg. However, in PE, $O_2^{\bullet-}$ production may dominate the neutrophil and EC NO production, resulting in EC injury [88].

ROS may be involved in the severe endoplasmic reticulum stress initiated by deficient remodeling of the uterine arteries during PE [124]. The decreased formation of spiral arteries causes them to spontaneously constrict, resulting in ischemia-reperfusion injury and increased in ROS, which decreases the amount of stored Ca^{2+} and intracellular ATP and thereby produces misfolded proteins. These misfolded proteins create endoplasmic reticulum stress, which the cell attempts to fix via the unfolded protein response (UPR). During low-grade endoplasmic reticulum stress, the UPR promotes phosphorylation of eukaryotic initiation factor 2, which prevents the binding of the initiator to the ribosome and reduces cell proliferation, and thereby maintains cell survival. However, if the endoplasmic reticulum stress is severe enough, the prolonged exposure to UPR during pregnancy makes the cells undergo apoptosis. As a result, syncytiotrophoblast microvillous membranes (STBM) particles enter the maternal circulation, impair vascular EC function, and lead to PE and IUGR [124].

$O_2^{\bullet-}$ and peroxynitrite ($ONOO^-$) may promote the vascular dysfunction in PE through stimulation of MMP-1, -2, and -9 [125] and degradation of HIF-1 α in VSM [126]. ROS increase VSM $[Ca^{2+}]_i$ by stimulating the influx of extracellular Ca^{2+} , inhibiting Ca^{2+} -ATPase activity, and promoting inositol trisphosphate (IP_3)-induced Ca^{2+} release from the intracellular stores. ROS also activate protein kinase C (PKC) and promote vasoconstriction by activating Rho-A/Rho-kinase signaling mechanisms [127].

Hypoxia Inducible Factors (HIF)

HIF-1 is a transcriptional activator that plays a role in the physiologic responses to hypoxia, and the pathophysiology of ischemic cardiovascular disease, PE and IUGR. HIF-1 is a

heterodimer consisting of an oxygen-regulated HIF1- α and HIF2- α subunits and a constitutively expressed HIF1- β subunit [128]. To date, more than 100 HIF-1 downstream genes with varying functions have been identified including VEGF, leptin, TGF- β 3, and NOS. Also, DNA microarrays have shown that more than 2% of all human genes are regulated by HIF-1 in arterial ECs, directly or indirectly [129]. HIF-1 activates the expression of these genes by binding to a 50-base pair *cis*-acting hypoxia response element located in their enhancer and promoter regions. Under normoxic conditions, the α -subunit of HIF-1 is rapidly degraded by ubiquitination and proteosomal degradation [130]. Because placental hypoxia may play a role in PE, the role of HIFs has been investigated. The expression of HIF-1 α and HIF-2 α , but not HIF-1 β , is selectively increased in PE placenta. Accumulation of HIF-1 α protein occurs as a result of both increased formation secondary to relative ischemia/hypoxia and reduced degradation after reperfusion/oxygenation due to proteosomal dysfunction [19]. The molecular mechanisms underlying the changes in HIF expression and the genes affected during PE are unclear. However, upregulation of HIF-1 has been shown to enhance the expression of VEGF [126]. Also, a HIF-1 α binding site exists in the promoter region of the ET-1 gene and may act to regulate ET-1 expression and consequently vasoconstriction [131].

2-Methoxy estradiol (2-ME) Deficiency

2-ME, a natural metabolite of E2, is elevated during the third trimester of Norm-Preg. Plasma levels of 2-ME are markedly lower in women with severe PE. Pregnant mice deficient in catechol-O-methyltransferase (COMT) demonstrate a PE-like phenotype resulting from the absence of 2-ME. 2-ME ameliorates PE-like features in the COMT-deficient pregnant mice and suppresses placental hypoxia, HIF-1 α expression and sFLT-1 elevation. Measurement of 2-ME in plasma and urine may be used as a diagnostic marker for PE, and 2-ME may prevent or treat PE [83].

Generalized Endotheliosis in PE

One of the major targets of the circulating vasoactive factors in PE is ECs. PE and HELLP syndrome may be associated with generalized endotheliosis and abnormal proliferation of ECs. Women with PE may develop glomerular endotheliosis, swelling of glomerular ECs and display fibrin deposit within EC and mesangial cells that could cause renal injury [132]. Microalbuminuria is an important manifestation of renal endothelial dysfunction, and two third of women with PE may show microalbuminuria 2 to 4 month after delivery [133]. In PE, the decrease in VEGF levels may be partly responsible for glomerular endotheliosis. VEGF is produced in the glomerulus by podocytes and is essential for the preservation of general endothelial wellness. VEGF also plays a role in the formation of fenestrae, the small pores in ECs that allow the transfer of molecules from the glomerular capillaries into the urinary space. In women with PE, the excessive production of sFlt-1 inhibits VEGF signaling, and the lack of fenestrae causes reduction in glomerular filtration rate (GFR) and eventually leads to proteinuria [134]. Also, vascular tone is regulated by both vasodilator and vasoconstrictor mediators, and generalized endotheliosis in PE could cause an imbalance in vascular mediators and lead to increased systemic vascular resistance and HTN. Endotheliosis could also affect the blood vessels of the brain leading to seizures, or those of the liver leading to HELLP syndrome.

Decreased Endothelium Derived Vasodilators in PE

Nitric Oxide (NO)

NO is a potent vasodilator and relaxant of VSM. NO is produced from the transformation of L-arginine to L-citrulline by neuronal nNOS, inducible iNOS, and endothelial eNOS. NOS expression and activity are increased in human uterine artery and NO production is enhanced

during Norm-Preg [135,136]. Also, the plasma concentration and urinary excretion of cGMP, a second messenger of NO and cellular mediator of VSM relaxation, are increased in Norm-Preg [137]. Because NO is an important vasodilator in Norm-Preg, NO deficiency during PE might be involved in the disease process.

Measurements of total NO production during HTN-Preg have not produced consistent findings [137]. Some studies have shown that the concentration of nitrate/nitrite, the stable end product of NO, is reduced in the sera of women with PE [138], while other studies have shown increased levels [139]. The difference in the nitrate/nitrite levels during PE could be related to dietary nitrate intake. While measurements of total nitrate/nitrite did not produce consistent findings, vascular endothelial NO production may be reduced during PE [140]. This is supported by report that NOS blockade with N^ω-nitro-L-arginine methyl ester (L-NAME) during mid- to late gestation in rats produces pathological changes similar to those observed in women with PE including increased BP, renal vasoconstriction, proteinuria, thrombocytopenia and IUGR [141]. Also, chronic RUPP in pregnant rats causes significant increase in BP, proteinuria, decreased GFR and renal plasma flow (RPF), and IUGR. Although the RUPP rat does not demonstrate significant reduction in whole-body NO synthesis as assessed by urinary excretion of nitrate/nitrite, significant reduction in the renal expression of nNOS may contribute to the changes in renal hemodynamics and the HTN in this experimental model of PE [98].

Prostacyclin (PGI₂)

PGI₂ is an anti-platelet aggregator and a vasodilator produced from the metabolism of arachidonic acid by the cyclooxygenase (COX)-1 and COX-2. Endothelium-derived PGI₂ may contribute to the hemodynamic and vascular changes during pregnancy. During Norm-Preg, the plasma and urinary levels of 6-keto-prostaglandin F1 α (PGF1 α), a hydration product of PGI₂, are increased [142]. Hypoxia causes downregulation of COX-1 and may alter PGI₂ production in PE [143]. Plasma and urinary concentrations of PGF1 α are lower during severe PE than in Norm-Preg, suggesting that overall PGI₂ synthesis is diminished. Endothelial PGI₂ production may also decrease in PE [144]. A recent study has shown that the release of PGI₂ is not different in apical and basal trophoblasts of PE compared with Norm-Preg women. On the other hand, the release of thromboxane A₂ (TXA₂) from basal trophoblast cells was increased in PE women, suggesting that it may contribute to the increased placental vasoconstriction in PE [145].

Endothelium-Derived Hyperpolarizing Factor (EDHF)

EDHF is an important relaxing factor particularly in the small resistance vessels. Also, while NO and PGI₂ are the dominant vasodilators in male mice, EDHF may be the predominant relaxing factor in female mice [146]. Studies in subcutaneous arteries from PE women demonstrated that vasodilatation of EDHF are mediated either by myoendothelial gap junctions alone or in combination with H₂O₂ or CYP450 epoxygenase metabolites of arachidonic acid [147]. Other studies on the uterine vessels of pregnant rats have suggested that EDHF release is activated by a delayed rectifier type of voltage-sensitive K⁺ channel [148]. Although NO, PGI₂ and EDHF have different vasodilator mechanisms, they may affect each other. For example, PGI₂ may upregulate eNOS and NO production in ECs, and activate the inward rectifier K⁺ channels through the release of EDHF [149,150].

Increased Endothelium-derived Vasoconstriction in PE

Endothelin-1 (ET-1)

ET-1 is produced by ECs and acts on VSM to cause vasoconstriction. Hypoxia induces the synthesis and secretion of ET-1 from ECs [131]. The plasma concentration of ET-1 could reach $\approx$ 2- to 3-fold higher in PE than Norm-Preg women [140]. Also, ET-1 secretion is 4- to 8-fold

higher in umbilical cord ECs of PE than Norm-Preg women [151]. The elevated plasma levels of ET-1 in PE women return to normal levels within 48 h of delivery, suggesting that ET-1 may contribute to the vasoconstriction and HTN in PE [152]. Typically, plasma levels of ET-1 are highest during the later stage of the disease, suggesting that ET-1 may not be involved in the initiation of PE, but rather in the progression of the disease into a malignant phase [93].

Although the circulating levels of ET-1 may not dramatically increase during PE, the role of ET-1 as a paracrine or autocrine agent in the vasculature should be considered. ET-1 interacts with ETAR and ETB₂R in VSM and leads to vascular contraction. ET-1 stimulates Ca²⁺ release from the intracellular stores, Ca²⁺ influx through voltage-dependent Ca²⁺ channels, and PKC-mediated Ca²⁺-sensitization pathways of VSM contraction [153]. ET-1 also activates ETB₁R in ECs and causes the release of vasodilator substances such as NO, PGI₂ and EDHF [154]. ET-1, *via* activation of ETB₁R, may mediate the reduced myogenic reactivity and vasodilation of renal arteries as well as hyperfiltration during pregnancy in rats [155]. Downregulation of ETBR may predispose women to PE by impairing trophoblast invasion. Also, excessive expression of ET-1 or ETAR or ETB₂R on VSM of renal arterioles may overwhelm the vasodilatory pathway initiated by the pregnancy hormone relaxin [156]. Studies have shown that increasing AT1-AA in pregnant rats to the levels observed in PE women increases BP, and that the HTN is attenuated by oral administration of the AT₁R antagonist losartan or an ETAR antagonist, supporting a role of ET-1 in PE [101].

Angiotensin II (AngII)

AngII is an important circulating hormone for BP regulation and electrolyte homeostasis. Also, ~40% of AngII is produced locally in the placenta by chymase, a chymotrypsin-like serine protease, which is non-angiotensin converting enzyme and produced mainly from villous syncytiotrophoblast [157]. AngII, *via* endothelial AT₂R, stimulates the release of NO through activation of eNOS [158] as well as the synthesis of PGI₂ [159], which oppose AngII-induced vasoconstriction. AngII, *via* AT₁R, causes vascular remodeling by promoting vasoconstriction, vascular growth, and inflammation. AngII increases VSM [Ca²⁺]_i and activates Rho/Rho-kinase, a prominent regulator of VSM contraction, the cytoskeleton and vascular remodeling [160]. TNF- α stimulates AngII production in the female reproductive tract [161] and IL-6 upregulates the expression of AT₁R in VSM cells [162].

Norm-Preg is associated with increased plasma renin and AngII levels, but decreased sensitivity and blunted response to AngII. The reduction in AngII sensitivity during Norm-Preg could be related to the relative expression of AT₁R and AT₂R, and the absence of adaptive changes in the AngII receptor expression could contribute to PE [163]. During Norm-Preg monomeric AT₁R are inactivated by ROS leading to lower AngII sensitivity. In PE, AT₁R forms a heterodimer with the bradykinin B2 receptor and thereby shows resistance to ROS inactivation and remains active and hyper-responsive to AngII [164]. AngII peptide levels, renin, and angiotensin converting enzyme (ACE) mRNA are also higher in the uterine placental bed in PE compared with Norm-Preg controls [165].

Agonistic autoantibodies to AT₁R (AT1-AA) may provide a link between placental ischemia and HTN-Preg [166]. AT1-AA induces signaling in vascular cells and activates protein-1, calcineurin, and nuclear factor kappa B (NF κ B) [166]. Also, activation of AT₁R by AT1-AA in human trophoblasts may increase PAI-1 production and cause shallow trophoblast invasion [167]. Administration of AT1-AA to pregnant rats increases ET-1 levels in renal cortex and placenta [101]. Also, AT1-AA can additively contribute, alongside AngII and local placental hypoxia, to the excess sFlt-1 secretion in PE [166].

Plasma hemopexin activity increases during Norm-Preg from 10 weeks onward and active hemopexin downregulates the AT₁R in human monocytes and ECs *in vitro*, and in aortic rings

of Norm-Preg rats. It has been suggested that inhibition of hemopexin activity during PE may enhance AT₁R expression and promote vasoconstriction [168].

Thromboxane A₂ (TXA₂)

TXA₂ is a potent stimulator of platelet aggregation, vasoconstriction, and VSM cell proliferation and mitogenesis [99,169]. PE is associated with deficient vascular production of PGI₂ and excessive production of TXA₂, and this imbalance might explain some of the manifestations of PE such as HTN and platelet aggregation. Measurements of urinary metabolites of TXB₂, markers of TXA₂ synthesis, and PGI₂ have shown an imbalance in their levels that predates the clinical symptoms of PE [122]. These findings led to the suggestion that antiplatelet agents such as low-dose aspirin and thromboxane modulators might be effective in preventing PE [170]. However, clinical trials on women at high risk for PE showed no benefit of low dose aspirin when used as a preventive measure [171]. Another study has shown that ozagrel, a thromboxane modulator, reduces the occurrence of PE, HTN-Preg and proteinuria [172], making it important to further examine the role of TXA₂ in PE.

