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Preeclampsia, a disease of the maternal endothelium: the role of anti-angiogenic factors and implications for later cardiovascular disease

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Clinical Features and Epidemiology of Preeclampsia

Preeclampsia is a clinical syndrome, defined as the new onset of hypertension and proteinuria during the second half of pregnancy.¹ It afflicts 3–5% of pregnancies and is a leading cause of maternal mortality, especially in developing countries.^{2–3} Since the only known remedy is delivery of the placenta, in developed countries preeclampsia is an important cause of premature delivery usually medically indicated for the benefit of the mother. This results in infant morbidity and substantial excess health care expenditure.⁴ Despite the considerable morbidity and mortality, the cause of preeclampsia has remained enigmatic.

Both hypertension and proteinuria implicate the endothelium as the target of the disease. The hypertension of preeclampsia is characterized by peripheral vasoconstriction and decreased arterial compliance.^{5–6} The proteinuria of preeclampsia is associated with a pathognomonic renal lesion known as glomerular endotheliosis, in which the endothelial cells of the glomerulus swell and endothelial fenestrations are lost.^{7–8} Podocyturia has been recently associated with preeclampsia during clinical disease⁹, however whether this is cause or effect of proteinuria is unknown. The glomerular filtration rate is decreased, compared to normotensive pregnant women; in rare cases, acute renal failure may develop.

Preeclampsia is a systemic vascular disorder which may also affect the liver and the brain in the mothers. When the liver is involved, women may present with abdominal pain, nausea, vomiting, and elevated liver enzymes. Pathologic examination of the liver reveals periportal and sinusoidal fibrin deposition, and in more extreme cases, hemorrhage and necrosis.¹⁰ The severe preeclampsia variant, HELLP syndrome (hemolysis, elevated liver enzymes, low platelets), occurs in approximately 20% of women with severe preeclampsia¹¹ and is named not only for the liver involvement but also for the disorder of the coagulation system which

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develops.¹² Approximately 20% of women with HELLP syndrome develop disseminated intravascular coagulation, which carries a poor prognosis for both mother and fetus.¹¹ Placental abruption, ascites, hepatic infarction, hepatic rupture, intra-abdominal bleeding, pulmonary edema, and acute renal failure are all severe clinical manifestations associated with preeclampsia which can result in maternal death.¹³ Perhaps the most feared complication of preeclampsia is eclampsia itself, defined by the presence of seizures, for which women with severe preeclampsia are often treated with magnesium sulfate prophylaxis.¹ The brain injury in eclampsia is associated with cerebral edema and characteristic white matter changes of reversible posterior leukoencephalopathy syndrome (RPLS), that is similar to what is noted in hypertensive encephalopathy and with cytotoxic immunosuppressive therapies.¹⁴ Cerebrovascular complications, including stroke and cerebral hemorrhage, are responsible for the majority of eclampsia related deaths.¹⁵ Complications affecting the developing fetus include indicated prematurity¹⁶, intrauterine fetal growth restriction (IUGR), oligohydramnios, bronchopulmonary dysplasia¹⁷ and increased risk of perinatal death.¹⁸

The risk factors for preeclampsia are varied and unique to this condition. Genetic factors are at least partially responsible, as both a maternal and paternal family history of the disease predisposes to preeclampsia.¹⁹ There is a 7-fold risk of recurrence for women who have had the disease in a previous pregnancy.²⁰ Multiple gestation is an additional risk factor, and triplet gestation carries a greater risk than twin, suggesting that increased placental mass plays some role.²⁰ Associations between preeclampsia and nulliparity,²⁰ change in paternity from a previous pregnancy,²¹ increased interpregnancy interval,²² use of barrier contraception,²³ and conception by intracytoplasmic sperm injection,²⁴ implicate limited recent exposure to paternal antigen as a predisposing factor. Notably, classic cardiovascular risk factors are associated with preeclampsia: maternal age greater than 40, insulin resistance, obesity, systemic inflammation, and preexisting hypertension, diabetes, or renal disease all increase the risk.^{20, 25–26} Consistent with this, women with a history of preeclampsia have an elevated risk for cardiovascular disease later in life (see discussion later in this review). Surprisingly, smoking during pregnancy protects against preeclampsia.²⁷

The diagnosis of preeclampsia is clinical. As defined by the American College of Obstetrics and Gynecology, the diagnosis requires blood pressures greater than 140/90 on two occasions, combined with urine protein excretion greater than 300 mg per day.¹ Edema, a classic feature of the disease, is no longer considered a diagnostic feature, given its lack of sensitivity or specificity.¹ Importantly, in 20% of cases, eclampsia may present without preceding hypertension or proteinuria, suggesting that the currently employed diagnostic criteria are imperfect.²⁸ Laboratory tests such as liver function tests, quantification of urine protein, and serum creatinine may be helpful in characterizing the degree of end organ damage, but none is specific for preeclampsia.¹ Hyperuricemia, more likely to be present in women with preeclampsia than in normotensive pregnant women, has been used as a diagnostic aid and to predict adverse outcomes in preeclampsia, but its predictive value is generally modest.^{29–30}

Recently work by our group and others has identified an imbalance of pro- and anti-angiogenic proteins as a key factor in the pathogenesis of the preeclampsia.^{31–38} Here, we review our current understanding of the biology underlying the disease. We first describe the role of the placenta in preeclampsia, then review the mechanisms of angiogenesis and its role in preeclampsia and the role of other contributory pathways in the pathogenesis of preeclampsia. Finally, we comment on the potential mechanisms by which the risk of cardiovascular disease is elevated in women with a history of preeclampsia.

The Preeclamptic Placenta

The placenta is the central organ in the pathogenesis of preeclampsia. Removal of the placenta abolishes the disease;³⁹ moreover, only the placenta, and not the fetus, is required for its development. This is best demonstrated by the case of molar pregnancy, which carries an elevated risk for preeclampsia.⁴⁰ Pathologic examination of placentas from women with severe preeclampsia reveals several abnormalities including infarcts, atherosclerosis, thrombosis, and chronic inflammation.⁴¹ Likely, some of the abnormalities seen in the preeclamptic placenta are consequences of the hypertension and endothelial injury induced by the disease. However, there are abnormalities of placental development which precede the maternal derangements.

