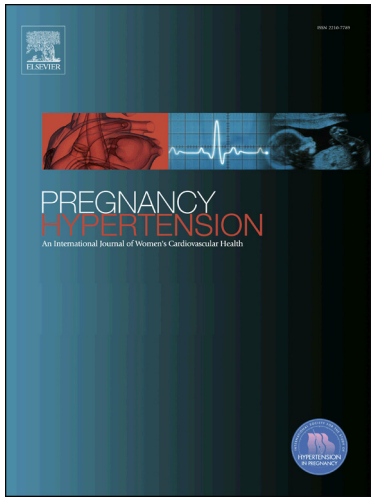
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**Placental Growth Factor: A review of literature and future applications**

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**Placental Growth Factor: A review of literature and future applications**

## Introduction:

Placental growth factor is an angiogenic protein, highly expressed during pregnancy, which correlates well with placental function. In this review, we highlight the origin, structure and function of Placental Growth Factor and its receptors. We discuss how their pro-angiogenic/anti-angiogenic synergism is critical for successful placentation and how their imbalance may be utilised as a diagnostic marker of disease or a potential therapeutic target for adverse pregnancy outcomes. .

## Discovery, Structure & Function

Placental growth factor (PlGF) is a member of the vascular endothelial growth factor (VEGF) family of proteins. Crystallography resolution at 2.0 Å resolution shows PlGF to have a three-dimensional structure comprising of 149–amino-acids. Comparison with that of VEGF-A shows a remarkable similarity between the two proteins, with 53% sequence identity between amino acids from positions 39-132 of PlGF and amino acids from positions 38-131 of VEGF-A. (1, 2) PlGF was the second member of the vascular endothelial growth factor (VEGF) family identified, VEGF-A having been described in 1989. (3-5) PlGF, like all proteins of the VEGF family, is a secreted dimeric glycoprotein with a distinctive cystine knot. This knot is characterised by a common motif of eight spatially conserved cysteines, which are involved in intra and inter molecular disulfide bonds. (6, 7) The two monomers are oriented side-by-side and head-to-tail and held together by one interchain disulfide bond. The dimeric structure is also stabilised by a hydrophobic core region. (1, 8) Its discovery is credited to Italian scientist Dr. Maria Graziella Persico who first described PlGF in 1991. She identified it while investigating the angiogenic potential of human term placental tissue which is why this protein was termed “the placental growth factor”. (9) In 1993 the location of the human PlGF gene on chromosome 14q24 was isolated and reported to consist of seven exons spanning 13.7 kb. (10)

PlGF can exist in multiple isoforms due to alternate splicing encoded by the human PlGF gene. Four isoforms of human PlGF are described, PlGF-1, PlGF-2, PlGF-3, and PlGF-4 composed of 131, 152, 203 and 224 amino acids respectively. PlGF-1 and PlGF-2 are believed to be the major isoforms. (10-14) Apart from in size, the PlGF isoforms differ in terms of both their secretion properties and their binding affinities. PlGF-1 and PlGF-3 are non-heparin binding diffusible isoforms while PlGF-2 and

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PlGF-4 have additional (highly basic 21 amino acids) heparin binding domains. (10, 12, 15) Both VEGF-A and PlGF can exist as homodimers and heterodimers (PlGF:PlGF, PlGF:VEGF-A, VEGF-A:VEGF-A), with PlGF:VEGF heterodimers displaying 20-50-fold less mitogenic activity than VEGF homodimers (Figure 1). (16, 17)

Initial studies conducted in healthy mice in the 1990's reported a lack of PlGF did not appear to confer any negative impact on vascular development. However, when mice deficient in PlGF (knockout) were subjected to pathological conditions such as ischaemia, inflammation or cancer, they demonstrated severely impaired angiogenic ability. Their inability to adapt and compensate to these pathological conditions highlighted the role of PlGF in pathological angiogenesis. (18)