Increased Vascular Smooth Muscle Mediators in HTN-Preg

VSM Ca²⁺

Ca²⁺ is a major determinant of VSM contraction and growth. VSM contraction is triggered by increases in [Ca²⁺]_i due to Ca²⁺ release from the intracellular stores and Ca²⁺ entry from the extracellular space. Ca²⁺ binds calmodulin to form a complex which activates myosin light chain (MLC) kinase, causes MLC phosphorylation, initiates actin-myosin interaction and produces VSM contraction [173]. Endothelium-derived relaxing factors act on VSM to inhibit phospholipase C, open K⁺ channels or stimulate Ca²⁺ extrusion, and thereby decrease [Ca²⁺]_i. EC dysfunction is associated with decreased release of relaxing factors, and decreased VSM Ca²⁺ extrusion mechanisms. EC dysfunction also causes an increase in VSM contracting factors, which stimulate Ca²⁺ mobilization. Studies in myometrial and subcutaneous resistance microvessels demonstrated that the reactivity to high KCl depolarizing solution, phenylephrine (PHE) and AngII is not increased in PE compared with Norm-Preg women, and suggested that this is an unlikely mechanism of the increased vascular resistance in PE [174]. On the other hand, the basal and agonist-stimulated [Ca²⁺]_i are reduced in renal arterial VSM cells from Norm-Preg compared with virgin rats, but significantly elevated in RUPP rats and pregnant rats treated with L-NAME [81,102]. AngII- and caffeine-induced transient contraction and [Ca²⁺]_i in Ca²⁺-free solution are not different in VSM cells from Norm-Preg and RUPP rats, suggesting that the pregnancy associated changes in VSM contraction may not involve the Ca²⁺ release from the intracellular stores. In contrast, the AngII- and KCl-induced maintained [Ca²⁺]_i in Ca²⁺-containing medium is greater in VSM of RUPP than Norm-Preg rats, suggesting that the Ca²⁺ entry mechanisms of VSM contraction are enhanced in animal models of HTN-Preg [102].

VSM Protein Kinase C (PKC)

Activation of PKC by phorbol esters causes sustained contraction of VSM with no change in [Ca²⁺]_i, suggesting that PKC increases the Ca²⁺ sensitivity of the contractile proteins. Vascular PKC activity does not change during early and mid-gestation, but is reduced in late pregnant compared with virgin rats, ewes and gilts [175,176]. The changes in vascular PKC activity during late pregnancy could be related to the increased NO and cGMP production. Vascular PKC activity is greater in L-NAME treated pregnant rats than virgin rats, suggesting that L-NAME treatment not only inhibits NO synthesis, but may also increase the synthesis of vasoactive compounds that increase PKC activity [175]. Increased VSM PKC expression/activity has been identified in HTN [177]. Also, the Ca²⁺ sensitivity of VSM contractile myofilaments is increased in women with PE, and PKC activation may be involved in the

vascular changes observed in PE [178]. PKC may also play a role in the changes in AngII and AT₁R-mediated signaling associated with PE. Studies on cultured neonatal rat cardiomyocytes have shown that IgG in plasma from PE women enhances AT₁R-mediated chronotropic response, and treatment of cardiomyocytes with the PKC inhibitor calphostin C prevented the stimulatory effect of PE IgG. Also, examination of VSM cells with confocal microscopy have shown colocalization of purified IgG from PE women and AT₁-AA, and suggested that PE women may develop stimulatory AT₁-AA via a PKC-mediated process [179]. The expression and activity of α - and δ -PKC are also enhanced in L-NAME treated pregnant compared with Norm-Preg rats, supporting a role of PKC in the increased vasoconstriction and vascular resistance observed in PE [175].

VSM Rho/Rho-Kinase

Rho is a family of small GTP-binding proteins that may regulate cell proliferation, migration, cytoskeletal reorganization and contraction. During cell activation by growth factors and vasoactive substances Rho-kinase is activated by GTP binding and inactivated by hydrolyzing GTP to GDP [180]. Rho-kinase may play a role in the increased Ca²⁺ sensitivity of the contractile proteins observed in subcutaneous resistance arteries from PE compared with Norm-Preg and nonpregnant women [178]. Also, stimulation of AT₁R induces upregulation of RhoA/Rho-kinase activity in hypertensive rats, and an increase in AngII activity during PE may activate Rho-kinase and promote vasoconstriction [181]. Other studies have shown that Rho-kinase mRNA expression is downregulated in umbilical arteries of PE women [182], making it important to further investigate the role of Rho-kinase in the vascular changes associated with PE.

Vascular Extracellular Matrix in PE

The extracellular matrix (ECM) provides the architectural framework of the arterial wall, and may regulate the behavior of the vascular cells, and their ability to migrate, proliferate and survive injury [183]. MMPs, a family of zinc- and Ca²⁺-dependent endopeptidases involved in remodeling and physiological homeostasis of ECM, are reliable indices of vascular ECM turnover [184]. MMPs include collagenases, gelatinases, stromelysins, and other subtypes [185,186]. MMP activity is modulated by tissue inhibitors of MMPs (TIMPs) [187]. Studies have shown that during pregnancy and chronic relaxin administration to nonpregnant rats for days, vascular MMP-2 is increased and mediates renal vasodilation, hyperfiltration, and inhibition of myogenic reactivity of small renal arteries. In contrast, vascular MMP-9 rather than MMP-2 plays a central role in the vasodilatory effect and the inhibition of myogenic reactivity in small renal arteries isolated from rats after short-term administration of relaxin for only several hours [188]. MMPs could also play a role in the vascular dysfunction in PE. MMP-2 is the main collagenolytic enzyme in umbilical cord artery (UCA) wall. MMP-2 cleaves big ET-1 into ET-1₁₋₃₂, which is a potent vasodilator. Some studies suggest that serum MMP-2 and TIMP-1 levels are increased in PE [89,189], while other studies suggest that reduced levels of MMP-2 in UCA reduces the breakdown of collagen in the arterial wall [190]. MMP-2 and -9 also decrease vascular [Ca²⁺]_i, through reduction of Ca²⁺ entry from extracellular spaces [191]. The relationship between MMP and VEGF may be bi-directional, whereby VEGF upregulates MMP expression, while membrane type-1 MMP promotes VEGF expression [192].

Predictors, Biomarkers, and Diagnosis of PE

PE has a relatively long preclinical phase before clinically manifesting in late gestation. One might argue that the ability to predict PE may be of limited benefit because neither the development of the disorder nor its progression from mild to severe disease can be prevented in most patients, and there is no cure except delivery. Nevertheless, the identification of women

at risk, early diagnosis, and prompt and appropriate management could improve the maternal and perinatal outcome. Also, accurate diagnostic tests allow the differentiation between true cases of PE and other disorders such as nephrotic syndrome in pregnancy or severe chronic HTN without proteinuria [193].

Circulating Biomarkers

Measurement of plasma levels of biomarkers such as VEGF, sFlt-1 and sEng might allow the stratification of PE patients in different categories according to the severity of symptoms and thus improve clinical management of the disease [194] (Table 5). Biomarkers also allow early disease assessment in asymptomatic pregnant women, in particular among target groups at increased risk based on their clinical history (PE or HTN in a previous pregnancy) or pre-pregnancy state (HTN, obesity, autoimmune disease).

Microarray analysis may help to screen the placental transcriptome for upregulated and downregulated genes in PE samples compared to healthy controls [195]. Studies have selected several target genes that are produced mainly by the placenta and that show increased protein concentrations in patients with PE. Based on the result, mRNA levels of plasminogen activator inhibitor-1 (SERPINE1), tissue-type plasminogen activator (PLAT), vascular endothelial growth factor (VEGFA), VEGFA receptor 1 (FLT1), endoglin, placenta-specific 1 (PLAC1) and selectin P (SELP) were assessed in the plasma of women with and without PE. These mRNA expressions were increased in plasma from pregnant women who later developed PE in comparison to Norm-Preg women. Also, analysis showed FLT1 as the marker with the highest detection rate and PLAC1 with the lowest detection rate (Table 5). The best multivariable model was obtained by the combination of all markers [196].

Despite the existence of many potential markers for PE, their reliability in predicting PE has not been consistent and the predictive value of different markers needs to be further assessed in order to identify the best marker combination for use in clinical settings.

Immune system

The status of the immune system can be useful in predicting the risk of PE in early pregnancy. The immune system fights off foreign pathogens partly through activation of the alternate complement pathway and the factor B-derived Bb fragment, a process that could also cause vascular injury and fetal demise. The maternal abnormal immune response to paternal antigens is prominent in PE. PE women show higher levels of Bb than Norm-Preg women [212]. Also, PE is more common during a first conception, after the mother has switched partners, in women who use barrier contraception, and in cases of donated gametes. Conversely, conditions associated with suppressed immune response such as HIV-related T-cell immune deficiency are associated with a low rate of PE [29].

An increase in inflammatory factors in PE may also represent an abnormal maternal immunological response, comprising a change in the role of monocytes and natural killer cells, altered release of circulating cytokines, and activation of pro-inflammatory AT₁R [29]. Neutrophils and other inflammatory cell profile could also be useful as biomarkers of PE. PE is characterized by leukocyte activation, which may account for the elevated cytokine levels found in the disease [213–216]. Therefore, it has been suggested that PE may represent an excess maternal inflammatory response to pregnancy [217]. In particular, it has been shown that maternal neutrophils are activated during their passage in the decidua of women with PE [216] and that neutrophil activation can be achieved by the conditioned medium of placental villous culture taken from PE pregnancies [218]. These findings further suggest that decidual/placental inflammatory factors are involved in the disease.

Platelet Count

The mean platelet volume (MPV) could be an important predictor of PE. Longitudinal studies have shown that women who develop PE have higher MPV 4.6 weeks prior to the appearance of symptoms when compared to Norm-Preg women [219]. Also, thrombocytopenia is a common abnormality in PE, and the degree of thrombocytopenia increases with the severity of the disease [9].

Uric Acid

Uric Acid is a marker of oxidative stress, tissue injury and renal dysfunction, and may be helpful in the prediction of PE [220]. During Norm-Preg, uric acid levels decrease initially, but then gradually increase over gestational time. Hypoxia and ischemia of the placenta and cytokines such as interferon induce the expression of xanthine oxidase and increase the production of uric acid and ROS [221]. During PE, hyperuricemia develops as early as 10 wk of gestation. Elevated levels of circulating uric acid may contribute to the pathogenesis of PE and may attenuate normal trophoblast invasion and spiral artery remodeling [222]. Elevated uric acid levels could contribute to the reduced production of NO and the altered endothelial function in PE women. Also, uric acid stimulates human monocytes to produce TNF- α , IL-6 and IL-1 β , which are elevated in PE [222]. Recent meta-analysis further supported that measurement of serum uric acid may be a useful test to predict maternal complications in management of PE [223].

Amniotic Fluid

Amniotic fluid derived by amniocentesis in the second trimester may be helpful in the prediction of PE. In a recent study, amniotic fluid samples were tested for insulin-like factor 3 (INSL3), a member of the insulin/relaxin family of peptide hormones made by the fetal testis and responsible for the first trans-abdominal phase of testicular descent. The results demonstrated that, in male fetus only, INSL3 was elevated in asymptomatic women who subsequently developed PE [224]. Other studies showed that the levels of amniotic fluid inhibin A, a glycoprotein produced by syncytiotrophoblast, in pregnant women who subsequently developed severe PE were higher than in Norm-Preg women [225]. Also, the level of sFlt1 is higher in the amniotic fluid of PE (51,040 pg/ml) than Norm-Preg women (33,490 pg/ml) [226]. Thus, careful monitoring of the hormones in the amniotic fluid could be a potential indicator of PE.

Uterine artery Doppler screening

Uterine artery Doppler screening at 23 weeks of pregnancy may predict PE as an abnormal early diastolic bilateral uterine artery notching in the waveform. However, about 50% of mothers with uterine artery notching do not develop PE. Plasma levels of fibrinolytic markers such as tissue-type plasminogen activator (t-PA), PAI-1, PAI-2, plasmin- α 2-antiplasmin (PAP) and D-dimers have been used in conjunction with uterine artery Doppler screening as predictors of PE. Uterine artery notching at 23 weeks of gestation is associated with increased PAI-1 levels. Within the uterine artery notching group, mothers who developed PE had increased t-PA levels and decreased PAI-2 levels. Thus while no single fibrinolytic marker may be helpful in determining pregnancy outcome in women with uterine artery notching, t-PA and PAI-2 are worthy of study [227].

Prevention of PE

Clinical trials examined various approaches to reduce the rate or severity of PE (Table 6). For example, Ca²⁺-supplementation was associated with reduced HTN and PE, particularly for those at high risk of the disease and with a low baseline dietary Ca²⁺ intake [37]. Other clinical

trials investigated the prevention of PE using low dose aspirin (500–1500 mg/L). Low-dose aspirin in pregnancy inhibits biosynthesis of platelet TXA₂ with little effect on vascular PGI₂ production, thus altering the balance in favor of PGI₂ and preventing the development of PE [11]. One systematic review, which included 51 trials (n=36500 subjects) of safety and effectiveness of aspirin for the prevention of PE has shown that the risk of PE fell 19% with the use of antiplatelet drugs, and that low-dose aspirin has little benefits when used to prevent PE [170]. Clinical trials have shown promising effects of supplements of L-arginine, a precursor of NO, in non-proteinuric gestational HTN [228], but little effects on the glomerular injury associated with PE [229].

Management of PE

Adequate prenatal care is most important in the management of PE and includes bed rest and anti-HTN drugs such as oral nifedipine or i.v. hydralazine or diazoxide, depending on the severity of HTN. Antihypertensive drugs decrease the risk of developing acute HTN, but may not have an effect on fetal mortality, premature birth or IUGR. Antihypertensives should be carefully selected particularly with regard to their contraindications [235]. Contraindicated antihypertensive medications during PE include angiotensin receptor blockers (ARBs) and angiotensin converting enzyme (ACE) inhibitors.

Among women with PE, signs of eclampsia include severe headache (especially occipital headache), brisk reflexes (3+), papilledema, and visual disturbances. If PE worsens into eclampsia, maintenance of airway patency and prevention of fluid aspiration should be the first measure, then anticonvulsants are given with Mg²⁺ sulfate infusion being the drug of choice.

Thus the management of PE is mainly symptomatic, and the most effective way to reverse the manifestations is induction of prompt delivery. The indications for delivery in women with PE or gestational HTN are inability to control BP, gestational age exceeding term, HELLP syndrome, deteriorating renal function, placental abruption, eclampsia, acute pulmonary edema, and severe IUGR [235]. Graduated compression stockings should be considered to decrease the risk of deep venous thrombosis. Low molecular weight heparin is indicated if there is nephrotic syndrome, provided that serum creatinine and platelet counts are normal [235]. Future approaches for management of PE should focus on preventing the disease and assessing the risk factors for PE at antenatal follow-up so that a suitable surveillance routine to detect PE can be planned for the rest of the pregnancy [236].

