The vascular endothelial growth factor family in adverse pregnancy outcomes

P.H. Andraweera¹,², G.A. Dekker¹,³, and C.T. Roberts¹,*
¹Discipline of Obstetrics and Gynaecology, Robinson Institute, University of Adelaide, Adelaide 5005, Australia ²Department of Anatomy and Human Genetics, Faculty of Medicine, University of Colombo, Colombo, Sri Lanka ³Lyell McEwin Hospital, Elizabeth Vale, SA, Australia

*Correspondence address. Tel: +61-8-8303-3118; Fax: +61-8-8303-4099; E-mail: claire.roberts@adelaide.edu.au

Submitted on June 20, 2011; resubmitted on February 23, 2012; accepted on March 9, 2012

Table of Contents

• Introduction
• Methods
• Results
  Biology of the VEGF family of angiogenic growth factors
  Roles of the VEGF family in pregnancy
  Roles of the VEGF family in adverse pregnancy outcomes
  VEGF family gene polymorphisms and adverse pregnancy outcomes
• Discussion

Background:
Pre-eclampsia, small-for-gestational-age infants, preterm birth and recurrent miscarriage complicate a significant number of pregnancies. The vascular endothelial growth factor (VEGF) family of angiogenic growth factors is implicated in the pathophysiology of these complications. We aimed to elucidate the role of these angiogenic factors in placentation and to evaluate the predictive value of their protein concentrations and genetic variations in pregnancy complications.

Methods:
We performed a systematic search of PubMed, and retrieved original articles. The search included a combination of terms such as VEGF-A, placental growth factor (PIGF), kinase insert domain receptor, fms-like-tyrosine-kinase receptor 1, soluble fms-like-tyrosine-kinase receptor 1, pre-eclampsia, small-for-gestational-age infants, preterm birth, recurrent miscarriage, placenta, prediction and polymorphisms.

Results:
This review summarizes the current knowledge of the roles of the VEGF family in early placentation and of the abnormalities in maternal plasma and placental expression of angiogenic proteins in adverse pregnancy outcomes compared with normal pregnancy. PIGF and sFLT-1 in combination with other clinical and biochemical markers in late first or second trimester appear to predict early-onset pre-eclampsia with a high sensitivity and specificity. However, VEGF family proteins do not have sufficient power to accurately predict late-onset pre-eclampsia, small-for-gestational age pregnancies or preterm birth. Functional polymorphisms in these angiogenic genes are implicated in pregnancy complications, but their contribution appears to be minor.

Conclusions:
Although the VEGF family has important roles in normal and complicated pregnancy, the current predictive value of the VEGF family as biomarkers appears to be limited to early-onset pre-eclampsia.

Key words: VEGF family / pre-eclampsia / small-for-gestational age / pregnancy / placenta

Introduction
Pre-eclampsia, small-for-gestational age (SGA) pregnancy, preterm birth and recurrent miscarriage together complicate ~17–29% of all pregnancies (Li et al., 2002; Sibai, 2003; Sibai et al., 2005; Goldenberg et al., 2008). Defects in early placentation processes, including trophoblast invasion, spiral artery remodelling and angiogenesis are implicated in the pathogenesis of these complications (Khong et al., 1986; Kim et al., 2002, 2003; Mayhew et al., 2004; Ball et al., 2006a, b). The vascular endothelial growth factor (VEGF) family of angiogenic growth factors

© The Author 2012. Published by Oxford University Press on behalf of the European Society of Human Reproduction and Embryology. All rights reserved. For Permissions, please email: journals.permissions@oup.com
are important molecules regulating early placental vascular changes. The key molecules VEGF-A and placental growth factor (PIGF) and the receptors VEGF receptor 1 (fms-like-tyrosine-kinase receptor 1, FLT-1) and VEGF receptor 2 (kinase insert domain receptor, KDR) are expressed in the human placenta throughout gestation, and the VEGF family is known to regulate placental angiogenesis and maternal spiral artery remodelling (Senger et al., 1996; Charnock-Jones et al., 2004). In addition to the two main membrane-bound receptors, a splice variant of FLT-1 designated soluble FLT-1 (sFLT-1) is expressed in the placenta and is known to have potent anti-angiogenic properties. Soluble FLT-1 antagonises both VEGF-A and PIGF and induces symptoms of pre-eclampsia in animal models (Maynard et al., 2003).