**Receptors**

All members of the VEGF family bind and activate one of the following three homologous tyrosine kinase receptors: VEGFR1 (also called fms-like-tyrosine-kinase receptor/**Flt-1**) VEGFR2 (also called **Flk-1**/KDR**)** or VEGFR3. Each of these receptors has a similar structure; a tyrosine kinase extracellular seven Ig-like domain connected to an intracellular tyrosine kinase domain via a single transmembrane helix. (19-21) Binding induces the mitogenic action of the cell with KDR being 10 fold stronger than Flt-1 in this regard. (22, 23) VEGF-A can bind to either Flt-1 or KDR (24). Despite the structural similarity with VEGF-A, PlGF has been shown to only bind to Flt-1, but it does so with a higher affinity than VEGF-A. (25) In 1996, a non-membrane bound soluble receptor known as soluble VEGFR-1 (**sFlt-1**) was identified. This endogenous protein, synthesised by placental cells amongst others, arises from alternative splicing of Flt-1. It retains structural similarity to Flt-1 except that it lacks its transmembrane helix and tyrosine kinase intracellular domain, meaning that it can circulate freely. (26) Levels of sFlt-1 rise under hypoxic conditions. (27) Circulating sFlt-1 binds PlGF and VEGF-A resulting in reduced levels of these proteins available to the anchored cell membrane receptors Flt-1 and Flk-1 (Figure 1).

**Function**

PlGF exerts its angiogenic effects by both direct and indirect mechanisms, inducing receptor dimerisation and phosphorylation. PlGF directly activates endothelial cells, macrophages and haematopoietic progenitor cells by binding to the membrane receptor anchored Flt-1, and in doing so may increase the sensitivity of the cell to VEGF-A. PlGF acts indirectly by displacing VEGF-A from Flt-1, allowing VEGF-A to bind instead to the more potent FDK. Lastly, by forming a heterodimer with VEGF in mutually expressed cells, PlGF antagonises the angiogenic action of VEGF. (13, 17, 18, 28-30) sFlt-1 has anti-angiogenic potential, binding and neutralising PlGF and VEGF in the circulation,

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reducing their bio-availability and thus interaction with the cell membrane bound Flt-1 and Flk-1. (31, 32) In vitro effects of sFlt-1 include vasoconstriction and endothelial dysfunction. (33)

**Pre-eclampsia**

As far back as the late 1980's it had been hypothesised that a circulating factor existed in pre-eclampsia, which was responsible for the widespread endothelial dysfunction observed. (34) Vasculogenesis and angiogenesis are two essential components in development of the utero-placental circulatory interface in early pregnancy. It has been proposed that early placentation in utero occurs in a relatively hypoxic environment with a partial pressure of oxygen as low as 18 mm. At approximately 10 weeks gestation, an increase in the partial pressure of oxygen up to 60 mm occurs, triggering the proliferation of the cytotrophoblast. This process facilitates invasion of uterine spiral arteries of the decidua and myometrium by the cytotrophoblast, allowing these vessels to become functionally capable of supplying the high volumes of well-oxygenated blood necessary for nourishing a growing fetus. (35, 36) Should this rise in oxygen pressure fail to occur, the hypoxic environment persists, compromising the invasion of the cytotrophoblast. This leads to impaired placentation and results in inadequate placental perfusion, hypoxia and potential clinical fetal and maternal manifestations: impaired fetal growth and preeclampsia later in pregnancy. (37, 38)

Studies investigating this circulating factor hypothesis reported significant damage occurring in cultured human umbilical vein endothelial cells (HUVECs) exposed to serum from pregnant women with pre-eclampsia compared with controls. (37-39)

Following the discovery of PlGF and its receptors, reports of increased levels of sFlt-1 and reduced levels of PlGF and VEGF in pre-eclamptic cases compared to controls were published. (40-43) Higher levels of sFlt-1 have also been reported in; first versus second pregnancies, multiples versus singletons, molar and trisomy 13 affected pregnancies, all of these being well established independent risk factors for development of pre-eclampsia. (44) Patients receiving VEGF antagonists for cancer treatment may develop hypertension and proteinuria, confirming the role of VEGF/PlGF blockade in endothelial cell dysfunction. (45, 46) In the early 2000's, a number of publications reported circulating levels of sFlt-1 and PlGF to be altered several weeks before the clinical onset of disease in pre-eclampsia. They also showed correlation of these angiogenic factors with the severity of disease. (47, 48) Hypoxia alone is enough to trigger sFlt-1 over expression by the placenta, in a self-defence type response, to VEGF-A produced by maternal decidual cells. (49) A three stage disorder has now been proposed for pre-eclampsia. Initially in early pregnancy from approximately