During normal placentation, the embryo-derived cytotrophoblast cells invade the maternal uterine wall. Post-invasion, cytotrophoblasts are found in the smooth muscle and endothelial layers of the maternal decidual arteries. This interaction acts to induce the remodeling of these maternal vessels into high capacitance, low resistance vessels which provide access to maternal oxygen and nutrients for the placenta and developing fetus.⁴² As part of this process, the cytotrophoblasts adopt an endothelial phenotype, expressing adhesion molecules classically found on the surface of endothelial cells.⁴³ In preeclampsia, this process is aberrant. The invasion of the cytotrophoblasts is incomplete, with cytotrophoblast cells present only in the superficial layers of the decidua.⁴³ The spiral arteries fail to be invaded or remodeled, resulting in constricted, high resistance vessels, visible upon pathologic examination of preeclamptic placentas. This shallow invasion has been shown to be related to a failure of the cytotrophoblasts to adopt an endothelial adhesion phenotype.⁴⁴ (see Figure 1)

Hypoxia may contribute to the abnormal placental development described above, as the failure of cytotrophoblasts to fully invade and to switch adhesion molecules can also be reproduced in vitro when cytotrophoblasts are cultured under hypoxic conditions.⁴⁶ Consistent with this, the risk for preeclampsia is higher in women living at high altitude.⁴⁷ However, hypoxia resulting from abnormal placentation also contributes to the fetal and maternal complications of the disease. Clinically, abnormal uterine artery Doppler wave forms herald the development of preeclampsia, suggesting decreased placental perfusion.⁴⁸ Decreased placental perfusion in its more extreme cases results in fetal growth restriction, oligohydramnios, or intrauterine fetal demise. Interestingly, pregnant rats and baboons develop hypertension and proteinuria in response to surgically induced uteroplacental ischemia,^{49–50} implicating placental hypoxia in the development of the maternal disease.

Pro-Angiogenic factors and Vascular Homeostasis

Abnormalities in the placenta and resulting consequences to the fetus are a hallmark of preeclampsia, but the maternal features of the disease have been its most mysterious feature. Recently, circulating anti-angiogenic proteins have been implicated in the pathogenesis of many of the maternal features of the disease (see Figure 2). Prior to describing how release of these factors into the circulation may lead to the maternal syndrome, we review the evidence for the role of pro-angiogenic growth factors and their receptors in vascular homeostasis.

Vascular Endothelial Growth Factors

The vascular endothelial growth factors are secreted dimeric glycoproteins involved in vasculogenesis (the process by which new blood vessels are formed in embryonic life) and angiogenesis (the process by which blood vessels branch to form new blood vessels). In humans and other mammals this family of growth factors includes VEGF-A (VEGF) and

placental growth factor (PlGF), among others. VEGF-A (hereafter VEGF), the first discovered and prototypical protein in this family, is a pro-angiogenic factor which promotes the proliferation and survival of endothelial cells and induces vascular permeability.^{51–52} PlGF is a VEGF homolog released by the placenta which also has pro-angiogenic activity.⁵³

VEGF family receptors present on vascular endothelial cells include Flt-1 (VEGFR-1) and KDR (VEGFR-2, murine Flk-1). While VEGF binds to both Flt-1 and KDR receptors, PlGF homodimers bind exclusively to Flt-1.⁵⁴ KDR is thought to be primarily responsible for VEGF's action on endothelial cells.^{55–57} KDR null-mice die at embryonic day 8.5–9.5 with an absence of organized blood vessels and widespread necrosis, suggesting lack of perfusion to vital structures.⁵⁸ Flt-1 null mice die in embryonic life, with death due to the overgrowth of endothelial cells and resultant blood vessel disarray.⁵⁸ Mice with Flt-1 lacking the tyrosine kinase domain but with intact ligand binding domain have normal blood vessels and survive,⁵⁹ implying that Flt-1 acts as a negative regulator of angiogenesis through sequestration of extracellular VEGF rather than through intracellular action. More recently work by Chappell et al suggest that the role of Flt1 gene may be to express sFlt-1 (a soluble VEGF signaling inhibitor) which acts by regulating guidance of emerging vessel sprouts by modulating local VEGF availability.⁶⁰

VEGF is essential for embryonic vasculogenesis and angiogenesis. The ablation of a single VEGF allele results in markedly abnormal vasculature including the placental vasculature with death at embryonic day 10–12.⁶¹ Besides its essential role in placental and embryonic vasculogenesis and angiogenesis, VEGF is involved in the survival of endothelial cells and vascular homeostasis in mature vessels and tissues. In adult mice VEGF is expressed by cell types located adjacent to fenestrated endothelia including the epithelial cells of the choroid plexus, renal podocytes, and hepatocytes.⁶² *In vitro*, VEGF induces endothelial fenestrations,⁶³ while inhibition of VEGF in adult mice reduces the density of so-called VEGF dependent fenestrated capillaries.⁶⁴ Accordingly, targeted inhibition of VEGF *in vivo* leads to pathology in many of the organs with fenestrated endothelia, also affected in preeclampsia. Specifically, in the mouse kidney, podocyte selective knockout of VEGF in early post-natal life results in proteinuria, nephrotic syndrome, endotheliosis and eventual disappearance of endothelial cells from the glomerular tuft, recapitulating the classic renal lesion seen in preeclampsia.⁶⁵ In the liver, inhibition of VEGF signaling in early post-natal life leads to abnormal liver development with small hepatocytes and immature sinusoidal vasculature.⁶⁶ In adult mice, activation of the Flt-1 receptor on liver sinusoidal endothelial cells by VEGF or PlGF leads to elaboration of hepatocyte growth factor and liver enlargement.⁶⁷ Additionally, in the brain, inhibition of VEGF signaling results in decreased perfusion of choroid plexus vasculature.⁶⁸

VEGF also seems to have a direct vasodilatory effect on the systemic vasculature, as infusion of VEGF leads to nitric oxide dependent vasorelaxation in the coronary arteries and other vessels in dogs and humans,⁶⁹ likely through upregulation of nitric oxide and prostacyclin in vascular endothelial cells.^{70–71} Suggesting a role for VEGF in control of systemic blood pressure, antagonism of the KDR receptor leads to elevations in mean arterial pressure in mice by a nitric oxide dependent mechanism.⁷² Most relevant, VEGF inhibition has a biological effect on endothelial function in adult men and women. Side effects of VEGF inhibition in patients undergoing anti-angiogenic cancer therapy are consistent with animal models suggesting VEGF's homeostatic role in the mature vasculature: hypertension, proteinuria, glomerular endothelial damage, hypothyroidism, and in rare cases the reversible posterior leukoencephalopathy syndrome^{73–78}

PlGF, which has approximately 53% homology with VEGF, is expressed at high levels by the human placenta. PlGF homodimers do not bind to the KDR receptor, but do bind to the

Flt-1 receptor with high affinity. PlGF has weak mitogenic activity and no effect on vascular permeability *in vitro* alone, but potentiates the actions of VEGF in cultured endothelial cells and in an *in vivo* vascular permeability model.⁵⁴ In contrast to VEGF knockout mice, PlGF null mice have normal vascular development with the exception of subtle defects in the retinal vasculature and in luteal vasculogenesis which do not seem to affect retinal or reproductive functioning.⁷⁹ However, PlGF null mice do exhibit defects in tumor angiogenesis, post-ischemic retinal and myocardial neovascularization, and wound healing, suggesting that PlGF plays a role in angiogenesis in pathological settings.^{79–80} Consistent with this, PlGF stimulates angiogenesis in ischemic myocardium and arterial collateral growth in ischemic limbs. PlGF may act by displacing VEGF from the Flt-1 receptor, allowing it to bind to the more active KDR receptor.⁵⁴ Other possible mechanisms include direct effects of Flt-1 signaling and the formation of VEGF/PlGF heterodimers.^{81–82} During pregnancy, the placenta releases PlGF at high amounts into the maternal circulation. Levels increase beginning in the 2nd trimester, peak during weeks 29–32 and decline thereafter. However, because most *in vivo* investigation of PlGF has been conducted in non-pregnant animals, the function of PlGF in the physiology of normal pregnancy has not been well elucidated.