In humans, placental expression and maternal serum concentrations of sFLT-1 are up-regulated, and maternal serum-free VEGF-A and PIGF are reduced, in preeclamptic women compared with normotensive pregnant women. Consistent evidence exists that these changes are detected several weeks prior to the clinical onset of symptoms of pre-eclampsia (Levine et al., 2004). Therefore, sFLT-1 and PIGF are considered as biomarkers in predicting pre-eclampsia early in pregnancy. Although these biomarkers in combination with other clinical and biochemical markers are demonstrated to have a strong predictive value for early onset (and consequently severe) pre-eclampsia, their value in predicting the more common late onset disease is questionable.

These biomarkers are also not restricted to pre-eclampsia, as several groups have shown their involvement in pregnancies to be complicated by small-for-gestational-age infants and to a lesser extent, in the pathophysiology of preterm birth and recurrent miscarriage. This review examines the role of VEGF family angiogenic growth factors in placentation and the implications of derangements in these molecules in the development of pre-eclampsia, as well as pregnancies complicated by small-for-gestational-age infants, preterm birth and recurrent miscarriage. We also evaluate the role of VEGF family proteins, as well as their genetic variations, in predicting these pregnancy complications.

**Methods**

An extensive online search of published articles on the VEGF family in normal and complicated pregnancy was undertaken. We used the Pubmed database employing combinations of the following search terms: pregnancy, VEGF, PIGF, FLT-1, KDR, sFLT-1, placenta, trophoblast invasion, spiral artery remodelling, angiogenesis, vasularization, pre-eclampsia, small-for-gestational-age infants, preterm birth, recurrent pregnancy loss, single nucleotide polymorphism, biomarker and prediction. The search was mainly focused on publications involving humans, but experimental studies with animals or cell culture models were included where appropriate. In addition, references cited in the selected articles and reviews were searched. Only articles written in English were considered. The review was conducted according to the roles of the VEGF family in placental development including trophoblast invasion and spiral artery remodelling, vascularization and implantation and their roles in pregnancy complications classified as pre-eclampsia, SGA pregnancy, preterm birth and recurrent pregnancy loss. Finally, we reviewed articles on polymorphisms in VEGF family genes in pregnancy complications.

**Results**

**Biology of the VEGF family of angiogenic growth factors**

The VEGF family (see Fig. 1) consists of VEGF-A, PIGF, VEGF-B, VEGF-C and VEGF-D as well as their receptors VEGFR-1 (also called FLT-1), VEGFR-2 (also called KDR, in humans and fetal liver kinase, Flk, in mice; Waltenberger et al., 1994) and VEGFR-3 (FLT-4), as well as the co-receptors, neuropilin-1 (NRP-1) and NRP-2.

**Vascular endothelial growth factor-A**

The human VEGF-A gene has been assigned to chromosome 6p12-p21.1 (Mattei et al., 1996) and is organized as eight exons separated by seven introns (Houck et al., 1992). Alternative exon splicing results in six different isoforms VEGF-A121, VEGF-A145, VEGF-A165, VEGF-A183, VEGF-A189 and VEGF-A206 having 121, 145, 165, 183, 189 and 206 amino acids, respectively. VEGF-A165 is the predominant isoform and native VEGF-A closely resembles VEGF-A165 (Houck et al., 1992). VEGF-A binds with high affinity to two related receptor tyrosine kinases expressed on vascular endothelial cells (de Vries et al., 1992; Terman et al., 1992), FLT-1 and KDR (Waltenberger et al., 1994). In addition, VEGF-A also binds to NRP-1 and NRP-2.