Conclusions and Future Directions

PE is a systemic vascular endothelial disorder. Genetic predisposition, immune and environmental factors affect the normal development of the placenta and may lead to placental ischemia/hypoxia and increase the release of bioactive anti-angiogenic factors, cytokines, ROS, HIF-1 and AT1-AA. The bioactive factors decrease the vasodilator mediators and increase the production of vasoconstrictor mediators leading to severe vasoconstriction, increased vascular resistance and HTN. Many challenges remain regarding the prediction, prevention, and management of PE. Future studies should focus on identifying the specific genes involved and their role in the regulation of placental angiogenesis and the systemic vascular health. These studies should provide a better understanding of the pathogenesis of PE, and identify potential biomarkers for early prediction, more specific therapeutic options, and new approaches to reduce the incidence of the disorder.

Recent studies identified a human-specific splicing variant of VEGF type 1 receptor (designated sFlt1–14) that is qualitatively different from sFlt1 and functions as a potent VEGF inhibitor [237]. sFlt1–14 is generated in a cell type-specific manner, primarily in nonendothelial cells such as VSM cells. The expression of sFlt1–14 is elevated in the placenta

of women with PE, and is specifically induced in abnormal clusters of degenerative syncytiotrophoblasts known as syncytial knots. Other studies have demonstrated that the ratio of circulating Cterminal glucose-regulated protein 78 (GRP78) over full length (FL) GRP78, an endoplasmic reticulum stress protein present on the cell surface of invasive cytotrophoblast, is lower in plasma of women who later develop PE. This could be used as an early biological marker of PE and might allow novel strategies early in pregnancy to improve placentation before the consequences of defective trophoblastic invasion become irreversible [238]. The levels of circulating soluble c-Met (sMet), a receptor for hepatocyte growth factor, are markedly lower at the early second trimester of severe PE than Norm-Preg women, and may serve as an early predictive biomarker for severe PE [203]. Well-powered studies of the genetics of PE did not confirm significant associations between a single nucleotide polymorphism in candidate genes and PE [239]. Advances in genotyping technology will facilitate genome-wide association studies and will likely identify novel candidate genes for PE. Most of the studies of the pathogenesis of PE suggest that the hypoxic placenta produces multiple cytotoxic factors that are released into the maternal circulation, and that the levels of these factors may be correlated with the onset or the severity of the disease. Whether these factors are the cause or the consequence of PE, and whether they act mutually or affect distinct pathogenic mechanisms is unclear. Since no concrete evidence has been provided for a single causative factor for PE, it is likely that concertive regulation may be involved in the pathogenesis and progression of the disease.

List of Abbreviations

AngII	angiotensin II
BMI	body mass index
BP	blood pressure
[Ca ²⁺] _i	intracellular free Ca ²⁺ concentration
cGMP	cyclic guanosine monophosphate
COX	cyclooxygenase
EC	endothelial cell
ECM	extracellular matrix
EDHF	endothelium-derived hyperpolarizing factor
eNOS	endothelial nitric oxide synthase
ET-1	endothelin-1
GFR	glomerular filtration rate
HIF	hypoxia-inducible transcription factor
HELLP	hemolysis elevated liver enzymes low platelets
HTN-Preg	hypertension in pregnancy
IL	interleukin
L-NAME	N ^o -nitro-L-arginine methyl ester
2-ME	2-methoxy estradiol
MMP	matrix metalloproteinase
NO	nitric oxide
Norm-Preg	normal pregnancy

O ₂ ^{-•}	superoxide anion
PE	preeclampsia
PGI ₂	prostacyclin
PHE	phenylephrine
PKC	protein kinase C
PIGF	placental growth factor
RAS	renin-angiotensin system
ROS	reactive oxygen species
RUPP	reduced uterine perfusion pressure
sEng	soluble endoglin
sFlt-1	soluble fms-like tyrosine kinase-1
STBM	syncytiotrophoblast microvillous membranes
TNF- α	tumor necrosis factor- α
TXA ₂	thromboxane A ₂
VEGF	vascular endothelial growth factor
VSM	vascular smooth muscle

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REFERENCES

1. Roberts JM, Gammill HS. Preeclampsia: recent insights. *Hypertension* 2005;46(6):1243–1249. [PubMed: 16230510]
2. Winn VD, Gormley M, Paquet AC, Kjaer-Sorensen K, Kramer A, Rumer KK, Haimov-Kochman R, Yeh RF, Overgaard MT, Varki A, Oxvig C, Fisher SJ. 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):452–462. [PubMed: 18818296]
3. Redman CW, Sargent IL. Latest advances in understanding preeclampsia. *Science* 2005;308(5728):1592–1594. [PubMed: 15947178]
4. ACOG practice bulletin. Diagnosis and management of preeclampsia and eclampsia. Number 33, January 2002. *Obstet Gynecol* 2002;99(1):159–167. [PubMed: 16175681]
5. Stennett AK, Khalil RA. Neurovascular mechanisms of hypertension in pregnancy. *Curr Neurovasc Res* 2006;3(2):131–148. [PubMed: 16719796]
6. Maynard S, Epstein FH, Karumanchi SA. Preeclampsia and angiogenic imbalance. *Annu Rev Med* 2008;59:61–78. [PubMed: 17937587]
7. Sholook MM, Gilbert JS, Sedeek MH, Huang M, Hester RL, Granger JP. Systemic hemodynamic and regional blood flow changes in response to chronic reductions in uterine perfusion pressure in pregnant rats. *Am J Physiol Heart Circ Physiol* 2007;293(4):H2080–H2084. [PubMed: 17644567]
8. Tinsley JH, Chiasson VL, South S, Mahajan A, Mitchell BM. Immunosuppression improves blood pressure and endothelial function in a rat model of pregnancy-induced hypertension. *Am J Hypertens* 2009;22(10):1107–1114. [PubMed: 19617880]
9. Sibai BM. Thrombophilia and severe preeclampsia: time to screen and treat in future pregnancies? *Hypertension* 2005;46(6):1252–1253. [PubMed: 16286582]

10. Haig D. Genetic conflicts in human pregnancy. *Q Rev Biol* 1993;68(4):495–532. [PubMed: 8115596]
11. Dekker G, Sibai B. Primary, secondary, and tertiary prevention of pre-eclampsia. *Lancet* 2001;357(9251):209–215. [PubMed: 11213110]
12. Basso O, Rasmussen S, Weinberg CR, Wilcox AJ, Irgens LM, Skjaerven R. Trends in fetal and infant survival following preeclampsia. *JAMA* 2006;296(11):1357–1362. [PubMed: 16985227]
13. Nilsson E, Salonen Ros H, Cnattingius S, Lichtenstein P. The importance of genetic and environmental effects for pre-eclampsia and gestational hypertension: a family study. *BJOG* 2004;111(3):200–206. [PubMed: 14961879]
14. Cnattingius S, Reilly M, Pawitan Y, Lichtenstein P. Maternal and fetal genetic factors account for most of familial aggregation of preeclampsia: a population-based Swedish cohort study. *Am J Med Genet A* 2004;130A(4):365–371. [PubMed: 15384082]
15. Cross JC. The genetics of pre-eclampsia: a feto-placental or maternal problem? *Clin Genet* 2003;64(2):96–103. [PubMed: 12859402]
16. Laivuori H, Lahermo P, Ollikainen V, Widen E, Haiva-Mallinen L, Sundstrom H, Laitinen T, Kaaja R, Ylikorkala O, Kere J. Susceptibility loci for preeclampsia on chromosomes 2p25 and 9p13 in Finnish families. *Am J Hum Genet* 2003;72(1):168–177. [PubMed: 12474145]
17. Founds SA, Conley YP, Lyons-Weiler JF, Jeyabalan A, Hogge WA, Conrad KP. Altered global gene expression in first trimester placentas of women destined to develop preeclampsia. *Placenta* 2009;30(1):15–24. [PubMed: 19027158]
18. Papazoglou D, Galazios G, Koukourakis MI, Kontomanolis EN, Maltezos E. Association of -634G/C and 936C/T polymorphisms of the vascular endothelial growth factor with spontaneous preterm delivery. *Acta Obstet Gynecol Scand* 2004;83(5):461–465. [PubMed: 15059159]
19. Rajakumar A, Michael HM, Daftary A, Jeyabalan A, Gilmour C, Conrad KP. Proteasomal activity in placentas from women with preeclampsia and intrauterine growth restriction: implications for expression of HIF-alpha proteins. *Placenta* 2008;29(3):290–299. [PubMed: 18222538]
20. Ciarmela P, Boschi S, Bloise E, Marozio L, Benedetto C, Castellucci M, Petraglia F. Polymorphisms of FAS and FAS ligand genes in preeclamptic women. *Eur J Obstet Gynecol Reprod Biol* 148(2):144–146. [PubMed: 19926197]
21. Muy-Rivera M, Ning Y, Frederic IO, Vadachkoria S, Luthy DA, Williams MA. Leptin, soluble leptin receptor and leptin gene polymorphism in relation to preeclampsia risk. *Physiol Res* 2005;54(2):167–174. [PubMed: 15544427]
22. Zafarmand MH, Franx A, Sabour S, van der Schouw YT, Grobbee DE, de Leeuw PW, Bots ML. The M235T variant of the angiotensinogen gene is related to development of self-reported hypertension during pregnancy: the Prospect-EPIC cohort study. *Hypertens Res* 2008;31(7):1299–1305. [PubMed: 18957799]
23. Duckers C, Simioni P, Spiezia L, Radu C, Gavasso S, Rosing J, Castoldi E. Low plasma levels of tissue factor pathway inhibitor in patients with congenital factor V deficiency. *Blood* 2008;112(9):3615–3623. [PubMed: 18695002]
24. Mayor-Olea A, Callejon G, Palomares AR, Jimenez AJ, Gaitan MJ, Rodriguez A, Ruiz M, Reyes-Engel A. Human genetic selection on the MTHFR 677C>T polymorphism. *BMC Med Genet* 2008;9:104. [PubMed: 19040733]
25. Piccoli JC, Gottlieb MG, Castro L, Bodanese LC, Manenti ER, Bogo MR, Peres A, Rocha MI, Cruz IB. Association between 894G>T endothelial nitric oxide synthase gene polymorphisms and metabolic syndrome. *Arq Bras Endocrinol Metabol* 2008;52(8):1367–1373. [PubMed: 19169496]
26. Nishizawa H, Pryor-Koishi K, Suzuki M, Kato T, Kogo H, Sekiya T, Kurahashi H, Udagawa Y. Increased levels of pregnancy-associated plasma protein-A2 in the serum of pre-eclamptic patients. *Mol Hum Reprod* 2008;14(10):595–602. [PubMed: 18805800]
27. Fitzpatrick E, Goring HH, Liu H, Borg A, Forrest S, Cooper DW, Brennecke SP, Moses EK. Fine mapping and SNP analysis of positional candidates at the preeclampsia susceptibility locus (PREG1) on chromosome 2. *Hum Biol* 2004;76(6):849–862. [PubMed: 15974297]
28. Brinkman-Van der Linden EC, Hurtado-Ziola N, Hayakawa T, Wiggleson L, Benirschke K, Varki A, Varki N. Human-specific expression of Siglec-6 in the placenta. *Glycobiology* 2007;17(9):922–931. [PubMed: 17580316]

29. Ward K. Searching for genetic factors underlying pre-eclampsia: recent progress and persistent challenges. *Minerva Ginecol* 2008;60(5):399–419. [PubMed: 18854807]
30. Tan CY, Chong YS, Loganath A, Chan YH, Ravichandran J, Lee CG, Chong SS. Possible gene-gene interaction of KIR2DL4 with its cognate ligand HLA-G in modulating risk for preeclampsia. *Reprod Sci* 2009;16(12):1135–1143. [PubMed: 19700612]
31. Lie RT, Rasmussen S, Brunborg H, Gjessing HK, Lie-Nielsen E, Irgens LM. Fetal and maternal contributions to risk of pre-eclampsia: population based study. *BMJ* 1998;316(7141):1343–1347. [PubMed: 9563982]
32. Esplin MS, Fausett MB, Fraser A, Kerber R, Mineau G, Carrillo J, Varner MW. Paternal and maternal components of the predisposition to preeclampsia. *N Engl J Med* 2001;344(12):867–872. [PubMed: 11259719]
33. Massardier J, Golfier F, Journet D, Frappart L, Zalaquett M, Schott AM, Lenoir VT, Dupuis O, Hajri T, Raudrant D. Twin pregnancy with complete hydatidiform mole and coexistent fetus: obstetrical and oncological outcomes in a series of 14 cases. *Eur J Obstet Gynecol Reprod Biol* 2009;143(2):84–87. [PubMed: 19193484]
34. Rosenberg TJ, Garbers S, Lipkind H, Chiasson MA. Maternal obesity and diabetes as risk factors for adverse pregnancy outcomes: differences among 4 racial/ethnic groups. *Am J Public Health* 2005;95(9):1545–1551. [PubMed: 16118366]
35. Caughey AB, Stotland NE, Washington AE, Escobar GJ. Maternal ethnicity, paternal ethnicity, and parental ethnic discordance: predictors of preeclampsia. *Obstet Gynecol* 2005;106(1):156–161. [PubMed: 15994632]
36. Adamova Z, Ozkan S, Khalil RA. Vascular and cellular calcium in normal and hypertensive pregnancy. *Curr Clin Pharmacol* 2009;4(3):172–190. [PubMed: 19500073]
37. Bucher HC, Guyatt GH, Cook RJ, Hatala R, Cook DJ, Lang JD, Hunt D. Effect of calcium supplementation on pregnancy-induced hypertension and preeclampsia: a meta-analysis of randomized controlled trials. *JAMA* 1996;275(14):1113–1117. [PubMed: 8601931]
38. Kumar A, Devi SG, Batra S, Singh C, Shukla DK. Calcium supplementation for the prevention of pre-eclampsia. *Int J Gynaecol Obstet* 2009;104(1):32–36. [PubMed: 18851852]
39. Levine RJ, Hauth JC, Curet LB, Sibai BM, Catalano PM, Morris CD, DerSimonian R, Esterlitz JR, Raymond EG, Bild DE, Clemens JD, Cutler JA. Trial of calcium to prevent preeclampsia. *N Engl J Med* 1997;337(2):69–76. [PubMed: 9211675]
40. Prada JA, Tsang RC, Clark KE. Hypocalcemia and pregnancy-induced hypertension produced by low-calcium diet. *Hypertension* 1994;23(6 Pt 1):695–702. [PubMed: 8206565]
41. Aiko A, Ito M, Okamura H, Araki H, Nishi K. Effect of a low calcium intake on the vascular sensitivity to angiotensin II in normotensive pregnant rats. *Artery* 1992;19(4):199–210. [PubMed: 1520073]
42. Ezimokhai M, Osman N. Calcium supplementation is associated with endothelium dependent attenuation of vascular smooth muscle reactivity in normotensive pregnant and nonpregnant rats. *Am J Hypertens* 1998;11(1 Pt 1):88–96. [PubMed: 9504455]
43. Roberts JM, Balk JL, Bodnar LM, Belizan JM, Bergel E, Martinez A. Nutrient involvement in preeclampsia. *J Nutr* 2003;133(5 Suppl 2):1684S–1692S. [PubMed: 12730485]
44. Frederick IO, Williams MA, Dashow E, Kestin M, Zhang C, Leisenring WM. Dietary fiber, potassium, magnesium and calcium in relation to the risk of preeclampsia. *J Reprod Med* 2005;50(5):332–344. [PubMed: 15971482]
45. Bodnar LM, Catov JM, Simhan HN, Holick MF, Powers RW, Roberts JM. Maternal vitamin D deficiency increases the risk of preeclampsia. *J Clin Endocrinol Metab* 2007;92(9):3517–3522. [PubMed: 17535985]
46. Halhali A, Diaz L, Avila E, Ariza AC, Garabedian M, Larrea F. Decreased fractional urinary calcium excretion and serum 1,25-dihydroxyvitamin D and IGF-I levels in preeclampsia. *J Steroid Biochem Mol Biol* 2007;103(3–5):803–806. [PubMed: 17254778]
47. Li YC, Kong J, Wei M, Chen ZF, Liu SQ, Cao LP. 1,25-Dihydroxyvitamin D(3) is a negative endocrine regulator of the renin-angiotensin system. *J Clin Invest* 2002;110(2):229–238. [PubMed: 12122115]
48. Holmes VA, McCance DR. Could antioxidant supplementation prevent pre-eclampsia? *Proc Nutr Soc* 2005;64(4):491–501. [PubMed: 16313693]