Transforming growth factor–beta (TGFβ)

The TGFβ family of proteins is made up of ubiquitous growth factors with diverse actions in many cell types. TGFβ is known to be involved in angiogenesis, however the mechanisms are not as well elucidated as those in the VEGF pathway. To initiate intracellular signaling, TGFβ and other proteins in this family must bind to both type I and type II receptors on the cell surface. TGFβ isoforms bind the TGFβ Type II receptor (TGFβII) initially with subsequent binding and activation of type I receptors. Mice null for the TGFβII receptor die at embryonic day 10.5 with defects in hematopoiesis and vasculogenesis,⁸³ implicating TGFβ in the development of the vasculature. Most cell types, including endothelial cells, express the activin-like kinase type I TGFβ receptor, ALK5, but endothelial cells alone express ALK1 type I receptor.⁸⁴ Like TGFβ, ALK1 is important in the development of blood vessels, as ALK1 null mice perish by embryonic day 11–12 with growth retardation with markedly reduced number of capillaries and dilation of larger vessels.⁸⁵ *In vitro*, TGFβ has differing effects on the activation state of endothelial cells dependent on the dose administered: at low TGFβ doses, ALK1 leads to proliferation and migration of endothelial cells; while at high TGFβ doses, ALK5 inhibits proliferation and migration of endothelial cells.^{84, 86} Moreover, TGFβ signaling regulates the expression of VEGF, connecting the two pathways and further linking TGFβ to angiogenesis.⁸⁷

TGFβ signaling in the vasculature also involves co-receptors which act to modulate TGFβ action. Endoglin is a TGFβ co-receptor expressed in endothelial cells and syncytiotrophoblasts of the placenta. Both human endoglin and ALK1 mutations independently cause hereditary hemorrhagic telangiectasia (Osler-Weber-Rendu disease), characterized by multisystemic vascular malformations.⁸⁸ Endoglin null mice die at embryonic day 10–12 with abnormal vasculature and internal bleeding.⁸⁹ On a molecular level, endoglin promotes and is required for TGFβ-ALK1 mediated endothelial cell proliferation and migration.⁸⁶

Besides being involved in angiogenesis in the embryonic phase, TGFβ likely functions in vascular homeostasis in mature vessels. In the mouse brain, TGFβ inhibition in combination with VEGF inhibition resulted in the loss of choroid plexus endothelial fenestrae with formation of periventricular edema detectable on magnetic resonance imaging (MRI).⁶⁸ Down regulation of TGFβ signaling in the retinal microvasculature leads to decreased perfusion, breakdown of the blood-retinal-barrier, reduced endothelium dependent

vasodilation, and increased endothelial cell apoptosis, suggesting a role for TGF β in the maintenance of the adult vasculature.⁹⁰

Anti-angiogenic Factors and Endothelial Dysfunction in Preeclampsia

In 1989 Roberts and Taylor advanced the hypothesis that preeclampsia results from the release of circulating factors by the placenta leading to widespread maternal vascular endothelial dysfunction.^{91–92} Several lines of evidence continue to support this understanding of the disease. The cardinal signs and symptoms of preeclampsia involve the vasculature, specifically areas of the vasculature with fenestrated endothelia. Furthermore, vessels isolated from the soft tissue of preeclamptic women demonstrate endothelial dysfunction, with impaired endothelium-dependent, but not endothelium-independent dilatation.^{93–94} Human studies have firmly established the presence of factors released by the injured or activated endothelium in the circulation of women with clinical preeclampsia. These include, among others, endothelin-1,⁹⁵ fibronectin,^{96–98} Von Willebrand factor,^{96, 99} thrombomodulin,^{100–101} markers of oxidative stress¹⁰², and inflammatory cytokines.¹⁰³ There is also evidence of deficiency of prostacyclin and nitric oxide, vasodilatory factors released by healthy vasculature, in the circulation of women with preeclampsia.^{104–106} Studies showing that serum from pregnant women with preeclampsia induces endothelial injury and dysfunction *in vitro* support the theory that a circulating factor causes the aforementioned endothelial dysfunction evident in the disease.^{92, 102, 107}

sFlt-1 and PlGF in the Pathogenesis of Preeclampsia

Because the vascular endothelium relies on pro-angiogenic factors, the release of anti-angiogenic factors by the placenta into the maternal circulation is a plausible cause of the endothelial dysfunction observed in preeclampsia. Investigation by our group and others has characterized two such anti-angiogenic proteins. Soluble fms-like tyrosine kinase (sFlt-1, also referred to as sVEGFR1), an anti-angiogenic protein, is a soluble form of the VEGF/PlGF receptor Flt-1 produced by alternative splicing¹⁰⁸. sFlt-1 was initially identified as a product of cultured human endothelial cells and subsequently shown to be produced by the placenta and released into the maternal circulation.^{108–110} sFlt-1 is a potent inhibitor of VEGF and PlGF activity: recombinant sFlt-1 inhibits endothelial tube formation and blocks the vasodilatory effect of VEGF and PlGF *in vitro*.¹⁰⁸ Our group identified elevated expression of sFlt-1 by gene expression profiling in placentas delivered from women with preeclampsia.³¹ Since that time several novel sFlt-1 variant isoforms have been identified and shown to be upregulated in preeclampsia.^{111–113}

Animal data supports a causal role for sFlt-1 in the pathogenesis of the maternal disease. Administration of sFlt-1 to pregnant rats using an adenoviral vector induced hypertension and proteinuria and caused glomerular endotheliosis, the classic renal lesion seen in preeclampsia.³¹ Other groups have also generated animal models which implicate sFlt-1 in the pathogenesis of preeclampsia. These include rat and baboon models in which uterine hypoxia induced elevated production of sFlt-1, hypertension, and proteinuria,^{50, 114} as well as a mouse model in which sFlt-1 expression in pregnant mice resulted in hypertension, decreased platelet count, and reduced fetal weight.¹¹⁵