VEGF-A mediates many functions in endothelial cells. VEGF-A promotes angiogenesis, induces the growth of vascular endothelial cells (Ferrara and Davis-Smyth, 1997), reduces apoptosis (Zhou et al., 2003), mediated via the KDR/Flk1 receptor through the PI3-kinase/Akt signal transduction pathway (Gerber et al., 1998), and increases vascular permeability (Dvorak et al., 1995). In addition, VEGF-A promotes vasodilatation via the endothelial-derived nitric oxide pathway. Hypoxia is a potent stimulus for the expression of VEGF-A mRNA and is mediated via hypoxia-inducible-factor-1α (Taylor et al., 1997; Semenza 2002). In addition, several growth factors including fibroblast growth factor, transforming growth factors (TGF-α and TGF-β), keratinocyte growth factor, insulin-like growth factor 1 (IGF-1) and platelet-derived growth factor, as well as the inflammatory cytokines, interleukin (IL)-1α and IL-6, are also known to up-regulate VEGF-A expression (Ferrara and Davis-Smyth, 1997; Neufeld et al., 1999).

**Placental growth factor**

PIGF demonstrates 42% amino acid sequence identity with VEGF-A (de Falco et al., 2002). PIGF has been assigned to human chromosome 14 and consists of seven exons (Maglione et al., 1993). Alternative mRNA splicing of the PIGF primary transcript results in four isoforms, PIGF-1 (PIGF131), PIGF-2 (PIGF152), PIGF-3 (PIGF203) and PIGF-4 (PIGF224) (Maglione et al., 1993) differing in secretion properties and binding affinities (Ribatti, 2008). PIGF homodimers bind FLT-1 and NRP-1 while PIGF/VEGF-A heterodimers bind KDR and FLT-1/KDR heterodimers in vitro. PIGF is predominantly expressed in the placenta, heart and lungs. The exact physiological actions of PIGF are still not clear, however, evidence suggests a pivotal role for PIGF in regulating VEGF-dependent angiogenesis under pathological conditions (Carmeliet et al., 2001). A few proposed mechanisms by which PIGF potentiates angiogenesis are by (i) stimulating endothelial cells via FLT-1, (ii) separating VEGF-A from FLT-1, allowing VEGF-A to activate KDR, (iii) recruiting monocytes/macrophages which have a
crucial role in vessel growth (Ribatti, 2008) and (iv) inducing the secretion of VEGF-A from monocytes (Bottomley et al., 2000).

Vascular endothelial growth factor-B
VEGF-B has structural similarities to VEGF-A and PlGF, and VEGF-B gene is localized to chromosome 11q13 (Paavonen et al., 1996). It is expressed as two isoforms VEGF-B167 and VEGF-B186, and is abundant in heart and skeletal muscle (Olofsson et al., 1996a, b). VEGF-B forms stable heterodimers with VEGF-A (Olofsson et al., 1996a, b) and is generally co-expressed with VEGF-A. VEGF-B binds to two of the VEGF receptors, FLT-1 (Olofsson et al., 1998) and NRP-1 (Makinen et al., 1999). VEGF-B is reported to behave as an endothelial cell mitogen (Olofsson et al., 1996a, b) but part of the mitogenic activity may be due to VEGF-A/VEGF-B heterodimers.

Vascular endothelial growth factor-C
VEGF-C has been recognized as a lymphatic system regulator during embryogenesis as well as in adult life, and the VEGF-C gene is localized to chromosome 4q34 (Paavonen et al., 1996). VEGF-C has a high affinity for both KDR and FLT-4 (Joukov et al., 1996). VEGF-C induces selective lymphangiogenesis without accompanying angiogenesis and is mainly expressed together with FLT-4 predominantly in regions where lymphatic vessels develop.

Vascular endothelial growth factor-D
VEGF-D is also a primarily lymphangiogenic growth factor and the VEGF-D gene has been localized to chromosome Xp22.31 (Tammela et al., 2005). VEGF-D binds to both KDR and FLT-4 and is present in many human tissues, most abundantly in skin and lung during embryogenesis. Although mainly a mediator of lymphangiogenesis, animal experiments have shown VEGF-D to possess strong angiogenic properties (Rissanen et al., 2003).