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week 8-18 abnormal remodelling of the spiral arterioles and trophoblast invasion occurs due to a deficiency in the pro-angiogenic PlGF and VEGF (Stage 1). The net result of this is impaired placental perfusion, which in turn leads to hypoxia and oxidative damage from 20 weeks gestation (Stage 2). The pathological placenta then induces apoptosis, inflammation, and releases anti-angiogenic factors (sFlt1) and other inflammatory agents such as cytokines in a bid to induce vasoconstriction and increase oxygen supply to the hypoxic placenta. The net result of the release of these factors is systemic endothelial cell dysfunction and end-organ ischemia, which leads to the classical clinical signs and symptoms of pre-eclampsia (Stage 3) which may occur from as early as 20 weeks gestation. (50, 51)

**Application**

In the last 15 years, the concept of using sFlt-1 or PlGF, either singly or in combination, as a potential screening tool or diagnostic marker for pre-eclampsia has been explored. Screening for pre-eclampsia appears to make sense. The ability to stratify women in early (11-13 weeks gestation) pregnancy as high risk and appropriately tailor antenatal care has huge clinical and economic benefits. Currently a huge amount of our antenatal resources are targeted towards routine clinic visits including measurement of blood pressure and urinalysis in women at low or moderate risk for pre-eclampsia. An effective early screening test would enable those at low risk to be identified, and stratified to community based care, with less frequent hospital review. Simultaneously, the early identification of women at risk of pre-eclampsia would enable their antenatal care to be appropriately tailored and hospital resources to be focused to them. In order to be effective, a screening test must demonstrate both clinical and health economic benefits. Clinically relevant outcomes would include both maternal and neonatal morbidity. A health economic model analysis should assess the cost-effectiveness of a screening strategy relative to no screening at all. (52)

The recently published ASPRE trial showed a reduction of more than 60% in rates of preterm pre-eclampsia when aspirin is commenced prior to 14 weeks in women at high risk of same. (53) Identification of who exactly is high risk is paramount. The ASPRE trial screened over 25,000 women between 11-13 weeks gestation and dichotomised them to high (> 1 in 100) or low risk (<1 in 100) of preterm pre-eclampsia. The predictive model used incorporated maternal serum PlGF, pregnancy associated plasma protein–A (PAPP-A), mean arterial pressure (MAP) and uterine artery pulsatility index (UtA-PI) as well as maternal factors. The authors reported a detection rate of 76.7% for preterm pre-eclampsia with a false positive rate (FPR) of 10%. This compares to a detection rate of just 39% using the traditional approach based on maternal characteristics and medical history alone recommended by leading international bodies The National Institute for Health and Clinical

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Excellence (NICE) and the Americana College of Obstetricians and Gynaecologists (ACOG). This study highlights the potential utility of PlGF as part of combined early pregnancy screening. However prior to the introduction of any screening test, both external validation and a health economic analyses are necessary, to confirm utility and reproducibility.

The main use of PlGF in pregnancy currently is in short term prediction of time to delivery in women with suspected pre-eclampsia. Having an effective diagnostic test for pre-eclampsia would eliminate protracted hospitalisations of women and allow resources to be better utilised. A systematic review in 2015 evaluated trials on placental growth factor (alone or in combination with sFlit-1) as an aid to the assessment of women with suspected pre-eclampsia. (54) Four prospective (cohort) studies were identified and examined. Meta-analysis was not possible because the studies employed different outcome measures, test cut-off points and gestational periods. The PELICAN study showed that the PlGF test alone had a very high accuracy for predicting pre-eclampsia requiring delivery within 14 days for women presenting with suspected pre-eclampsia between 20 - 35 weeks of gestation. For a test cut-off <100 pg/mL, PlGF alone showed 96% sensitivity (95% CI, 89–99), 56% specificity (95% CI, 49–63), 44% positive predictive value (PPV) (95% CI, 36–52), and 98% negative predictive value (NPV) (95% CI, 93–100) (55). The PROGNOSIS study evaluated whether the sFlt-1:PlGF ratio is predictive of the short-term absence or presence of pre-eclampsia in women with suspicion of pre-eclampsia between 24 and 36+6 weeks of pregnancy. It reported a sFlt-1:PlGF ratio ≤ 38 had a NPV in the subsequent week of 99.3% (95% confidence interval [CI] 97.9–99.9). In 2016 NICE published guidance on the use of PlGF testing based on this review. The Triage PlGF test and the Elecsys immunoassay sFlt-1:PlGF ratio, used with standard clinical assessment and subsequent clinical follow-up, were recommended to help rule-out pre-eclampsia in women presenting with suspected pre-eclampsia between 20 weeks and 34 weeks plus 6 days of gestation.