Soluble Endoglin in the Pathogenesis of Preeclampsia

Notably absent from the phenotype of rats administered sFlt-1 is the liver dysfunction and cerebral changes seen in women with severe preeclampsia. Soluble endoglin (sEng) is another anti-angiogenic protein identified by gene expression profiling of placentas from women with preeclampsia. sEng may combine with sFlt-1 to induce features of severe preeclampsia including liver dysfunction, fetal growth restriction, coagulation, and

neurologic abnormalities.^{32, 68} Our group identified the 65kDa soluble endoglin monomer produced by placentas from preeclamptic women at a level 4 fold higher than placentas from women with normal pregnancies. Subsequent *in vitro* studies demonstrated that sEng reduces the binding of TGF β 1 to its receptor and blocks TGF β 1 induced vasodilation of rat vessels, likely through down regulation of nitric oxide synthase.³² Furthermore, sEng reduced endothelial tube formation *in vitro* and led to increased capillary permeability in mouse lung, liver, and kidney. Importantly, when pregnant rats were injected with both sFlt-1 and sEng, a condition reminiscent of severe preeclampsia develops with hypertension, nephrotic range proteinuria, low platelet count, elevated liver enzymes, and reduced fetal weight.³² Thus, most, if not all, clinical manifestations of preeclampsia can be explained by the anti-angiogenic actions of sFlt-1 and sEng on the maternal endothelium. More recent studies have shown that mice injected with both sFlt-1 and sEng expressing adenoviruses, but not those injected with adenoviruses expressing either molecule alone, exhibit not only decreased cerebral perfusion and vascular thrombi but also loss of choroid plexus endothelial fenestrae, choroid plexus endothelial swelling, and cerebral edema on brain MRI.⁶⁸ While women with eclampsia are known to have brain edema and white matter lesions on MRI, it remains to be seen whether the histopathologic changes in women with preeclampsia with neurologic involvement are similar to those seen in sFlt-1 and sEng injected mice.

Human Studies of sFLT-1, PlGF, and sEng in Preeclampsia

Epidemiologic studies have revealed that blood levels of sFlt-1 and PlGF are altered in women with preeclampsia both during and prior to clinical signs and symptoms of the disease, consistent with a pathogenic role for these angiogenic factors in preeclampsia. sFlt-1 is present at relatively high concentrations in the serum of normal pregnant women at term¹¹⁶, but declines to non-pregnant levels 48 hours after delivery.³¹ In preeclampsia, sFlt-1 levels begin to rise at least 5 weeks prior to the onset of clinical disease and remain elevated compared to non-affected women.^{34, 36, 117} Alterations in sFlt1 are more dramatic in patients who have early onset preeclampsia (PE<37 weeks).³⁴ Levels of sFlt-1 also correlate with the severity of the disease.³⁷ In pregnancies afflicted by severe IUGR without preeclampsia, there may also be a modest elevation of sFlt-1 levels.¹¹⁸

Consistent with the pathophysiology suggested by animal models, levels of free PlGF are depressed in women with preeclampsia. In fact, low PlGF levels in the first trimester, prior to the sFlt-1 rise, are a risk factor for subsequent preeclampsia.^{34, 119} PlGF can also be measured in the urine of women destined to develop preeclampsia, where levels are depressed compared to normotensive pregnant women beginning at 25 weeks. The degree of suppression of urine PlGF levels is correlated with the severity of the disease.¹²⁰ In contrast, although circulating free VEGF levels are low in preeclampsia, it is not useful clinically since the majority of patients have levels below the detection limit of the currently available ELISA kits. Of note, the ratio of sFlt-1 to PlGF is a better marker of preeclampsia than either measure alone.^{121–122} This implies that it is an imbalance of anti- and pro-angiogenic factors rather than the level of either sFlt-1 or PlGF alone leads to preeclampsia.³⁴

Studies of sEng levels in women with and without preeclampsia are consistent with the animal studies, supporting a role for elevated sEng in the pathogenesis of severe preeclampsia. sEng levels in women with normal pregnancies are stable until approximately week 33 of pregnancy, when they rise, peaking at delivery.³³ In women with preeclampsia beginning before 37 weeks of gestation (preterm) levels of sEng begin to rise earlier, by 20 weeks gestation and rise more steeply after 33 weeks. Women with preeclampsia beginning after 37 weeks of gestation (term) also have elevated 3rd trimester sEng levels, but a slower rise, beginning at 25 weeks gestation and rising steeply around 33 weeks of gestation.³³ The

combination of sFlt-1, PlGF and sEng level characterize preeclampsia better than any single analyte, linking the combined action of several angiogenic factors to clinical preeclampsia.^{33, 123} High circulating levels of both sEng and sFlt-1/PlGF are usually observed before onset of preterm preeclampsia.^{33–34} Consistent with this, more recent data suggests that alterations in sFlt-1, PlGF, and sEng in women with preeclampsia are associated with maternal vascular dysfunction and impaired nitric oxide formation.^{106, 35} In preeclampsia-associated placental abruption, sFlt-1, PlGF, and sEng levels have all been shown to be altered.^{124–125} In eclampsia, sFlt-1, PlGF, and sEng are also altered to a similar degree as in patients with severe preeclampsia, reiterating the combined role of these factors in both of these conditions.¹²⁶

Other Contributory Factors to the Development of Preeclampsia

Several factors that are upstream to the angiogenic proteins have been tied to the pathogenesis of preeclampsia (see Figure 3). Many of these factors have also been shown to influence sFlt-1 or sEng expression; they may be modulators of their ultimate concentrations in the maternal circulation.

Placental Hypoxia

Because placental hypoxia is thought to be involved in the pathogenesis of preeclampsia (see prior discussion in this review), the effect of hypoxia on sFlt-1 expression has been an area of active investigation. *In vitro*, culture of cytotrophoblast cells at low oxygen tension has been shown to induce the expression and release of sFlt-1.^{127–128} sFlt-1 has also been studied in pregnant animal models of preeclampsia in which surgical uteroplacental ischemia is induced and causes hypertension and proteinuria. In both rat¹¹⁴ and baboon⁵⁰ surgical uterine ischemia has been shown to induce elevated circulating sFlt-1 levels in addition to the preeclampsia-like syndrome. Overexpression of the stabilized form of hypoxia inducible factor 1- α (HIF-1 α) during mouse pregnancy is associated with a preeclampsia-like phenotype including elevated levels of sFlt-1 and sEng.¹²⁹ However, hypoxia studies in animals are associated with enhanced trophoblast invasion¹³⁰ suggesting that abnormal placentation is the primary event that leads to placental hypoxia which in turn may liberate soluble factors necessary for the maternal syndrome.