Fms-like tyrosine kinase receptor-1
Fms-like tyrosine kinase receptor (FLT-1) is composed of seven extracellular immunoglobulin homology domains, a single transmembrane region and an intracellular tyrosine kinase sequence that is interrupted by a kinase-insert domain (Shibuya et al., 1990). The FLT-1 gene is localized to chromosomes 13q12-q13 (Tammela et al., 2005). FLT-1 binds VEGF-A, VEGF-B and PlGF with high affinity and is expressed in many human tissues including monocytes/macrophages and placental trophoblasts, and its expression is up-regulated by hypoxia (Gerber et al., 1997).

Alternative splicing of the pre-mRNA which encodes FLT-1 results in the production of sFLT-1 comprising the ligand-binding domain of FLT-1 but lacking the membrane-spanning and intracellular domains (He et al., 1999). Soluble FLT-1 is secreted by endothelial cells, monocytes and the placenta (Shibuya, 2006). Soluble FLT-1 acts as a potent
antagonist of VEGF-A and PIGF, by inhibiting their binding to cell surface receptors (Kendall and Thomas, 1993) as well as by forming heterodimers with KDR (Kendall et al., 1996), and is considered an anti-angiogenic factor. Recently, a human-specific splice variant of FLT-1 was discovered to produce a soluble receptor designated sFLT-14 (Sela et al., 2008). Soluble FLT-14 is primarily expressed in non-endothelial cells, notably vascular smooth muscle cells. Placental expression of the two isoforms of sFLT changes over time with sFLT-1 being the dominant form during the first trimester to almost exclusive sFLT-14 production by term. Major sites of placental sFLT-14 expression are degenerative syncytiotrophoblasts known as syncytiot knots. Soluble FLT-14 is qualitatively different from sFLT-1, but is a potent inhibitor of VEGF-A signalling, with its inhibitory activity being comparable to that of sFLT-1 (Sela et al., 2008).

**Kinase insert domain receptor**

KDR has a structure similar to FLT-1 and the KDR gene is localized to chromosomes 4q11-4q12 (Tammela et al., 2005). KDR binds VEGF-A, VEGF-C and VEGF-D. Although the binding affinity of VEGF-A for KDR is lower than that of FLT-1, it has been shown that KDR is the primary receptor transmitting VEGF-A signals in endothelial cells (Gille et al., 2001). Despite this, other cell types including neuronal cells and megakaryocytes also express KDR. The expression of KDR is auto-regulated, being up-regulated by VEGF-A, VEGF-C and VEGF-D.

A soluble form of the KDR receptor has also been detected in human plasma and is suggested to be secreted by endothelial cells (Ebos et al., 2004). Alternative mRNA splicing or proteolytic cleavage of the membrane-bound receptor is hypothesized as the probable mechanism of generation of this receptor, but the mechanism remains to be elucidated (Ebos et al., 2004). Soluble KDR is also considered an anti-angiogenic protein (Maynard et al., 2003), but its mechanism of action remains unclear.

**Fms-like tyrosine kinase-4**

FLT-4 has only six immunoglobulin-like domains and the FLT4 gene is localized to chromosomes 5q33-qter (Tammela et al., 2005). FLT-4 binds VEGF-C and VEGF-D. FLT-4 is present on all endothelia during development, but becomes restricted to lymphatic endothelial cells and certain fenestrated vascular endothelial cells in adult life (Kaipainen et al., 1995; Partanen et al., 2000).

**NRP-1 and NRP-2**

NRP-1 binds VEGF-A, VEGF-B and PIGF while NRP-2 binds VEGF-A, VEGF-C and PIGF (Klagsbrun et al., 2002). In endothelial cells, NRPs are thought to increase VEGF signalling by ensuring optimal presentation of ligands to the receptors and by stabilizing VEGF/VEGFR complexes. Interaction of VEGF-A with NRP-1 is required for VEGF-A binding to KDR, activation of KDR and the downstream signalling and biological actions. Similarly, interaction of VEGF-A or VEGF-C with NRP-2 increases the phosphorylation threshold of KDR and promotes endothelial cell survival and motility (Sulpice et al., 2008).