49. Title LM, Ur E, Giddens K, McQueen MJ, Nassar BA. Folic acid improves endothelial dysfunction in type 2 diabetes--an effect independent of homocysteine-lowering. *Vasc Med* 2006;11(2):101–109. [PubMed: 16886840]
50. Wen SW, Chen XK, Rodger M, White RR, Yang Q, Smith GN, Sigal RJ, Perkins SL, Walker MC. Folic acid supplementation in early second trimester and the risk of preeclampsia. *Am J Obstet Gynecol* 2008;198(1):45, e41–47. [PubMed: 18166303]
51. Flegal KM, Carroll MD, Ogden CL, Johnson CL. Prevalence and trends in obesity among US adults, 1999–2000. *JAMA* 2002;288(14):1723–1727. [PubMed: 12365955]
52. Villamor E, Cnattingius S. Interpregnancy weight change and risk of adverse pregnancy outcomes: a population-based study. *Lancet* 2006;368(9542):1164–1170. [PubMed: 17011943]
53. Stone JL, Lockwood CJ, Berkowitz GS, Alvarez M, Lapinski R, Berkowitz RL. Risk factors for severe preeclampsia. *Obstet Gynecol* 1994;83(3):357–361. [PubMed: 8127525]
54. Clement K, Viguerie N, Poitou C, Carette C, Pelloux V, Curat CA, Sicard A, Rome S, Benis A, Zucker JD, Vidal H, Laville M, Barsh GS, Basdevant A, Stich V, Cancellato R, Langin D. Weight loss regulates inflammation-related genes in white adipose tissue of obese subjects. *FASEB J* 2004;18(14):1657–1669. [PubMed: 15522911]
55. Hendler I, Blackwell SC, Mehta SH, Whitty JE, Russell E, Sorokin Y, Cotton DB. The levels of leptin, adiponectin, and resistin in normal weight, overweight, and obese pregnant women with and without preeclampsia. *Am J Obstet Gynecol* 2005;193(3 Pt 2):979–983. [PubMed: 16157097]
56. Leddy MA, Power ML, Schulkin J. The impact of maternal obesity on maternal and fetal health. *Rev Obstet Gynecol* 2008;1(4):170–178. [PubMed: 19173021]
57. Montoro MN, Kjos SL, Chandler M, Peters RK, Xiang AH, Buchanan TA. Insulin resistance and preeclampsia in gestational diabetes mellitus. *Diabetes Care* 2005;28(8):1995–2000. [PubMed: 16043744]
58. Bartha JL, Gonzalez-Bugatto F, Fernandez-Macias R, Gonzalez-Gonzalez NL, Comino-Delgado R, Hervias-Vivancos B. Metabolic syndrome in normal and complicated pregnancies. *Eur J Obstet Gynecol Reprod Biol* 2008;137(2):178–184. [PubMed: 17681419]
59. Becker T, Vermeulen MJ, Wyatt PR, Meier C, Ray JG. Prepregnancy diabetes and risk of placental vascular disease. *Diabetes Care* 2007;30(10):2496–2498. [PubMed: 17586740]
60. Carpenter MW. Gestational diabetes, pregnancy hypertension, and late vascular disease. *Diabetes Care* 2007;30 Suppl 2:S246–S250. [PubMed: 17596480]
61. Wang JX, Knottnerus AM, Schuit G, Norman RJ, Chan A, Dekker GA. Surgically obtained sperm, and risk of gestational hypertension and pre-eclampsia. *Lancet* 2002;359(9307):673–674. [PubMed: 11879865]
62. Saito S, Shiozaki A, Nakashima A, Sakai M, Sasaki Y. The role of the immune system in preeclampsia. *Mol Aspects Med* 2007;28(2):192–209. [PubMed: 17433431]
63. Tafuri A, Alferink J, Moller P, Hammerling GJ, Arnold B. T cell awareness of paternal alloantigens during pregnancy. *Science* 1995;270(5236):630–633. [PubMed: 7570020]
64. Koelman CA, Coumans AB, Nijman HW, Doxiadis II, Dekker GA, Claas FH. Correlation between oral sex and a low incidence of preeclampsia: a role for soluble HLA in seminal fluid? *J Reprod Immunol* 2000;46(2):155–166. [PubMed: 10706945]
65. Robertson SA, Bromfield JJ, Tremellen KP. Seminal 'priming' for protection from pre-eclampsia-a unifying hypothesis. *J Reprod Immunol* 2003;59(2):253–265. [PubMed: 12896827]
66. Hanna J, Goldman-Wohl D, Hamani Y, Avraham I, Greenfield C, Natanson-Yaron S, Prus D, Cohen-Daniel L, Arnon TI, Manaster I, Gazit R, Yutkin V, Benharroch D, Porgador A, Keshet E, Yagel S, Mandelboim O. Decidual NK cells regulate key developmental processes at the human fetal-maternal interface. *Nat Med* 2006;12(9):1065–1074. [PubMed: 16892062]
67. Sibai B, Dekker G, Kupferminc M. Pre-eclampsia. *Lancet* 2005;365(9461):785–799. [PubMed: 15733721]
68. Le Bouteiller P, Pizzato N, Barakonyi A, Solier C. HLA-G, pre-eclampsia, immunity and vascular events. *J Reprod Immunol* 2003;59(2):219–234. [PubMed: 12896824]
69. von Dadelszen P, Magee LA, Kraiden M, Alasaly K, Popovska V, Devarakonda RM, Money DM, Patrick DM, Brunham RC. Levels of antibodies against cytomegalovirus and Chlamydia

pneumoniae are increased in early onset pre-eclampsia. *BJOG* 2003;110(8):725–730. [PubMed: 12892683]

70. Trogstad LI, Eskild A, Bruu AL, Jeansson S, Jenum PA. Is preeclampsia an infectious disease? *Acta Obstet Gynecol Scand* 2001;80(11):1036–1038. [PubMed: 11703202]
71. Kusanovic JP, Romero R, Hassan SS, Gotsch F, Edwin S, Chaiworapongsa T, Erez O, Mittal P, Mazaki-Tovi S, Soto E, Than NG, Friel LA, Yoon BH, Espinoza J. Maternal serum soluble CD30 is increased in normal pregnancy, but decreased in preeclampsia and small for gestational age pregnancies. *J Matern Fetal Neonatal Med* 2007;20(12):867–878. [PubMed: 17853188]
72. Geva E, Ginzinger DG, Zaloudek CJ, Moore DH, Byrne A, Jaffe RB. Human placental vascular development: vasculogenic and angiogenic (branching and nonbranching) transformation is regulated by vascular endothelial growth factor-A, angiopoietin-1, and angiopoietin-2. *J Clin Endocrinol Metab* 2002;87(9):4213–4224. [PubMed: 12213874]
73. McMaster MT, Zhou Y, Fisher SJ. Abnormal placentation and the syndrome preeclampsia. *Semin Nephrol* 2004;24(6):540–547. [PubMed: 15529288]
74. Genbacev O, DiFederico E, McMaster M, Fisher SJ. Invasive cytotrophoblast apoptosis in pre-eclampsia. *Hum Reprod* 1999;14 Suppl 2:59–66. [PubMed: 10690801]
75. Chaddha V, Viero S, Huppertz B, Kingdom J. Developmental biology of the placenta and the origins of placental insufficiency. *Semin Fetal Neonatal Med* 2004;9(5):357–369. [PubMed: 15691771]
76. Levine RJ, Lam C, Qian C, Yu KF, Maynard SE, Sachs BP, Sibai BM, Epstein FH, Romero R, Thadhani R, Karumanchi SA. Soluble endoglin and other circulating antiangiogenic factors in preeclampsia. *N Engl J Med* 2006;355(10):992–1005. [PubMed: 16957146]
77. Gilbert JS, Gilbert SA, Arany M, Granger JP. Hypertension produced by placental ischemia in pregnant rats is associated with increased soluble endoglin expression. *Hypertension* 2009;53(2):399–403. [PubMed: 19075097]
78. Chen W, Khalil RA. Differential $[Ca^{2+}]_i$ signaling of vasoconstriction in mesenteric microvessels of normal and reduced uterine perfusion pregnant rats. *Am J Physiol Regul Integr Comp Physiol* 2008;295(6):R1962–R1972. [PubMed: 18843089]
79. Walsh SK, English FA, Johns EJ, Kenny LC. Plasma-mediated vascular dysfunction in the reduced uterine perfusion pressure model of preeclampsia: a microvascular characterization. *Hypertension* 2009;54(2):345–351. [PubMed: 19564546]
80. Orshal JM, Khalil RA. Reduced endothelial NO-cGMP-mediated vascular relaxation and hypertension in IL-6-infused pregnant rats. *Hypertension* 2004;43(2):434–444. [PubMed: 14707155]
81. Murphy JG, Fleming JB, Cockrell KL, Granger JP, Khalil RA. $[Ca^{2+}]_i$ signaling in renal arterial smooth muscle cells of pregnant rat is enhanced during inhibition of NOS. *Am J Physiol Regul Integr Comp Physiol* 2001;280(1):R87–R99. [PubMed: 11124138]
82. Crews JK, Novak J, Granger JP, Khalil RA. Stimulated mechanisms of Ca^{2+} entry into vascular smooth muscle during NO synthesis inhibition in pregnant rats. *Am J Physiol* 1999;276(2 Pt 2):R530–R538. [PubMed: 9950934]
83. Kanasaki K, Palmsten K, Sugimoto H, Ahmad S, Hamano Y, Xie L, Parry S, Augustin HG, Gattone VH, Folkman J, Strauss JF, Kalluri R. Deficiency in catechol-O-methyltransferase and 2-methoxyoestradiol is associated with pre-eclampsia. *Nature* 2008;453(7198):1117–1121. [PubMed: 18469803]
84. Shaarawy M, Al-Sokkary F, Sheba M, Wahba O, Kandil HO, Abdel-Mohsen I. Angiogenin and vascular endothelial growth factor in pregnancies complicated by preeclampsia. *Int J Gynaecol Obstet* 2005;88(2):112–117. [PubMed: 15694084]
85. Stepan H, Faber R, Dornhofer N, Huppertz B, Robitzki A, Walther T. New insights into the biology of preeclampsia. *Biol Reprod* 2006;74(5):772–776. [PubMed: 16421233]
86. Hayashi M, Ueda Y, Yamaguchi T, Sohma R, Shibazaki M, Ohkura T, Inaba N. Tumor necrosis factor- α in the placenta is not elevated in pre-eclamptic patients despite its elevation in peripheral blood. *Am J Reprod Immunol* 2005;53(3):113–119. [PubMed: 15727564]
87. Casart YC, Tarrazzi K, Camejo MI. Serum levels of interleukin-6, interleukin-1 β and human chorionic gonadotropin in pre-eclamptic and normal pregnancy. *Gynecol Endocrinol* 2007;23(5):300–303. [PubMed: 17558690]