Immune Factors

Risk factors for preeclampsia related to paternal antigen exposure suggest that immunologic dysfunction at the fetal-maternal interface may contribute to preeclampsia. Moreover, women without an intact immune system, due to untreated HIV, rarely develop preeclampsia, while HIV treatment and resulting immune reconstitution brings the risk of preeclampsia to levels seen in the general population.¹³¹ Normal placentation requires an immune tolerance for fetal antigen which may be altered in preeclampsia, as pathological examination of preeclamptic placentas reveals increased dendritic cell and macrophage infiltration as well as signs of chronic inflammation.^{41, 132–133, 134} Dysregulated complement system has also been proposed as a regulator of placental angiogenesis in animal models.¹³⁵ Decidual NK cells, which promote angiogenesis and are involved in trophoblast invasion may contribute to the abnormal placental development seen in the disease.¹³⁶ These cells are further implicated by genetic studies finding associations between polymorphisms in killer immunoglobulin receptors (KIR, present on NK cells), HLA-C (KIR ligands present on trophoblasts) and preeclampsia.¹³⁴ These studies provide compelling evidence that immune dysregulation is involved in preeclampsia pathogenesis, but the mechanisms by which this occurs have so far not been well elucidated.

Renin-Angiotensin Aldosterone Pathway

Normal pregnancy is characterized by resistance to the vasoconstrictive effects of angiotensin II.¹²⁴ Levels of renin, angiotensin, and aldosterone are increased despite an overall decrease in systemic vascular resistance.^{137–139} In pregnancy induced hypertension (preeclampsia or gestational hypertension), this resistance is blunted, resulting in increased sensitivity to angiotensin II when compared to normotensive pregnant women.¹²⁴ Circulating angiotensin receptor AT1 activating autoantibodies levels are elevated may explain the hypersensitivity to the effects of angiotensin in preeclampsia.¹⁴⁰ When injected into pregnant mice, these auto-antibodies lead to hypertension, proteinuria, glomerular endothelial damage, and elevated levels of sFlt-1 and sEng, and thus may contribute to the pathogenesis of preeclampsia.¹⁴¹ Since in some women with a history of preeclampsia auto-antibodies remain elevated, they may also contribute to the development of hypertension in later life.¹⁴² Recently, a novel form of circulating oxidized angiotensinogen, which enhances angiotensin formation has been found in the circulation of preeclamptic subjects.¹⁴³ However, circulating angiotensin II and aldosterone are suppressed in preeclamptic subjects (and not elevated as one might predict). Studies are needed to evaluate if this oxidized form of angiotensinogen is altered prior to clinical disease.

Alterations in Placental Enzymes

Recently, genetic knockout of the catechol-O-methyltransferase (COMT) enzyme has been shown to recapitulate some signs and symptoms of preeclampsia.¹⁴⁴ COMT knockdown leads to a deficiency of 2-methoxyestradiol (2-ME), an inhibitor of the HIF 1 α , a transcription factor which acts as a mediator of the cellular response to hypoxia. COMT knockout mice develop placental hypoxia, hypertension, proteinuria and modestly elevated levels of sFlt-1 in contrast to wild type mice. Deficiency of 2-ME is also demonstrable in the serum of women with preeclampsia, thus in some women decreased COMT expression or 2-ME deficiency may be proximal to the elevated levels of sFlt-1.¹⁴⁴ Heme oxygenase-1 (HO-1), a placental enzyme whose product is carbon monoxide (CO), is thought to be a negative regulator of sFlt-1 production. *In vitro* studies demonstrate that overexpression of HO-1 or CO production inhibits sFlt-1 release from placental explants.¹⁴⁵ Consistent with these *in vitro* studies, end tidal CO levels are lower in women with preeclampsia.¹⁴⁶ The suppression of sFlt-1 by CO may also explain the lower risk for preeclampsia in smokers.¹⁴⁷

Oxidative Stress/Placental Debris

In preeclampsia, oxidative stress is demonstrable both in the placenta and in the maternal circulation.^{148–149} Preeclamptic placentas produce greater quantities of superoxide and have less antioxidant capacity compared with normal placentas. Maternal serum from preeclamptic pregnancy shows evidence of oxidative modification of protein and lipoprotein particles. Blood levels of antioxidants have also been reported to be decreased in women with preeclampsia. Unfortunately, large randomized controlled trials did not show an effect of antioxidants vitamin C and vitamin E on the risk of preeclampsia.^{150–154} The shedding of placental debris has also been suggested to cause elevated oxidative stress and endothelial dysfunction in preeclampsia. Placental abnormalities and uteroplacental ischemia may induce the shedding of placental microparticles into the maternal circulation and these particles may lead to inflammation and vascular damage.¹⁵⁵ Consistent with this, women with preeclampsia have elevated circulating levels of placental debris.^{156–157} Interestingly, these microparticles have been shown to be associated with sFlt-1 in the maternal circulation, and thus may be an additional source of circulating sFlt-1 in preeclampsia.¹⁵⁸

Each of these factors may have a role in the regulation and release of anti-angiogenic factors into the maternal circulation, but none has been shown to be primarily responsible for the imbalance of angiogenic proteins found in preeclampsia. It is possible that the dysregulation

of these factors has multiple etiologies, with over production of anti-angiogenic factors the final common pathway to preeclampsia. Alternatively, the imbalance in angiogenic proteins may be the primary derangement, since angiogenic factors are intimately involved in early placental development (reviewed in Khankin et al).¹⁵⁹ The regulation of the expression of angiogenic proteins in the placenta is an area of active investigation.

Implications for Diagnosis and Treatment

Currently there is no laboratory test which provides a reliable diagnosis of preeclampsia. This is problematic as hypertension and proteinuria are not specific to preeclampsia and the treatment for preeclampsia (delivery) puts the preterm fetus at risk. Many women present with "atypical" preeclampsia, without either hypertension or proteinuria, and some of these women go on to have unexpected severe disease.²⁸ Since the initial reports that sFlt-1 may be intimately involved in preeclampsia pathogenesis, several studies have demonstrated the ability of the ratio of sFlt-1 and PlGF to distinguish women with and without preeclampsia, using newly developed automated assays with sensitivities and specificities greater than 95% for preterm preeclampsia.^{121, 160–161} Moreover, the measurement of anti-angiogenic proteins seems to distinguish preeclampsia in women with chronic diseases who may have hypertension or proteinuria for other reasons, including diabetes and systemic lupus erythematosus.^{162–163} Levels of anti-angiogenic factors similarly differentiate women with HELLP from women with low platelets due to other conditions including thrombotic thrombocytopenic purpura.¹⁶⁴ However, not all patients with preeclampsia have been reported to have altered sFlt1 and PlGF.¹⁶⁵ Whether these patients presenting with low levels of sFlt1 and signs/symptoms of preeclampsia represent a non-angiogenic form of the disease or are simply misdiagnosed remains unknown. One possibility is that women with underlying vascular disease may develop preeclamptic signs/symptoms at relatively lower levels of sFlt-1.¹⁶⁶ Future prospective studies and clinical trials will define how best to use levels of angiogenic proteins in clinical management. The implication of sFlt-1 in the pathogenesis of preeclampsia also opens the possibility for the development of new targeted therapies. Administration of VEGF rescues the phenotype produced by either sFlt-1 administration^{167–168} or uterine hypo-perfusion in rats,¹⁶⁹ suggesting that VEGF itself or compounds which mimic its actions such as PlGF may hold promise as treatments for preeclampsia and related conditions. Levels of anti-angiogenic factors may also serve as a useful intermediate outcome in initial trials of potential preeclampsia therapies in humans and animals.