NICE recommended that these tests should not yet be used to diagnose pre-eclampsia until further research is available, specifically on how an abnormal PlGF result would affect management decisions regarding timing and gestation of delivery and the outcomes associated with this. (56) Interventional studies were recommended to confirm the clinical utility of the results to date. Some smaller cohort studies (MAPPLE) with unblinded PlGF testing have reported a lowering of gestational age at delivery and an increase in neonatal prematurity related morbidity. This highlights the importance of conducting appropriately powered trials before PlGF testing is routinely adapted into clinical practice. (57) A number of randomised controlled trials are currently ongoing, the UK PARROT trial and the PARROT Ireland trial among these, with results expected in 2019. (58)

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The prospect of using PlGF as a therapeutic agent has also begun to be considered. Recent studies in mice have demonstrated that administration of VEGF early in pregnancy prevents the development of pre-eclampsia (59) and reduction in circulating sFlt-1 alleviated pre-eclampsia like symptoms again in a mouse model. (60) A pilot study on the safety and efficacy of therapeutic apheresis for preterm pre-eclampsia was conducted on 11 pregnant women with pre-eclampsia ranging from 23 to 32 weeks gestation. A reduction in levels of circulating sFlt-1 was achieved with combinations of single or multiple plasma apheresis. Overall a prolongation of pregnancy without major adverse maternal or fetal consequences was seen. (61) A case controlled prospective study is currently ongoing, collecting maternal plasma and serum from patients with both pre-eclampsia and normal pregnancy for in vitro validation of new therapeutics based on extra-corporal removal of sFlt-1 (APHERESE) (62)" Also currently recruiting is an interventional trial of a medical apheresis device for Flt-1 in pre-eclamptic women (SAVE). (63)

These studies suggest the potential utility of early pro-angiogenic therapies in treating pre-eclampsia in the future.

**Assays**

A variety of different assays are now commercially available for quantification of PlGF alone or in combination with sFlt-1; Triage PlGF test, Elecsys immunoassay sFlt-1/PlGF ratio, DELFIA Xpress PlGF 1-2-3 test, The Quantikine Human PlGF Immunoassay (R&D systems) and BRAHMS sFlt-1 Kryptor/BRAHMS PlGF plus Kryptor PE ratio. Most of these are laboratory based and require significant infrastructure available to use while the Triage PlGF test is point of care and could be easily integrated to antenatal clinical care algorithms in developing countries. Importantly to note, the normal reference values of PlGF obtained with one platform may not be interchangeable with others. Validation studies, head-to-head comparative studies, and cost effective analyses comparing these platforms are required as the performance and costs of these may differ between assays.

**Outside Pregnancy**

PlGF is more than just a pregnancy specific biomarker. Although originally identified in the placenta, PlGF is also expressed in heart, lung, thyroid, adipose tissue and skeletal muscle. Its absence impairs angiogenesis and arteriogenesis during tumour growth and heart, limb and ocular ischaemia. (18, 64-69) Patients with sickle cell disease are noted to have increased levels of PlGF, expressed by bone marrow erythroid cells under hypoxic conditions, with levels of PlGF correlating with degree of disease activity. An association between PlGF and β-thalassaemia has also been reported, with levels of PlGF positively correlating with other markers of haemolysis such as lactate dehydrogenase, uric

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acid and reticulocyte counts in this group. (70-72) Whether PlGF may act as a biomarker of disease activity or have a role in potential targeted therapies in patients with haemaglobinopathies remains the subject of ongoing research. (73) More recently, PlGF has been identified as a possible contributor in haematologic malignancies, with both PlGF and sFlt-1 expression increased in samples from patients with chronic myeloid leukaemia (CML), acute myeloid leukaemia (AML) and acute lymphoid leukaemia (ALL). This is in contrast to the increased PlGF and reduced sFlt-1 expression seen in patients with pre-eclampsia. The exact mechanism of action of the angiogenic PlGF and anti-angiogenic sFlt-1 in these malignancies is as yet not fully understood, but the potential of targeted anti-PlGF therapy is being explored. (74-76) A recent study on thyroid carcinoma found significantly higher levels of PlGF in metastatic disease, suggesting that antagonising PlGF in this setting may be a promising therapy to suppress cancer metastasis (77). In the pathological models studied, the absence of PlGF impairs the associated inflammation and angiogenesis and confers a general reduction in pathological changes. (78)

**Conclusion**

PlGF plays an integral role in the development of a normal pregnancy and aberrations in PlGF concentrations are associated with adverse pregnancy outcomes, in particular pre-eclampsia. The universal integration of PlGF into antenatal assessment of women with suspected preterm pre-eclampsia is dependent on results of current RCTs. The role of PlGF as a as a biomarker of disease outside of pregnancy shows promise while its potential as a possible therapeutic for pre-eclampsia and some malignancies warrants further research.