88. Tsukimori K, Fukushima K, Tsushima A, Nakano H. Generation of reactive oxygen species by neutrophils and endothelial cell injury in normal and preeclamptic pregnancies. *Hypertension* 2005;46(4):696–700. [PubMed: 16172429]
89. Montagnana M, Lippi G, Albiero A, Scevarolli S, Salvagno GL, Franchi M, Guidi GC. Evaluation of metalloproteinases 2 and 9 and their inhibitors in physiologic and preeclamptic pregnancy. *J Clin Lab Anal* 2009;23(2):88–92. [PubMed: 19288452]
90. Ranta V, Viinikka L, Halmesmaki E, Ylikorkala O. Nitric oxide production with preeclampsia. *Obstet Gynecol* 1999;93(3):442–445. [PubMed: 10074996]
91. Mills JL, DerSimonian R, Raymond E, Morrow JD, Roberts LJ 2nd, Clemens JD, Hauth JC, Catalano P, Sibai B, Curet LB, Levine RJ. Prostacyclin and thromboxane changes predating clinical onset of preeclampsia: a multicenter prospective study. *JAMA* 1999;282(4):356–362. [PubMed: 10432033]
92. Kenny LC, Baker PN, Kendall DA, Randall MD, Dunn WR. Differential mechanisms of endothelium-dependent vasodilator responses in human myometrial small arteries in normal pregnancy and preeclampsia. *Clin Sci (Lond)* 2002;103(1):67–73. [PubMed: 12095405]
93. Clark BA, Halvorson L, Sachs B, Epstein FH. Plasma endothelin levels in preeclampsia: elevation and correlation with uric acid levels and renal impairment. *Am J Obstet Gynecol* 1992;166(3):962–968. [PubMed: 1532292]
94. Brosnihan KB, Neves LA, Anton L, Joyner J, Valdes G, Merrill DC. Enhanced expression of Ang-(1–7) during pregnancy. *Braz J Med Biol Res* 2004;37(8):1255–1262. [PubMed: 15273828]
95. Maynard SE, Min JY, Merchan J, Lim KH, Li J, Mondal S, Libermann TA, Morgan JP, Sellke FW, Stillman IE, Epstein FH, Sukhatme VP, Karumanchi SA. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. *J Clin Invest* 2003;111(3):649–658. [PubMed: 12618519]
96. LaMarca BD, Ryan MJ, Gilbert JS, Murphy SR, Granger JP. Inflammatory cytokines in the pathophysiology of hypertension during preeclampsia. *Curr Hypertens Rep* 2007;9(6):480–485. [PubMed: 18367011]
97. Gilbert JS, Ryan MJ, LaMarca BB, Sedeek M, Murphy SR, Granger JP. Pathophysiology of hypertension during preeclampsia: linking placental ischemia with endothelial dysfunction. *Am J Physiol Heart Circ Physiol* 2008;294(2):H541–H550. [PubMed: 18055511]
98. Alexander BT, Kassab SE, Miller MT, Abram SR, Reckelhoff JF, Bennett WA, Granger JP. Reduced uterine perfusion pressure during pregnancy in the rat is associated with increases in arterial pressure and changes in renal nitric oxide. *Hypertension* 2001;37(4):1191–1195. [PubMed: 11304523]
99. Wang YP, Walsh SW, Guo JD, Zhang JY. Maternal levels of prostacyclin, thromboxane, vitamin E, and lipid peroxides throughout normal pregnancy. *Am J Obstet Gynecol* 1991;165(6 Pt 1):1690–1694. [PubMed: 1750461]
100. LaMarca BB, Cockrell K, Sullivan E, Bennett W, Granger JP. Role of endothelin in mediating tumor necrosis factor-induced hypertension in pregnant rats. *Hypertension* 2005;46(1):82–86. [PubMed: 15928030]
101. LaMarca B, Parrish M, Ray LF, Murphy SR, Roberts L, Glover P, Wallukat G, Wenzel K, Cockrell K, Martin JN Jr, Ryan MJ, Dechend R. Hypertension in response to autoantibodies to the angiotensin II type I receptor (AT1-AA) in pregnant rats: role of endothelin-1. *Hypertension* 2009;54(4):905–909. [PubMed: 19704104]
102. Murphy JG, Herrington JN, Granger JP, Khalil RA. Enhanced $[Ca^{2+}]_i$ in renal arterial smooth muscle cells of pregnant rats with reduced uterine perfusion pressure. *Am J Physiol Heart Circ Physiol* 2003;284(1):H393–H403. [PubMed: 12388289]
103. Ferrara N, Houck K, Jakeman L, Leung DW. Molecular and biological properties of the vascular endothelial growth factor family of proteins. *Endocr Rev* 1992;13(1):18–32. [PubMed: 1372863]
104. Serraf A, Aznag H, Baudet B, Detruit H, Seccatore F, Mazmanian MG, Planche C. Pulmonary vascular endothelial growth factor and nitric oxide interaction during total cardiopulmonary bypass in neonatal pigs. *J Thorac Cardiovasc Surg* 2003;125(5):1050–1057. [PubMed: 12771878]
105. Vincenti V, Cassano C, Rocchi M, Persico G. Assignment of the vascular endothelial growth factor gene to human chromosome 6p21.3. *Circulation* 1996;93(8):1493–1495. [PubMed: 8608615]

106. Kupferminc MJ, Daniel Y, Englender T, Baram A, Many A, Jaffa AJ, Gull I, Lessing JB. Vascular endothelial growth factor is increased in patients with preeclampsia. *Am J Reprod Immunol* 1997;38(4):302–306. [PubMed: 9352019]
107. Eremina V, Jefferson JA, Kowalewska J, Hochster H, Haas M, Weisstuch J, Richardson C, Kopp JB, Kabir MG, Backx PH, Gerber HP, Ferrara N, Barisoni L, Alpers CE, Quaggin SE. VEGF inhibition and renal thrombotic microangiopathy. *N Engl J Med* 2008;358(11):1129–1136. [PubMed: 18337603]
108. Sugimoto H, Hamano Y, Charytan D, Cosgrove D, Kieran M, Sudhakar A, Kalluri R. Neutralization of circulating vascular endothelial growth factor (VEGF) by anti-VEGF antibodies and soluble VEGF receptor 1 (sFlt-1) induces proteinuria. *J Biol Chem* 2003;278(15):12605–12608. [PubMed: 12538598]
109. Ahmad S, Ahmed A. Elevated placental soluble vascular endothelial growth factor receptor-1 inhibits angiogenesis in preeclampsia. *Circ Res* 2004;95(9):884–891. [PubMed: 15472115]
110. Clark DE, Smith SK, He Y, Day KA, Licence DR, Corps AN, Lammoglia R, Charnock-Jones DS. A vascular endothelial growth factor antagonist is produced by the human placenta and released into the maternal circulation. *Biol Reprod* 1998;59(6):1540–1548. [PubMed: 9828203]
111. Maynard SE, Moore Simas TA, Solitro MJ, Rajan A, Crawford S, Soderland P, Meyer BA. Circulating angiogenic factors in singleton vs multiple-gestation pregnancies. *Am J Obstet Gynecol* 2008;198(2):200, e201–e207. [PubMed: 18226624]
112. Bdolah Y, Lam C, Rajakumar A, Shivalingappa V, Mutter W, Sachs BP, Lim KH, Bdolah-Abram T, Epstein FH, Karumanchi SA. Twin pregnancy and the risk of preeclampsia: bigger placenta or relative ischemia? *Am J Obstet Gynecol* 2008;198(4):428, e421–e426. [PubMed: 18191808]
113. Bdolah Y, Palomaki GE, Yaron Y, Bdolah-Abram T, Goldman M, Levine RJ, Sachs BP, Haddow JE, Karumanchi SA. Circulating angiogenic proteins in trisomy 13. *Am J Obstet Gynecol* 2006;194(1):239–245. [PubMed: 16389038]
114. Cheifetz S, Bellon T, Cales C, Vera S, Bernabeu C, Massague J, Letarte M. Endoglin is a component of the transforming growth factor-beta receptor system in human endothelial cells. *J Biol Chem* 1992;267(27):19027–19030. [PubMed: 1326540]
115. Venkatesha S, Toporsian M, Lam C, Hanai J, Mammoto T, Kim YM, Bdolah Y, Lim KH, Yuan HT, Libermann TA, Stillman IE, Roberts D, D'Amore PA, Epstein FH, Sellke FW, Romero R, Sukhatme VP, Letarte M, Karumanchi SA. Soluble endoglin contributes to the pathogenesis of preeclampsia. *Nat Med* 2006;12(6):642–649. [PubMed: 16751767]
116. Osol G, Celia G, Gokina N, Barron C, Chien E, Mandala M, Luksha L, Kublickiene K. Placental growth factor is a potent vasodilator of rat and human resistance arteries. *Am J Physiol Heart Circ Physiol* 2008;294(3):H1381–H1387. [PubMed: 18192215]
117. Kim SY, Ryu HM, Yang JH, Kim MY, Han JY, Kim JO, Chung JH, Park SY, Lee MH, Kim DJ. Increased sFlt-1 to PlGF ratio in women who subsequently develop preeclampsia. *J Korean Med Sci* 2007;22(5):873–877. [PubMed: 17982238]
118. Conrad KP, Miles TM, Benyo DF. Circulating levels of immunoreactive cytokines in women with preeclampsia. *Am J Reprod Immunol* 1998;40(2):102–111. [PubMed: 9764352]
119. Hashii K, Fujiwara H, Yoshioka S, Kataoka N, Yamada S, Hirano T, Mori T, Fujii S, Maeda M. Peripheral blood mononuclear cells stimulate progesterone production by luteal cells derived from pregnant and non-pregnant women: possible involvement of interleukin-4 and interleukin-10 in corpus luteum function and differentiation. *Hum Reprod* 1998;13(10):2738–2744. [PubMed: 9804222]
120. Alexander BT, Cockrell KL, Massey MB, Bennett WA, Granger JP. Tumor necrosis factor-alpha-induced hypertension in pregnant rats results in decreased renal neuronal nitric oxide synthase expression. *Am J Hypertens* 2002;15(2 Pt 1):170–175. [PubMed: 11863253]
121. Takagi Y, Nikaido T, Toki T, Kita N, Kanai M, Ashida T, Ohira S, Konishi I. Levels of oxidative stress and redox-related molecules in the placenta in preeclampsia and fetal growth restriction. *Virchows Arch* 2004;444(1):49–55. [PubMed: 14574573]
122. Walsh SW. Eicosanoids in preeclampsia. *Prostaglandins Leukot Essent Fatty Acids* 2004;70(2):223–232. [PubMed: 14683695]

123. Nakamura M, Sekizawa A, Purwosunu Y, Okazaki S, Farina A, Wibowo N, Shimizu H, Okai T. Cellular mRNA expressions of anti-oxidant factors in the blood of preeclamptic women. *Prenat Diagn* 2009;29(7):691–696. [PubMed: 19399816]
124. Yung HW, Calabrese S, Hynx D, Hemmings BA, Cetin I, Charnock-Jones DS, Burton GJ. Evidence of placental translation inhibition and endoplasmic reticulum stress in the etiology of human intrauterine growth restriction. *Am J Pathol* 2008;173(2):451–462. [PubMed: 18583310]
125. Egi K, Conrad NE, Kwan J, Schulze C, Schulz R, Wildhirt SM. Inhibition of inducible nitric oxide synthase and superoxide production reduces matrix metalloproteinase-9 activity and restores coronary vasomotor function in rat cardiac allografts. *Eur J Cardiothorac Surg* 2004;26(2):262–269. [PubMed: 15296881]
126. Chang TC, Huang CJ, Tam K, Chen SF, Tan KT, Tsai MS, Lin TN, Shyue SK. Stabilization of hypoxia-inducible factor-1 {alpha} by prostacyclin under prolonged hypoxia via reducing reactive oxygen species level in endothelial cells. *J Biol Chem* 2005;280(44):36567–36574. [PubMed: 16115891]
127. Jin L, Ying Z, Webb RC. Activation of Rho/Rho kinase signaling pathway by reactive oxygen species in rat aorta. *Am J Physiol Heart Circ Physiol* 2004;287(4):H1495–H1500. [PubMed: 15371261]
128. Semenza GL. Hypoxia-inducible factor 1: control of oxygen homeostasis in health and disease. *Pediatr Res* 2001;49(5):614–617. [PubMed: 11328942]
129. Ke Q, Costa M. Hypoxia-inducible factor-1 (HIF-1). *Mol Pharmacol* 2006;70(5):1469–1480. [PubMed: 16887934]
130. Cockman ME, Masson N, Mole DR, Jaakkola P, Chang GW, Clifford SC, Maher ER, Pugh CW, Ratcliffe PJ, Maxwell PH. Hypoxia inducible factor-alpha binding and ubiquitylation by the von Hippel-Lindau tumor suppressor protein. *J Biol Chem* 2000;275(33):25733–25741. [PubMed: 10823831]
131. Yamashita K, Discher DJ, Hu J, Bishopric NH, Webster KA. Molecular regulation of the endothelin-1 gene by hypoxia. Contributions of hypoxia-inducible factor-1, activator protein-1, GATA-2, AND p300/CBP. *J Biol Chem* 2001;276(16):12645–12653. [PubMed: 11278891]
132. Suzuki S, Gejyo F, Ogino S, Maruyama Y, Ueno M, Nishi S, Kimura H, Arakawa M. Postpartum renal lesions in women with pre-eclampsia. *Nephrol Dial Transplant* 1997;12(12):2488–2493. [PubMed: 9430839]
133. Bar J, Kaplan B, Wittenberg C, Erman A, Boner G, Ben-Rafael Z, Hod M. Microalbuminuria after pregnancy complicated by pre-eclampsia. *Nephrol Dial Transplant* 1999;14(5):1129–1132. [PubMed: 10344350]
134. Stillman IE, Karumanchi SA. The glomerular injury of preeclampsia. *J Am Soc Nephrol* 2007;18(8):2281–2284. [PubMed: 17634433]
135. Sladek SM, Magness RR, Conrad KP. Nitric oxide and pregnancy. *Am J Physiol* 1997;272(2 Pt 2):R441–R463. [PubMed: 9124465]
136. Nelson SH, Steinsland OS, Wang Y, Yallampalli C, Dong YL, Sanchez JM. Increased nitric oxide synthase activity and expression in the human uterine artery during pregnancy. *Circ Res* 2000;87(5):406–411. [PubMed: 10969039]
137. Conrad KP, Kerchner LJ, Mosher MD. Plasma and 24-h NO(x) and cGMP during normal pregnancy and preeclampsia in women on a reduced NO(x) diet. *Am J Physiol* 1999;277(1 Pt 2):F48–F57. [PubMed: 10409297]
138. Mutlu-Turkoglu U, Aykac-Toker G, Ibrahimoglu L, Ademoglu E, Uysal M. Plasma nitric oxide metabolites and lipid peroxide levels in preeclamptic pregnant women before and after delivery. *Gynecol Obstet Invest* 1999;48(4):247–250. [PubMed: 10592426]
139. Schiessl B, Mylonas I, Kuhn C, Kunze S, Schulze S, Friese K, Jeschke U. Expression of estrogen receptor-alpha, estrogen receptor-beta and placental endothelial and inducible NO synthase in intrauterine growth-restricted and normal placentals. *Arch Med Res* 2006;37(8):967–975. [PubMed: 17045112]
140. Roberts JM. Endothelial dysfunction in preeclampsia. *Semin Reprod Endocrinol* 1998;16(1):5–15. [PubMed: 9654603]
141. Khalil RA, Crews JK, Novak J, Kassab S, Granger JP. Enhanced vascular reactivity during inhibition of nitric oxide synthesis in pregnant rats. *Hypertension* 1998;31(5):1065–1069. [PubMed: 9576115]