Implications for Later Cardiovascular Disease

Delivery of the placenta cures preeclampsia, yet affected women continue to have an elevated risk of cardiovascular disease many years postpartum. Large retrospective epidemiologic studies have consistently demonstrated an elevated risk for many types of cardiovascular disease in women with a history of preeclampsia.^{170–173} According to a recent meta-analysis, the prevalence of hypertension in women with previous preeclampsia is greater than 50% an average of 14 years after pregnancy, 3–4 times the risk found in women without preeclampsia.¹⁷⁴ Similarly, the risk of death from cardiovascular disease and cerebrovascular disease is about 2-fold greater in women with a history of preeclampsia. Women who have had preeclampsia before 34 weeks or preeclampsia combined with preterm birth, have even higher risk of death from cardiovascular disease, 4–8 times the risk of women who had a normal pregnancy.^{172–174}

The mechanisms which account for an increased risk of cardiovascular disease in women with a history of preeclampsia are not yet well understood, but endothelial dysfunction, which has been linked to atherosclerosis, persists in formerly preeclamptic women many

years after an affected pregnancy. Three months up to at least 3 years postpartum, women with prior preeclampsia demonstrate a decrement in endothelium dependent dilatation.¹⁷⁵ Women with a history of preeclampsia also have been reported to be sensitive to angiotensin II and salt.¹⁷⁶ In addition, markers of endothelial activation including VCAM-1 and ICAM-1 appear to be higher more than 15 years after pregnancy in women with previous preeclampsia, independent of body mass index and smoking.¹⁷⁷ It is possible that shared risk factors may jointly predispose to preeclampsia, endothelial dysfunction, and cardiovascular disease. In this regard, diabetes mellitus, chronic hypertension, and renal disease prior to pregnancy all confer an elevated risk for preeclampsia. Even subclinical insulin resistance and inflammation, well known to elevate the risk of cardiovascular disease, predispose women to preeclampsia and persist up to 30 years after the disease.^{16, 26, 178–181} Endothelial dysfunction and cardiovascular disease following preeclampsia may be attributable to these preexisting risk factors and others yet unknown. A recent study of cardiovascular risk factors present before and after pregnancy suggests that nearly half of the elevated risk for future hypertension after preeclampsia can be explained by pre-pregnancy risk factors.¹⁸² Therefore, pregnancy may be viewed as a stress test that can reveal subclinical cardiovascular disease phenotypes long before overt disease (see Figure 4) However, further investigation will be necessary to determine whether preeclampsia itself may injure the endothelium and thereby increase the risk of atherosclerosis and cardiovascular disease.

Although levels of sFlt-1 decline after delivery of the placenta, a persistent and subtle anti-angiogenic milieu may contribute to lasting endothelial dysfunction and an elevated risk of cardiovascular disease in women with a history of preeclampsia. Some^{176, 179}, but not all studies^{35, 184–185} have shown that levels of sFlt-1 remained higher in women with a history of preeclampsia compared to those without preeclampsia an average of 18 months postpartum, independent of body mass index, blood pressure, and smoking. The source of sFlt-1 in non pregnant individuals may be peripheral blood mononuclear cells, as monocytes in women with preeclampsia produce elevated levels of sFlt-1 compared to those from control subjects.^{186–187} Persistent alterations in the levels of anti-angiogenic proteins may explain not only the elevated cardiovascular disease risk but also the observed lower risk of malignancy,¹⁷⁴ and higher risk of acquired hypothyroid function¹⁸⁸ in women with a history of preeclampsia. The role of anti-angiogenic proteins in the pathophysiology of cardiovascular disease has not been well worked out. In fact, angiogenesis has been viewed as both a pathogenic and therapeutic factor in cardiovascular disease (reviewed by Khurana et al).¹⁸⁹ A recent study in 130 patients with chronic kidney disease (CKD), suggests that elevated levels of sFlt-1 in this group of patients may contribute to endothelial dysfunction and cardiovascular disease risk. Serum from these patients had anti-angiogenic activity compared to control serum, which could be attenuated with the administration of an anti-sFlt-1 antibody,¹⁸⁷ and sFlt-1 levels were greater in subjects who had a history of myocardial infarction or stroke. Relatively high sFlt-1 levels are also associated with carotid intima-media thickness and progression of atherosclerosis in hypertensive subjects.¹⁹⁰ Studies of cardiovascular function and atherogenic potential in animal models expressing high levels of sFlt-1 chronically would add insight as to whether anti-angiogenic molecules are possible contributors to cardiovascular disease in women. Pulmonary and systemic vascular dysfunction in young offspring of mothers with preeclampsia have also been recently described, however the mechanisms mediating these phenotypes are largely unknown.¹⁹¹

Conclusions

Preeclampsia is a disease which begins in the placenta and ends at the maternal endothelium. As reviewed here, evidence suggests that the maternal disease is attributable to anti-

angiogenic factors sFlt-1 and sEng, released by an abnormal placenta. These anti-angiogenic factors antagonize the effects of pro-angiogenic factors VEGF, PlGF, and TGF β , which are important in the maintenance of the vascular endothelium. While these anti-angiogenic proteins likely cause the maternal disease and may prove to be a useful diagnostic aid, the primary cause of the placental disease is an active area of investigation. Preeclampsia portends future cardiovascular disease, and research into mechanisms by which this increased risk occurs, including the possible role of pro-angiogenic and anti-angiogenic factors, may lead to new insights into the pathogenesis of cardiovascular disease in women.