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The authors report no conflict of interest.

**Figures**

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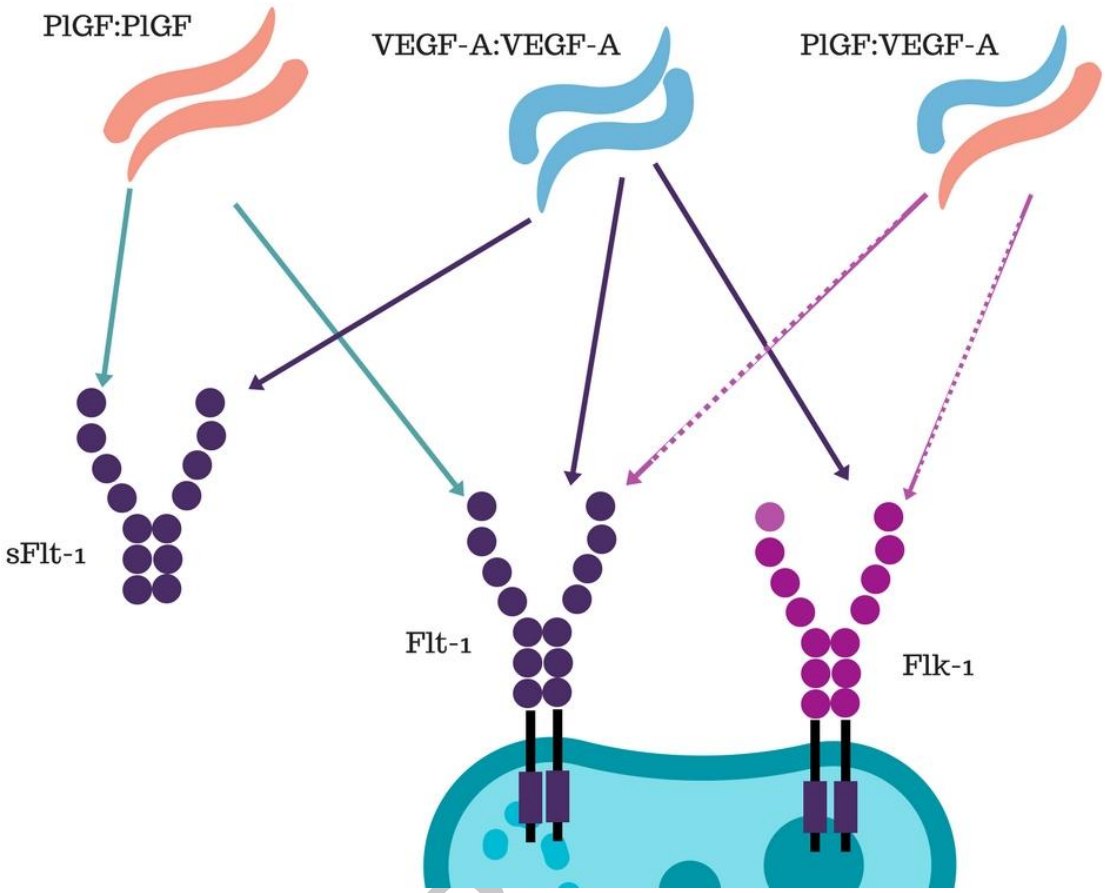



***Figure 1:*** *PlGF and VEGF hetero and homodimer protein structures and their respective membrane bound receptors Flt-1 ad Flk-1 and the freely circulating receptor sFlt-1*

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**Highlights:**

- PlGF plays an integral role in the development of a normal pregnancy
- Aberrations in PlGF are associated with adverse pregnancy outcomes, in particular pre-eclampsia
- The integration of PlGF into clinical care is dependent on results of current RCTs
- The role of PlGF as a as a biomarker of disease or therapeutic agent outside of pregnancy shows promise

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