142. Suzuki Y, Hattori T, Kajikuri J, Yamamoto T, Suzumori K, Itoh T. Reduced function of endothelial prostacyclin in human omental resistance arteries in pre-eclampsia. *J Physiol* 2002;545(Pt 1):269–277. [PubMed: 12433966]
143. Fike CD, Kaplowitz MR, Pfister SL. Arachidonic acid metabolites and an early stage of pulmonary hypertension in chronically hypoxic newborn pigs. *Am J Physiol Lung Cell Mol Physiol* 2003;284(2):L316–L323. [PubMed: 12388340]
144. Bowen RS, Zhang Y, Gu Y, Lewis DF, Wang Y. Increased phospholipase A2 and thromboxane but not prostacyclin production by placental trophoblast cells from normal and preeclamptic pregnancies cultured under hypoxia condition. *Placenta* 2005;26(5):402–409. [PubMed: 15850645]
145. Zhao S, Gu Y, Lewis DF, Wang Y. Predominant basal directional release of thromboxane, but not prostacyclin, by placental trophoblasts from normal and preeclamptic pregnancies. *Placenta* 2008;29(1):81–88. [PubMed: 17936899]
146. Scotland RS, Madhani M, Chauhan S, Moncada S, Andresen J, Nilsson H, Hobbs AJ, Ahluwalia A. Investigation of vascular responses in endothelial nitric oxide synthase/cyclooxygenase-1 double-knockout mice: key role for endothelium-derived hyperpolarizing factor in the regulation of blood pressure in vivo. *Circulation* 2005;111(6):796–803. [PubMed: 15699263]
147. Luksha L, Nisell H, Luksha N, Kublickas M, Hultenby K, Kublickiene K. Endothelium-derived hyperpolarizing factor in preeclampsia: heterogeneous contribution, mechanisms, and morphological prerequisites. *Am J Physiol Regul Integr Comp Physiol* 2008;294(2):R510–R519. [PubMed: 18032472]
148. Fulep EE, Vedernikov YP, Saade GR, Garfield RE. The role of endothelium-derived hyperpolarizing factor in the regulation of the uterine circulation in pregnant rats. *Am J Obstet Gynecol* 2001;185(3):638–642. [PubMed: 11568792]
149. Niwano K, Arai M, Tomaru K, Uchiyama T, Ohyama Y, Kurabayashi M. Transcriptional stimulation of the eNOS gene by the stable prostacyclin analogue beraprost is mediated through cAMP-responsive element in vascular endothelial cells: close link between PGI2 signal and NO pathways. *Circ Res* 2003;93(6):523–530. [PubMed: 12919953]
150. Orié NN, Fry CH, Clapp LH. Evidence that inward rectifier K⁺ channels mediate relaxation by the PGI2 receptor agonist cicaprost via a cyclic AMP-independent mechanism. *Cardiovasc Res* 2006;69(1):107–115. [PubMed: 16183044]
151. Kourembanas S, McQuillan LP, Leung GK, Faller DV. Nitric oxide regulates the expression of vasoconstrictors and growth factors by vascular endothelium under both normoxia and hypoxia. *J Clin Invest* 1993;92(1):99–104. [PubMed: 8326022]
152. Taylor RN, Varma M, Teng NN, Roberts JM. Women with preeclampsia have higher plasma endothelin levels than women with normal pregnancies. *J Clin Endocrinol Metab* 1990;71(6):1675–1677. [PubMed: 2229324]
153. McNair LL, Salamanca DA, Khalil RA. Endothelin-1 promotes Ca²⁺ antagonist-insensitive coronary smooth muscle contraction via activation of epsilon-protein kinase C. *Hypertension* 2004;43(4):897–904. [PubMed: 14981072]
154. Giardina JB, Green GM, Rinewalt AN, Granger JP, Khalil RA. Role of endothelin B receptors in enhancing endothelium-dependent nitric oxide-mediated vascular relaxation during high salt diet. *Hypertension* 2001;37(2 Part 2):516–523. [PubMed: 11230328]
155. Conrad KP, Gandley RE, Ogawa T, Nakanishi S, Danielson LA. Endothelin mediates renal vasodilation and hyperfiltration during pregnancy in chronically instrumented conscious rats. *Am J Physiol* 1999;276(5 Pt 2):F767–F776. [PubMed: 10330059]
156. Debrah DO, Novak J, Matthews JE, Ramirez RJ, Shroff SG, Conrad KP. Relaxin is essential for systemic vasodilation and increased global arterial compliance during early pregnancy in conscious rats. *Endocrinology* 2006;147(11):5126–5131. [PubMed: 16873529]
157. Yang G, Chang L, Alexander JS, Groome LJ, Yuping W. Chymotrypsin-like protease (chymase) mediates endothelial activation by factors derived from preeclamptic placentas. *Reprod Sci* 2009;16(9):905–913. [PubMed: 19494363]
158. Cheng ZJ, Vapaatalo H, Mervaala E. Angiotensin II and vascular inflammation. *Med Sci Monit* 2005;11(6):RA194–RA205. [PubMed: 15917731]

159. Askari B, Ferreri NR. Regulation of prostacyclin synthesis by angiotensin II and TNF-alpha in vascular smooth muscle. *Prostaglandins Other Lipid Mediat* 2001;63(4):175–187. [PubMed: 11305695]
160. Wesselman JP, De Mey JG. Angiotensin and cytoskeletal proteins: role in vascular remodeling. *Curr Hypertens Rep* 2002;4(1):63–70. [PubMed: 11790294]
161. Wijayagunawardane MP, Gabler C, Killian G, Miyamoto A. Tumor necrosis factor alpha in the bovine oviduct during the estrous cycle: messenger RNA expression and effect on secretion of prostaglandins, endothelin-1, and angiotensin II. *Biol Reprod* 2003;69(4):1341–1346. [PubMed: 12801985]
162. Wassmann S, Stumpf M, Strehlow K, Schmid A, Schieffer B, Bohm M, Nickenig G. Interleukin-6 induces oxidative stress and endothelial dysfunction by overexpression the angiotensin II type 1 receptor. *Circ Res* 2004;94(4):534–541. [PubMed: 14699015]
163. Anguiano-Robledo L, Reyes-Melchor PA, Bobadilla-Lugo RA, Perez-Alvarez VM, Lopez-Sanchez P. Renal angiotensin-II receptors expression changes in a model preeclampsia. *Hypertens Pregnancy* 2007;26(2):151–161. [PubMed: 17469005]
164. AbdAlla S, Abdel-Baset A, Lothar H, el Massiery A, Qwitterer U. Mesangial AT1/B2 receptor heterodimers contribute to angiotensin II hyperresponsiveness in experimental hypertension. *J Mol Neurosci* 2005;26(2–3):185–192. [PubMed: 16012192]
165. Anton L, Merrill DC, Neves LA, Diz DI, Corthorn J, Valdes G, Stovall K, Gallagher PE, Moorefield C, Gruver C, Brosnihan KB. The uterine placental bed Renin-Angiotensin system in normal and preeclamptic pregnancy. *Endocrinology* 2009;150(9):4316–4325. [PubMed: 19520788]
166. Zhou CC, Ahmad S, Mi T, Abbasi S, Xia L, Day MC, Ramin SM, Ahmed A, Kellems RE, Xia Y. Autoantibody from women with preeclampsia induces soluble Fms-like tyrosine kinase-1 production via angiotensin type 1 receptor and calcineurin/nuclear factor of activated T-cells signaling. *Hypertension* 2008;51(4):1010–1019. [PubMed: 18259044]
167. Bobst SM, Day MC, Gilstrap LC 3rd, Xia Y, Kellems RE. Maternal autoantibodies from preeclamptic patients activate angiotensin receptors on human mesangial cells and induce interleukin-6 and plasminogen activator inhibitor-1 secretion. *Am J Hypertens* 2005;18(3):330–336. [PubMed: 15797649]
168. Bakker WW, Henning RH, van Son WJ, van Pampus MG, Aarnoudse JG, Niezen-Koning KE, Borghuis T, Jongman RM, van Goor H, Poelstra K, Navis G, Faas MM. Vascular contraction and preeclampsia: downregulation of the Angiotensin receptor by hemopexin in vitro. *Hypertension* 2009;53(6):959–964. [PubMed: 19414647]
169. Koba S, Pakala R, Watanabe T, Katagiri T, Benedict CR. Synergistic interaction between thromboxane A2 and mildly oxidized low density lipoproteins on vascular smooth muscle cell proliferation. *Prostaglandins Leukot Essent Fatty Acids* 2000;63(6):329–335. [PubMed: 11133169]
170. Knight M, Duley L, Henderson-Smart DJ, King JF. Antiplatelet agents for preventing and treating pre-eclampsia. *Cochrane Database Syst Rev*. 2000;(2) CD000492.
171. Coomarasamy A, Honest H, Papaioannou S, Gee H, Khan KS. Aspirin for prevention of preeclampsia in women with historical risk factors: a systematic review. *Obstet Gynecol* 2003;101(6):1319–1332. [PubMed: 12798543]
172. Seki H, Kuromaki K, Takeda S, Kinoshita K, Satoh K. Trial of prophylactic administration of TXA2 synthetase inhibitor, ozagrel hydrochloride, for preeclampsia. *Hypertens Pregnancy* 1999;18(2):157–164. [PubMed: 10476617]
173. Horowitz A, Menice CB, Laporte R, Morgan KG. Mechanisms of smooth muscle contraction. *Physiol Rev* 1996;76(4):967–1003. [PubMed: 8874491]
174. Wimalasundera RC, Thom SA, Regan L, Hughes AD. Effects of vasoactive agents on intracellular calcium and force in myometrial and subcutaneous resistance arteries isolated from preeclamptic, pregnant, and nonpregnant woman. *Am J Obstet Gynecol* 2005;192(2):625–632. [PubMed: 15696013]
175. Kanashiro CA, Cockrell KL, Alexander BT, Granger JP, Khalil RA. Pregnancy-associated reduction in vascular protein kinase C activity rebounds during inhibition of NO synthesis. *Am J Physiol Regul Integr Comp Physiol* 2000;278(2):R295–R303. [PubMed: 10666128]

176. Farley DB, Ford SP. Evidence for declining extracellular calcium uptake and protein kinase C activity in uterine arterial smooth muscle during gestation in gilts. *Biol Reprod* 1992;46(3):315–321. [PubMed: 1319752]
177. Salamanca DA, Khalil RA. Protein kinase C isoforms as specific targets for modulation of vascular smooth muscle function in hypertension. *Biochem Pharmacol* 2005;70(11):1537–1547. [PubMed: 16139252]
178. VanWijk MJ, Boer K, van der Meulen ET, Bleker OP, Spaan JA, VanBavel E. Resistance artery smooth muscle function in pregnancy and preeclampsia. *Am J Obstet Gynecol* 2002;186(1):148–154. [PubMed: 11810101]
179. Wallukat G, Homuth V, Fischer T, Lindschau C, Horstkamp B, Jupner A, Baur E, Nissen E, Vetter K, Neichel D, Dudenhausen JW, Haller H, Luft FC. Patients with preeclampsia develop agonistic autoantibodies against the angiotensin AT1 receptor. *J Clin Invest* 1999;103(7):945–952. [PubMed: 10194466]
180. Hu E. Recent patents on Rho signaling pathway as therapeutic target for cardiovascular diseases. *Recent Pat Cardiovasc Drug Discov* 2006;1(3):249–263. [PubMed: 18221090]
181. Kataoka C, Egashira K, Inoue S, Takemoto M, Ni W, Koyanagi M, Kitamoto S, Usui M, Kaibuchi K, Shimokawa H, Takeshita A. Important role of Rho-kinase in the pathogenesis of cardiovascular inflammation and remodeling induced by long-term blockade of nitric oxide synthesis in rats. *Hypertension* 2002;39(2):245–250. [PubMed: 11847192]
182. Friel AM, Sexton DJ, O'Reilly MW, Smith TJ, Morrison JJ. Rho A/Rho kinase: human umbilical artery mRNA expression in normal and pre eclamptic pregnancies and functional role in isoprostan-induced vasoconstriction. *Reproduction* 2006;132(1):169–176. [PubMed: 16816342]
183. Raines EW. The extracellular matrix can regulate vascular cell migration, proliferation, and survival: relationships to vascular disease. *Int J Exp Pathol* 2000;81(3):173–182. [PubMed: 10971738]
184. Tayebjee MH, Lip GY, Blann AD, Macfadyen RJ. Effects of age, gender, ethnicity, diurnal variation and exercise on circulating levels of matrix metalloproteinases (MMP)-2 and -9, and their inhibitors, tissue inhibitors of matrix metalloproteinases (TIMP)-1 and -2. *Thromb Res* 2005;115(3):205–210. [PubMed: 15617743]
185. Sternlicht MD, Werb Z. How matrix metalloproteinases regulate cell behavior. *Annu Rev Cell Dev Biol* 2001;17:463–516. [PubMed: 11687497]
186. Raffetto JD, Qiao X, Koledova VV, Khalil RA. Prolonged increases in vein wall tension increase matrix metalloproteinases and decrease constriction in rat vena cava: Potential implications in varicose veins. *J Vasc Surg* 2008;48(2):447–456. [PubMed: 18502086]
187. McCawley LJ, Matrisian LM. Matrix metalloproteinases: they're not just for matrix anymore! *Curr Opin Cell Biol* 2001;13(5):534–540. [PubMed: 11544020]
188. Jeyabalan A, Novak J, Doty KD, Matthews J, Fisher MC, Kerchner LJ, Conrad KP. Vascular matrix metalloproteinase-9 mediates the inhibition of myogenic reactivity in small arteries isolated from rats after short-term administration of relaxin. *Endocrinology* 2007;148(1):189–197. [PubMed: 17053025]
189. Kelly BA, Bond BC, Poston L. Aortic adaptation to pregnancy: elevated expression of matrix metalloproteinases-2 and -3 in rat gestation. *Mol Hum Reprod* 2004;10(5):331–337. [PubMed: 15044600]
190. Galewska Z, Bankowski E, Romanowicz L, Jaworski S. Pre-eclampsia (EPH-gestosis)-induced decrease of MMP-s content in the umbilical cord artery. *Clin Chim Acta* 2003;335(1–2):109–115. [PubMed: 12927692]
191. Chew DK, Conte MS, Khalil RA. Matrix metalloproteinase-specific inhibition of Ca²⁺ entry mechanisms of vascular contraction. *J Vasc Surg* 2004;40(5):1001–1010. [PubMed: 15557917]
192. Wang H, Olszewski B, Rosebury W, Wang D, Robertson A, Keiser JA. Impaired angiogenesis in SHR is associated with decreased KDR and MT1-MMP expression. *Biochem Biophys Res Commun* 2004;315(2):363–368. [PubMed: 14766216]
193. MacKay AP, Berg CJ, Atrash HK. Pregnancy-related mortality from preeclampsia and eclampsia. *Obstet Gynecol* 2001;97(4):533–538. [PubMed: 11275024]
194. von Dadelszen P, Magee LA, Roberts JM. Subclassification of preeclampsia. *Hypertens Pregnancy* 2003;22(2):143–148. [PubMed: 12908998]