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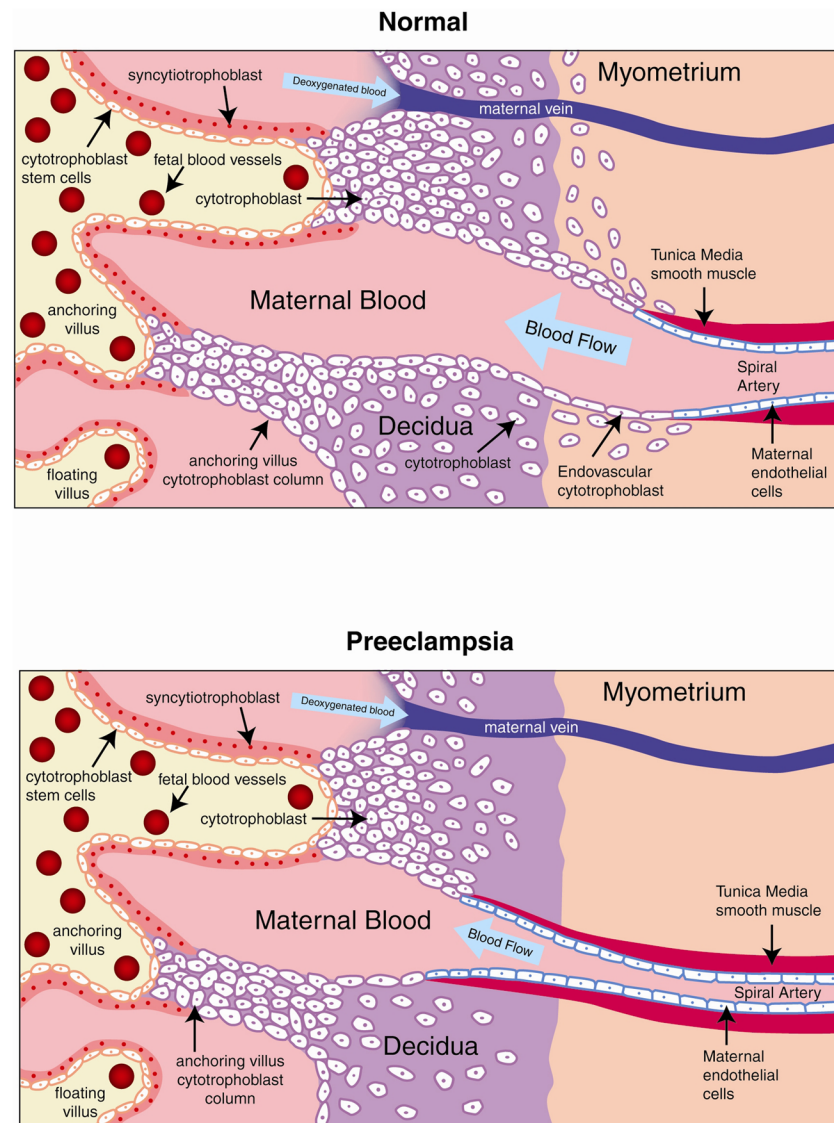


Figure 1. Abnormal Placentation in Preeclampsia

In normal placental development, invasive cytotrophoblasts of fetal origin invade the maternal spiral arteries, transforming them from small-caliber resistance vessels to high-caliber capacitance vessels capable of providing placental perfusion adequate to sustain the growing fetus. During the process of vascular invasion, the cytotrophoblasts differentiate from an epithelial phenotype to an endothelial phenotype, a process referred to as “pseudovasculogenesis” or “vascular mimicry” (Upper Panel). In preeclampsia, cytotrophoblasts fail to adopt an invasive endothelial phenotype. Instead, invasion of the spiral arteries is shallow and they remain small caliber, resistance vessels (Lower Panel). Figure reproduced with permission from Lam et al.⁴⁵

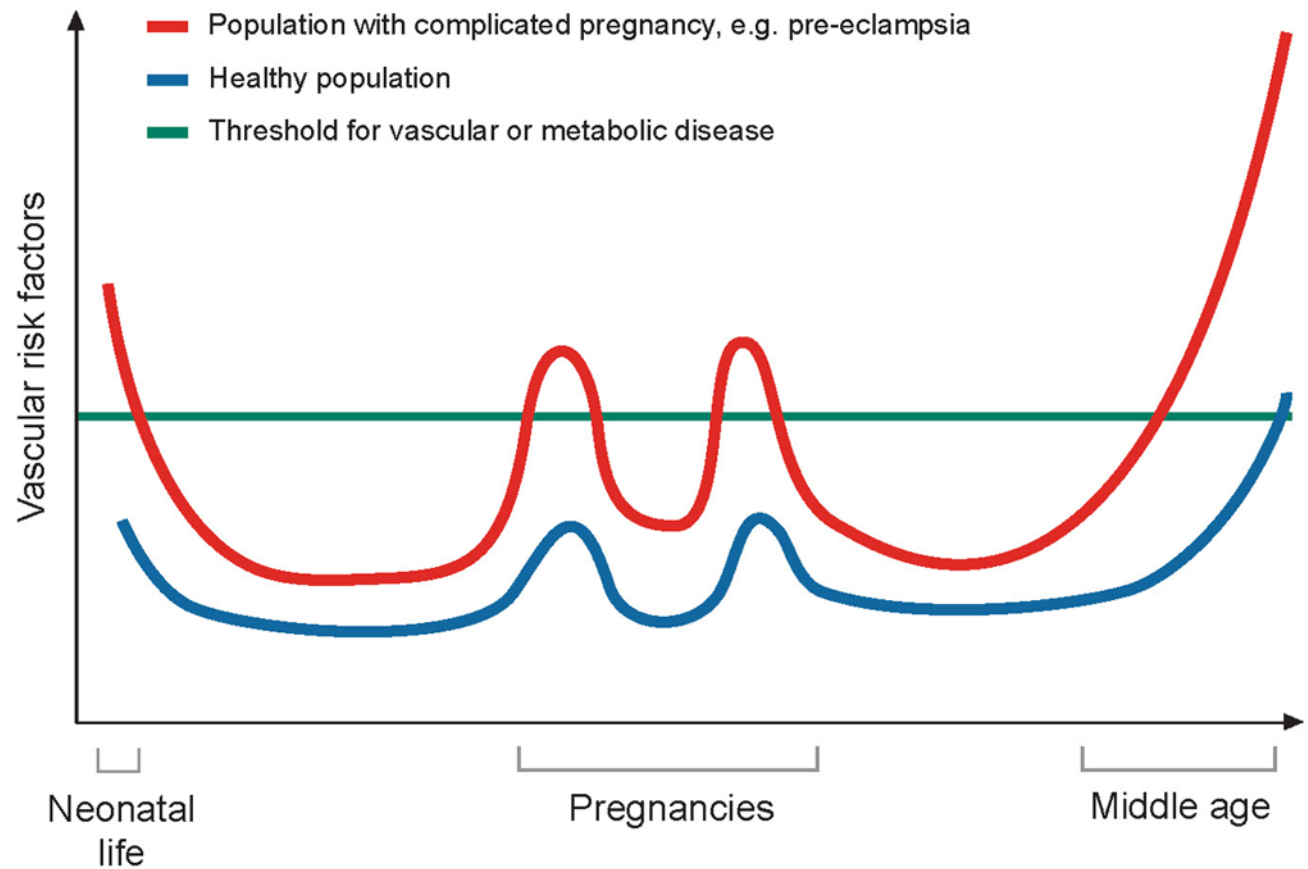


Figure 2. sFlt1 and sEng Causes Endothelial Dysfunction by Antagonizing VEGF and TGF- β 1 signaling

There is mounting evidence that VEGF and TGF- β 1 are required to maintain endothelial health in several tissues including the kidney and perhaps the placenta. During normal pregnancy, vascular homeostasis is maintained by physiological levels of VEGF and TGF- β 1 signaling in the vasculature. In preeclampsia, excess placental secretion of sFlt1 and sEng (two endogenous circulating anti-angiogenic proteins) inhibits VEGF and TGF- β 1 signaling respectively in the vasculature. This results in endothelial cell dysfunction, including decreased prostacyclin, nitric oxide production and release of procoagulant proteins.

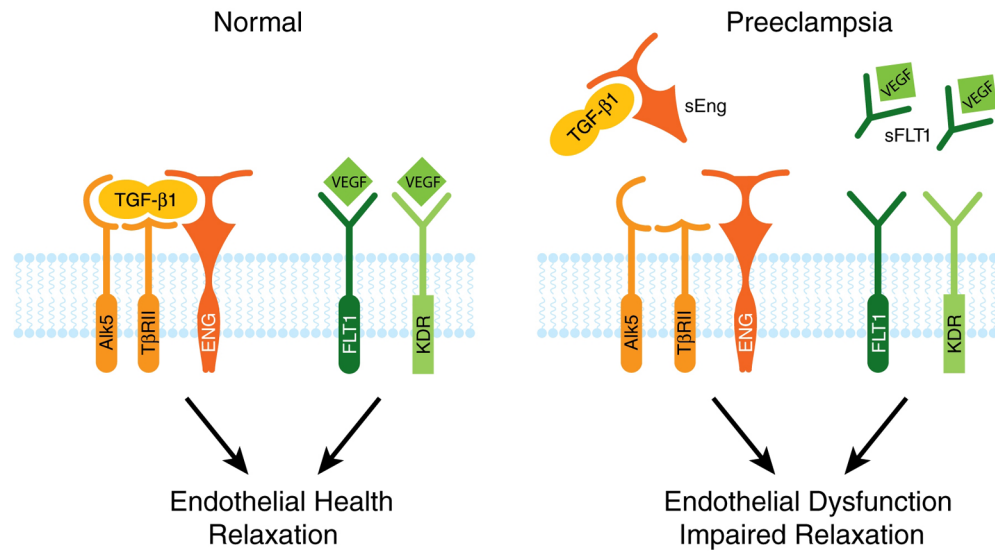


Figure 3. Summary of the pathogenesis of preeclampsia

AT_1 -AA, immunological factors, oxidative stress and other factors (such as decreased hemoxygenase expression) may cause placental dysfunction which in turn leads to the release of anti-angiogenic factors (such as sFlt1 and sEng) and other inflammatory mediators to induce preeclampsia

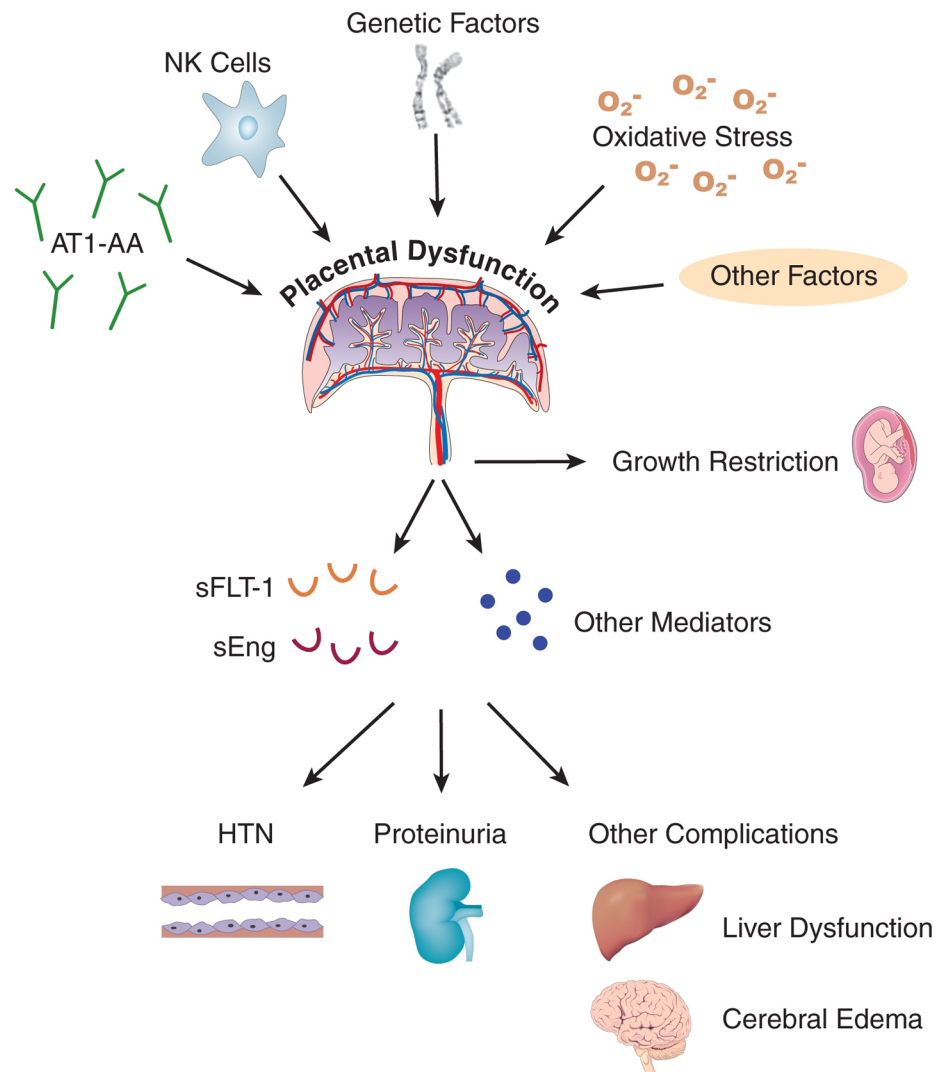


Figure 4.

Model for pregnancy as a stress test for long term cardiovascular disease. This model suggests that the women who develop preeclampsia (red line) have greater underlying vascular risk. This manifests during pregnancy (two pregnancies are represented) in the form of preeclampsia as well as later in life in the form of hypertension, heart disease, stroke, and other forms of cardiovascular disease. In women without these underlying vascular risk factors (blue line), pregnancy may increase vascular risk slightly, but not enough to lead to preeclampsia. These women have a lower cardiovascular risk both during pregnancy and later in life. Figure adapted from Sattar et al ¹⁸³