195. Grill S, Rusterholz C, Zanetti-Dallenbach R, Tercanli S, Holzgreve W, Hahn S, Lapaire O. Potential markers of preeclampsia--a review. *Reprod Biol Endocrinol* 2009;7:70. [PubMed: 19602262]
196. Purwosunu Y, Sekizawa A, Okazaki S, Farina A, Wibowo N, Nakamura M, Rizzo N, Saito H, Okai T. Prediction of preeclampsia by analysis of cell-free messenger RNA in maternal plasma. *Am J Obstet Gynecol* 2009;200(4):386, e381–e387. [PubMed: 19217595]
197. Lim JH, Kim SY, Park SY, Yang JH, Kim MY, Ryu HM. Effective prediction of preeclampsia by a combined ratio of angiogenesis-related factors. *Obstet Gynecol* 2008;111(6):1403–1409. [PubMed: 18515525]
198. Baumann MU, Bersinger NA, Mohaupt MG, Raio L, Gerber S, Surbek DV. First-trimester serum levels of soluble endoglin and soluble fms-like tyrosine kinase-1 as first-trimester markers for late-onset preeclampsia. *Am J Obstet Gynecol* 2008;199(3):266, e261–e266. [PubMed: 18771978]
199. Verlohren S, Galindo A, Schlembach D, Zeisler H, Herraiz I, Moertl MG, Pape J, Dudenhausen JW, Denk B, Stepan H. An automated method for the determination of the sFlt-1/PlGF ratio in the assessment of preeclampsia. *Am J Obstet Gynecol* 2012;206(2):161, e161–e161, e111. [PubMed: 19850276]
200. Tripathi R, Rath G, Ralhan R, Saxena S, Salhan S. Soluble and membranous vascular endothelial growth factor receptor-2 in pregnancies complicated by pre-eclampsia. *Yonsei Med J* 2009;50(5):656–666. [PubMed: 19881969]
201. Banzola I, Farina A, Concu M, Sekizawa A, Purwosunu Y, Strada I, Arcelli D, Simonazzi G, Caramelli E, Rizzo N. Performance of a panel of maternal serum markers in predicting preeclampsia at 11–15 weeks' gestation. *Prenat Diagn* 2007;27(11):1005–1010. [PubMed: 17654753]
202. Chavarria ME, Lara-Gonzalez L, Garcia-Paleta Y, Vital-Reyes VS, Reyes A. Adhesion molecules changes at 20 gestation weeks in pregnancies complicated by preeclampsia. *Eur J Obstet Gynecol Reprod Biol* 2008;137(2):157–164. [PubMed: 17706337]
203. Zeng X, Sun Y, Yang HX, Li D, Li YX, Liao QP, Wang YL. Plasma level of soluble c-Met is tightly associated with the clinical risk of preeclampsia. *Am J Obstet Gynecol* 2009;201(6):618, e611–e617. [PubMed: 19766985]
204. Spencer K, Cowans NJ, Stamatopoulou A. ADAM12s in maternal serum as a potential marker of pre-eclampsia. *Prenat Diagn* 2008;28(3):212–216. [PubMed: 18264967]
205. Poon LC, Maiz N, Valencia C, Plasencia W, Nicolaides KH. First-trimester maternal serum pregnancy-associated plasma protein-A and pre-eclampsia. *Ultrasound Obstet Gynecol* 2009;33(1):23–33. [PubMed: 19090499]
206. Adali E, Yildizhan R, Kolusari A, Kurdoglu M, Bugdayci G, Sahin HG, Kamaci M. Increased visfatin and leptin in pregnancies complicated by pre-eclampsia. *J Matern Fetal Neonatal Med* 2009;22(10):873–879. [PubMed: 19488934]
207. Hu W, Wang Z, Wang H, Huang H, Dong M. Serum visfatin levels in late pregnancy and pre-eclampsia. *Acta Obstet Gynecol Scand* 2008;87(4):413–418. [PubMed: 18382866]
208. Senna AA, Zedan M, el-Salam GE, el-Mashad AI. Study of plasma adrenomedullin level in normal pregnancy and preclampsia. *Medscape J Med* 2008;10(2):29. [PubMed: 18382699]
209. Khalil A, Cowans NJ, Spencer K, Goichman S, Meiri H, Harrington K. First trimester maternal serum placental protein 13 for the prediction of pre-eclampsia in women with a priori high risk. *Prenat Diagn* 2009;29(8):781–789. [PubMed: 19418482]
210. Spencer K, Cowans NJ, Nicolaides KH. Maternal serum inhibin-A and activin-A levels in the first trimester of pregnancies developing pre-eclampsia. *Ultrasound Obstet Gynecol* 2008;32(5):622–626. [PubMed: 18816493]
211. Armstrong RA, Reynolds RM, Leask R, Shearing CH, Calder AA, Riley SC. Decreased serum levels of kisspeptin in early pregnancy are associated with intra-uterine growth restriction and pre-eclampsia. *Prenat Diagn* 2009;29(10):982–985. [PubMed: 19582702]
212. Lynch AM, Murphy JR, Byers T, Gibbs RS, Neville MC, Giclas PC, Salmon JE, Holers VM. Alternative complement pathway activation fragment Bb in early pregnancy as a predictor of preeclampsia. *Am J Obstet Gynecol* 2008;198(4):385, e381–e389. [PubMed: 18221926]
213. Greer IA, Haddad NG, Dawes J, Johnstone FD, Calder AA. Neutrophil activation in pregnancy-induced hypertension. *Br J Obstet Gynaecol* 1989;96(8):978–982. [PubMed: 2775697]

214. Crocker IP, Wellings RP, Fletcher J, Baker PN. Neutrophil function in women with pre-eclampsia. *Br J Obstet Gynaecol* 1999;106(8):822–828. [PubMed: 10453833]
215. Sabatier F, Bretelle F, D'Ercole C, Boubli L, Sampol J, Dignat-George F. Neutrophil activation in preeclampsia and isolated intrauterine growth restriction. *Am J Obstet Gynecol* 2000;183(6):1558–1563. [PubMed: 11120528]
216. Mellembakken JR, Aukrust P, Olafsen MK, Ueland T, Hestdal K, Videm V. Activation of leukocytes during the uteroplacental passage in preeclampsia. *Hypertension* 2002;39(1):155–160. [PubMed: 11799095]
217. Redman CW, Sacks GP, Sargent IL. Preeclampsia: an excessive maternal inflammatory response to pregnancy. *Am J Obstet Gynecol* 1999;180(2 Pt 1):499–506. [PubMed: 9988826]
218. Wang Y, Gu Y, Philibert L, Lucas MJ. Neutrophil activation induced by placental factors in normal and pre-eclamptic pregnancies in vitro. *Placenta* 2001;22(6):560–565. [PubMed: 11440544]
219. Dundar O, Yoruk P, Tutuncu L, Erikci AA, Muhcu M, Ergur AR, Atay V, Mungen E. Longitudinal study of platelet size changes in gestation and predictive power of elevated MPV in development of pre-eclampsia. *Prenat Diagn* 2008;28(11):1052–1056. [PubMed: 18973157]
220. Powers RW, Bodnar LM, Ness RB, Cooper KM, Gallaher MJ, Frank MP, Daftary AR, Roberts JM. Uric acid concentrations in early pregnancy among preeclamptic women with gestational hyperuricemia at delivery. *Am J Obstet Gynecol* 2006;194(1):160. [PubMed: 16389026]
221. Many A, Hubel CA, Roberts JM. Hyperuricemia and xanthine oxidase in preeclampsia, revisited. *Am J Obstet Gynecol* 1996;174(1 Pt 1):288–291. [PubMed: 8572024]
222. Bainbridge SA, Roberts JM, von Versen-Hoynck F, Koch J, Edmunds L, Hubel CA. Uric acid attenuates trophoblast invasion and integration into endothelial cell monolayers. *Am J Physiol Cell Physiol* 2009;297(2):C440–C450. [PubMed: 19535510]
223. Koopmans CM, van Pampus MG, Groen H, Aarnoudse JG, van den Berg PP, Mol BW. Accuracy of serum uric acid as a predictive test for maternal complications in preeclampsia: bivariate meta-analysis and decision analysis. *Eur J Obstet Gynecol Reprod Biol* 2009;146(1):8–14. [PubMed: 19540647]
224. Anand-Ivell R, Ivell R, Driscoll D, Manson J. Insulin-like factor 3 levels in amniotic fluid of human male fetuses. *Hum Reprod* 2008;23(5):1180–1186. [PubMed: 18310050]
225. Kim SY, Ryu HM, Yang JH, Kim MY, Ahn HK, Shin JS, Choi JS, Park SY, Kim JM, Lee BY, Kim DJ. Maternal serum and amniotic fluid inhibin A levels in women who subsequently develop severe preeclampsia. *J Korean Med Sci* 2006;21(3):452–456. [PubMed: 16778388]
226. Staff AC, Braekke K, Harsem NK, Lyberg T, Holthe MR. Circulating concentrations of sFlt1 (soluble fms-like tyrosine kinase 1) in fetal and maternal serum during preeclampsia. *Eur J Obstet Gynecol Reprod Biol* 2005;122(1):33–39. [PubMed: 15935542]
227. Hunt BJ, Missfelder-Lobos H, Parra-Cordero M, Fletcher O, Parmar K, Lefkou E, Lees CC. Pregnancy outcome and fibrinolytic, endothelial and coagulation markers in women undergoing uterine artery Doppler screening at 23 weeks. *J Thromb Haemost* 2009;7(6):955–961. [PubMed: 19320824]
228. Facchinetti F, Saade GR, Neri I, Pizzi C, Longo M, Volpe A. L-arginine supplementation in patients with gestational hypertension: a pilot study. *Hypertens Pregnancy* 2007;26(1):121–130. [PubMed: 17454224]
229. Hladunewich MA, Derby GC, Lafayette RA, Blouch KL, Druzin ML, Myers BD. Effect of L-arginine therapy on the glomerular injury of preeclampsia: a randomized controlled trial. *Obstet Gynecol* 2006;107(4):886–895. [PubMed: 16582128]
230. Kulier R, de Onis M, Gulmezoglu AM, Villar J. Nutritional interventions for the prevention of maternal morbidity. *Int J Gynaecol Obstet* 1998;63(3):231–246. [PubMed: 9989892]
231. Olsen SF, Secher NJ, Bjornsson S, Weber T, Atke A. The potential benefits of using fish oil in relation to preterm labor: the case for a randomized controlled trial? *Acta Obstet Gynecol Scand* 2003;82(11):978–982. [PubMed: 14616269]
232. Kupferminc MJ, Fait G, Many A, Lessing JB, Yair D, Bar-Am A, Eldor A. Low-molecular-weight heparin for the prevention of obstetric complications in women with thrombophilias. *Hypertens Pregnancy* 2001;20(1):35–44. [PubMed: 12044312]

233. Rumbold AR, Crowther CA, Haslam RR, Dekker GA, Robinson JS. Vitamins C and E and the risks of preeclampsia and perinatal complications. *N Engl J Med* 2006;354(17):1796–1806. [PubMed: 16641396]
234. Magee LA, Abalos E, von Dadelszen P, Sibai B, Walkinshaw SA. Control of hypertension in pregnancy. *Curr Hypertens Rep* 2009;11(6):429–436. [PubMed: 19895754]
235. Lowe SA, Brown MA, Dekker GA, Gatt S, McLintock CK, McMahon LP, Mangos G, Moore MP, Muller P, Paech M, Walters B. Guidelines for the management of hypertensive disorders of pregnancy 2008. *Aust N Z J Obstet Gynaecol* 2009;49(3):242–246. [PubMed: 19566552]
236. Duckitt K, Harrington D. Risk factors for pre-eclampsia at antenatal booking: systematic review of controlled studies. *BMJ* 2005;330(7491):565. [PubMed: 15743856]
237. Sela S, Itin A, Natanson-Yaron S, Greenfield C, Goldman-Wohl D, Yagel S, Keshet E. A novel human-specific soluble vascular endothelial growth factor receptor 1: cell-typespecific splicing and implications to vascular endothelial growth factor homeostasis and preeclampsia. *Circ Res* 2008;102(12):1566–1574. [PubMed: 18515749]
238. Laverriere A, Landau R, Charvet I, Irion O, Bischof P, Morales M, Cohen M. GRP78 as a marker of pre-eclampsia: an exploratory study. *Mol Hum Reprod* 2009;15(9):569–574. [PubMed: 19482905]
239. Disentangling fetal and maternal susceptibility for pre-eclampsia: a British multicenter candidate-gene study. *Am J Hum Genet* 2005;77(1):127–131. [PubMed: 15889386]

Fig. 1.

Risk factors and pathogenesis of preeclampsia (PE). Genetic, environmental and immune factors cause shallow placentation and RUPP during late pregnancy and trigger the release of circulating factors, which affect the systemic blood vessels and lead to generalized vasoconstriction, increased vascular resistance and HTN-Preg. The bioactive factors could injure the kidney leading to increased plasma volume and severe HTN, as well as glomerular endotheliosis and proteinuria. Increased cerebral vascular permeability and edema lead to seizures and life-threatening eclampsia.

Fig. 2.

Circulating bioactive factors and vascular mediators during PE. Placental hypoxia/ischemia triggers the release of circulating factors in the plasma and cause a decrease in the release of vasodilators from the endothelium, an increase in vasoconstriction in VSM and the expression of MMPs and vascular remodeling in the adventitia. sEng is a TGF-β antagonist that blocks the TβRII receptor, and sFlt-1 is a VEGF antagonist that blocks the Flt-1 receptor. Solid arrows indicate stimulation. Interrupted arrows indicate inhibition.

Table 1

Representative Maternal Genes Associated with PE

Gene symbol	Full Name	Gene Map Locus	Function	Polymorphism	MIM Number	Ref
AGT	Angiotensinogen	1q42-q43	Converted to AngI then AngII, which maintains blood pressure: Linked to pathogenesis of HTN	M235T	106150	[22]
F5	Coagulation factor V	1q23	Produces proteins for hemostasis; prevents activation of protein C	R485K,M385T	612309	[23]
MTHFR	5,10-methylene-tetrahydrofolate reductase, NADPH	1p36.3	Produces enzyme that converts homocysteine to methionine	T677C	607093	[24]
NOS3	Nitric Oxide Synthase 3	7q36	Synthesizes NO in endothelium and maintains vascular homeostasis	C786T, T894G (linked to metabolic syndrome), Introns 4 a/b VNTR	163729	[25]
PAPP-A2	Pappalysin-2 Pregnancy-associated plasma protein A2	1q23-q25	Encodes proteases that cleave insulin-like growth factor (IGF)-binding proteins, resulting in local activation of IGF signaling pathways			[2,26]
PEE1 PEE2 PEE3	PE/eclampsia1 PE/eclampsia2 PE/eclampsia 3	2p13 2p25 9p13	Genome scans in Australia, Iceland and Finland suggest linkage to PE; Exact function of gene still unknown		189800 609402 609403	[27]
Siglec-6	Sialic acid binding I g-like lectin 6	19q13.3	Receptor for leptin; Rate of expression is related to progression of labor		604405	[28]

Table 2

Representative Animal Models of HTN-Preg

Model / Species	Technique	Manifestations				Other PE-Relevant Findings	Ref
		BP (mmHg)	Proteinuria (mg/24hr)	Pup Weight (g)	Litter size (# of pups)		
RUPP Sprague-Dawley Rat	Two groups: Norm-Preg RUPP On gestation day 14, a silver clip (0.203mm ID) is placed around the abdominal aorta and another clip (0.1mm ID) is placed on the main uterine branches of the ovarian arteries.	99±3 132±4	n/a	3.2±0.1 1.76±±.08	12.7±0.5 7.7±1.3	sFlt-1 ~70 vs. ~675pg/ml TNF- α ~8 vs. ~18pg/ml. sEng 0.10±0.02 vs. 0.05±0.01 apu HIF- α 1.42±0.25 vs. 0.68±0.09 apu Hemeoxygenase (HO-1) 1.4±0.3 vs. 2.5±0.1 apu Bradykinin relaxation 62±2 vs. 40±2% Ach relaxation 92±1 vs. 70±3% Cardiac output 65±5 vs. 104 ±7 ml/min. Cardiac index (CI) 348±19 vs. 246 ±20 ml/min. Total peripheral resistance 0.98±0.08 vs. 2.15±0.02 mmHg/ml/min	[7,77-79]
TNF- α , IL-6 Sprague-Dawley Rat	Two Groups: Norm-Preg Cytokine-infused On gestation day 14, rats are infused iv with TNF- α or IL-6 200ng/kg/day for 5 days.	96±3 123±3	15.6±2.4 88.2±9.6	10.4±0.6 2.6±0.2	10.4±0.6 7.3±0.5	Cytokine-infused pregnant rats show increased vascular responses to vasoconstrictors and inhibition of endothelium-dependent NO-cGMP vascular relaxation.	[80]
L-NAME Treated Sprague-Dawley Rat	Four groups: Virgin Virgin+L-NAME Preg Preg+L-NAME On gestation day 15, pregnant and virgin rats received L-NAME4 mg/kg/day in drinking water.	118±3 125±6 113±5 172±6	n/a	n/a	n/a	Reduction of NO production in late pregnancy is associated with increased contraction and [Ca ²⁺] _i in VSM and may lead to the increased vascular resistance associated with HTN-Preg.	[81,82]

Model / Species	Technique	Manifestations				Other PE-Relevant Findings	Ref
		BP (mmHg)	Proteinuria (mg/24hr)	Pup Weight (g)	Litter size (# of pups)	Norm-Preg vs. HTN-Preg	
DOCA-Treated pregnant Sprague-Dawley Rat	<i>Five Groups:</i> Virgin Preg Preg+DOCA Preg+DOCA+MMF Preg+DOCA+AZA	106±2 116±4 133±4 112±6 122±2	Urinary Albumin/Cr (mg/mg;% of control) 100±48 91±23 369±47 121±31 169±36	Litter # - 14.0±0.6 10.2±0.7 17.1±0.3 13.2±0.8	Malformed Litter # - 0.0±0.0 1.0±0.3 17.1±0.3 3.9±1.5	Serum Th1-type cytokines IL-2, IL-12, IFN γ , and RANTES were elevated in HTN-Preg rats. Th2-type cytokine IL-4 was elevated in Norm-Preg rats. MMF attenuated the HTN, proteinuria, and endothelial dysfunction and the increased proinflammatory Th1 cytokine profile in preg rats treated with DOCA/salt	[8]
2-ME Deficient Mouse	<i>Four groups:</i> COMT ^{+/+} COMT ^{+/-} +RO41-0960 (COMT inhibitor, S Q, 24 mg/kg/day) COMT ^{-/-} COMT ^{-/-} + 2-ME (SQ, 10 ng/day)	105 122 121 108	Albumin/Cr 1.0 1.48 1.50 1.20	Placenta Weight 0.094 0.085 0.085 0.090	# Dead embryos 1 - 2.5 1	Decreased levels of 2-ME are associated with symptoms of PE. 2-ME may have utility as a plasma and urine diagnostic marker of PE, and may serve as a therapeutic supplement to prevent or treat PE.	[83]

apu, arbitrary pixel unit; n/a, not available; AZA, azathioprine (immunosuppressant); DOCA, deoxycorticosterone acetate; MMF, mycophenolate mofetil (immunosuppressant); 2-ME, 2- methoxy estradiol, COMT, catechol-O-methyl transferase

Plasma Levels and Effects of Bioactive Factors and Vascular Mediators during Normal Pregnancy (Norm-Preg) and Preeclampsia (PE) in Humans

Table 3

Bioactive Factor	Norm-Preg	PE	Site of Action	Effect	Reference
VEGF (pg/mL)	184	396	Endothelium	Vasorelaxation	[84]
sFlt-1 (pg/mL)	339.4	7247	Endothelium	Anti VEGF	[85]
sEng (ng/mL)	9.8	46.4	Endothelium	Anti TGF- β	[76]
TNF- α (pg/mL)	3.31	4.68	Endothelium	EC Dysfunction	[86]
IL-6 (pg/mL)	4.9 $\pm$ 1.1	16.5 $\pm$ 2.1	Endothelium	EC Dysfunction	[87]
ROS (O $_2^{\cdot-}$ release by neutrophils)	$\approx$ 3.5nmol/10 6 cell s/5min $\uparrow$	$\approx$ 6.2nmol/10 6 cell s/5min $\uparrow\uparrow$	Endothelium	EC Dysfunction	[88]
HIF	$\uparrow$	$\uparrow\uparrow$	DNA	Gene Transcription	[19]
MMP-2 (ng/mL)	669	834	ECM	Remodeling	[89]
MMP-9 (ng/mL)	390	290			
TIMP-1 (ng/mL)	148	213			
TIMP-2 (ng/mL)	228	232			
2-ME (ng/mL)	$\sim$ 1.5	$\sim$ 0.9	Estrogen receptor	Vasodilator	[83]
Vascular Mediator					
NO (plasma nitrite/nitrate μ mol/L)	13.0 $\pm$ 4.3	18.1 $\pm$ 6.2 $\uparrow$, $\rightarrow$, $\downarrow$	VSM	Vasorelaxation	[90]
PGI $_2$ (urinary metabolite pg/mg Creatinine)	1452	1354	VSM	Vasorelaxation	[91]
EDHF	$\uparrow$	$\downarrow$	VSM	Vasorelaxation	[92]
ET-1 (pmol/L)	12 $\pm$ 1.0	22.6 $\pm$ 2.0	VSM Endothelium	Vasoconstriction Vasorelaxation	[93]

Bioactive Factor	Norm-Preg	PE	Site of Action	Effect	Reference
AngII (fmol/L)	30.2	53.3	VSM	Vasoconstriction	[94]

↑↑ Greatly increased
↑ Increased
↓ decreased. Data represent means±SEM.

Plasma and Vascular Tissue Levels, and Effects of Bioactive Factors and Vascular Mediators in Norm-Preg and HTN-Preg in rats

Table 4

Bioactive Factor	Norm-Preg	HTN-Preg	Site of Action	Effect	Reference
VEGF (pg/mL)	830±33	594±34	Endothelium	Vasorelaxation	[95]
sFlt-1 (pg/mL)	82±26	660±270	Endothelium	Anti VEGF	[77]
sEng (apu)	0.05±0.01	0.10±0.02	Endothelium	Anti TGF-β	[77]
TNF-α (pg/mL)	8±1	48±13	Endothelium	EC Dysfunction	[96]
IL-6 (pg/mL)	36.6±7	104.5±28.6	Endothelium	EC Dysfunction	
ROS (placental levels of 8-isoprostane ng/g tissue)	0.8±0.1 ↑	1.9±0.4 ↑↑	Endothelium	EC Dysfunction	[97]
Hypoxia-Inducible Factor (HIF)	↑	↑↑	DNA	Gene Transcription	[97]
Vascular Mediator					
NO (urinary nitrite/nitrate) (μmol/24hr)	46.4±5.3	49.8±6.4 ↑, -, ↓	VSM	Vasorelaxation	[98]
PGI ₂	↑	↓	VSM	Vasorelaxation	[99]
ET-1 (pmol/L)	~1.7	~2.2	VSM	Vasoconstriction	[100]
AngII (ATI-AA)	0.6±3U	15.3±1.6U	VSM	Vasoconstriction	[101]
VSM Ca ²⁺ (nM)	63±5	109±8	Myofilaments Myofilaments Myofilaments	Basal Tone Contraction Contraction	[81,102]
Basal PHE (10 ⁻⁵ M)	149±8	234±1			
AngII (10 ⁻⁷ M)	149±8	225±90			

↑↑ Greatly increased

↑ Increased

↓ Decreased; apu, arbitrary pixel unit. Data represent means±SEM.

Table 5
Biomarkers in maternal peripheral blood for the prediction (1st, 2nd and 3rd Trimester) and detection (Manifest PE) of PE

Biomarker	Norm-Preg Trimester			PE Trimester			Manifest PE	Reference
	1 st	2 nd	3 rd	1 st	2 nd	3 rd		
sFlt-1 (pg/ml)	1445	1576	4400	1764 ±757	4945.1 ±329.4	>12981	↑	[197–199]
sEng (pg/ml)	5010 ±1010	4905.9 ±1332.1	9800	5570 ±1180	11509.2 ±6108.2	46400	↑	[76,197,198]
PIGF (pg/ml)	62.8	135	232	↓	99.6 ±52.2	↓	↓	[197–199]
sVEGFR-2 (pg/ml)	--	7645.8 ±1938.0	--	--	6541.6 ±3285.8	--	↓	[200]
TGF-β1 (pg/ml)	--	4718.6 ±2371.9	--	--	2901.1 ±1308.4	--	↓	[197]
P-Selectin	1±0.86 (MoM)	82.5±1.2 ng/ml	--	2.49±1.94 (MoM)	91.2±2.0 ng/ml	--	↑	[201,202]
L-Selectin (ng/ml)	--	1761 ±31.3	--	--	1194 ±43.2	--	↓	[201,202]
s-Met (ng/ml)	259.1 ±13.3	356.2 ±18.3	293.7 ±20.6	182.5 ±6.8	189.9 ±9.7	235.1 ±11.7	↓	[203]
ADAM12 (MoM)	1.00	--	--	0.50 – 0.71	--	--	↓	[204]
PAPP-A (MoM)	1.002	--	--	0.555 to 0.911	--	--	↓	[205]
Visfatin (adipokinin ng/ml)) Leptin (ng/ml)	-- --	↑ ↓	695.9 ±92.5 26.8 ± 6.47	-- --	↓ ↑ ↑	308.3 ±80.0 62.1 ± 23.8	↓ ↑	[206,207]
Adrenomedullin (pg/ml)	53.4 ±17.3	139.8 ±20.9	232.6 ±17.2	--	302.1 ±46.0	302.1 ±46.0	↑	[208]
PP-13 (pg/ml)	≈136.6	--	--	≈50.00	--	--	↓	[209]
Inhibin-A (μg/ml)	180 ±105	--	--	220 ±113	--	--	↑	[198]
Activin-A (ng/ml)	2.16	--	--	2.522	--	--	↑	[210]
Kisspeptin (pg/ml)	--	1188 ±365	--	--	1109 ±449	--	↓	[211]

Biomarker	Norm-Preg Trimester			PE Trimester			Manifest PE	Reference
	1 st	2 nd	3 rd	1 st	2 nd	3 rd		
Cell-free mRNA FLT1 (copies/ml)	--	1.90 (0.32)	--	--	2.39 (0.32)	--	↑	[196]

sVEGFR-2, soluble vascular endothelial growth factor receptor-2; PP-13, placental protein 13; ADAM12, a disintegrin and metalloprotease 12; PTX3, pentraxin 3; PAPP-A, pregnancy-associated plasma protein A; TGF-β1: Transforming growth factor-β1 MoM: Multiple of Mean; s-Met: soluble c-Met ; FLT1, VEGFA receptor 1

Table 6

Clinical Trials to Prevent Preeclampsia

Clinical Trial	Pregnancy Outcome	Recommendation	Reference
Magnesium or Zinc supplementation	No reduction in PE	Insufficient evidence to recommend	[230]
Fish-oil supplementation and other sources of fatty acids (FOTIP trial)	No effect in low-risk or high-risk populations	Insufficient evidence to recommend	[231]
Ca²⁺ supplementation (Multiple trials)	Reduced PE in those at high risk and with low baseline dietary Ca ²⁺ intake. No effect on perinatal outcome	Recommended for women at high risk of gestational HTN, and in communities with low dietary Ca ²⁺ intake	[11]
Low-dose aspirin (Multiple trials)	19% reduction in risk of PE. 16% reduction in fetal or neonatal death	Consider in high-risk populations	[170]
Heparin or low-molecular-weight heparin	Reduced PE in women with renal disease and in women with thrombophilia	Lack of data from randomized clinical trials. Not recommended	[232]
Antioxidant vitamins C, E (ACTS trial)	Did not reduce the risk of PE in nulliparous women	Insufficient evidence to recommend	[233]
Antihypertensive medications in women with chronic HTN (CHIPS trial)	May decrease transient severe maternal HTN, but may also impair fetal growth and perinatal health	There is no consensus regarding management of nonsevere HTN	[234]