**Roles of the VEGF family in pregnancy**

The VEGF family and implantation

Successful implantation depends on the development of healthy functional embryos during the preimplantation period. VEGFA mRNA expression is detected from the unfertilized oocyte to the blastocyst stage and VEGF-A protein is detected from the 3-cell stage to the blastocyst stage in human embryos (Hwu et al., 2006), but knowledge on the role of VEGF family in preimplantation embryos is at present limited. A recent study of porcine preimplantation embryos demonstrated that VEGF-A mRNA was expressed in oocytes, 2, 4 and 8-cell embryos, morulae and blastocyst and that KDR and FLT1 mRNA were expressed from the I-cell to the blastocyst stage (Biswas et al., 2011). VEGF-A expression has also been shown to be up-regulated in Day 6 rabbit blastocysts (Saenz-de-Juano et al., 2011). VEGF-A supplementation during porcine and bovine embryonic development is known to improve the rate of blastocyst formation (Einspanier et al., 2002; Biswas et al., 2011) and the blastocyst cell number (Biswas et al., 2011). As there is no angiogenesis occurring in the early embryos, these findings suggest that VEGF-A may regulate functions other than angiogenesis during the preimplantation period that need to be further explored. Since VEGF-A is known for its role in increasing vascular permeability, it is possible that in the early embryos it may be involved in fluid transport.

Implantation is the complex process where the developing embryo establishes contact with the maternal endometrium initiating the development of the placenta. Endometrial VEGF-A is also likely to be a critical molecule regulating implantation (Smith, 2000). The most frequently expressed VEGF-A splice variants VEGF-A121 and VEGF-A165 are expressed in the endometrium (Charnock-Jones et al., 1993). Uterine expression of VEGF-A appears to be cycle dependent, with increased levels of both VEGF-A mRNA and protein levels reported during the mid-secretory period (Shifren et al., 1996; Torry et al., 1996). In contrast, increased mid-secretory VEGF-A expression is not evident in women experiencing repeated IVF failures (Jee et al., 2009) suggesting that VEGF-A may have a role in successful implantation. In support of this hypothesis, Hannan and colleagues recently demonstrated that VEGF-A levels were significantly reduced in uterine fluid during the mid-secretory phase in women with unexplained infertility compared with fertile women. They also demonstrated that culturing mouse embryos with either mid-secretory phase uterine fluid from fertile women or recombinant human VEGF-A enhanced blastocyst outgrowth, and that treatment of human endometrial epithelial cells with uterine fluid from fertile women or recombinant human VEGF-A increased endometrial epithelial cell adhesion (Hannan et al., 2011). These findings suggest novel mechanisms by which VEGF-A may regulate implantation.

The VEGF family and the placenta

During pregnancy, the placenta expresses the VEGF family angiogenic growth factors, but the literature is somewhat controversial. While some report that VEGF-A mRNA is expressed in villous and extravillous trophoblasts (EvTs), Hofbauer cells and maternal decidual cells (Sharkey et al., 1993; Jackson et al., 1994; Clark et al., 1996; Khaliq et al., 1996; Schiessl et al., 2008) throughout pregnancy, others report that the expression is seen in villous mesenchyme, decidual macrophages and decidual glands but not in trophoblasts (Vuorela et al., 1997; Clark et al., 1998). In the first trimester of pregnancy, PIGF is mainly expressed in EVT cells within the maternal decidua, but towards term the expression is shown to be abundant in villous trophoblasts (Khaliq et al., 1996; Vuorela et al., 1997; Clark et al., 1998). The inconsistent results on VEGF-A expression are attributed...
to the probes used in in situ hybridization studies that may cross-react with PI GF in the trophoblast layer resulting in false-positive results (Clark et al., 1998). The expression pattern of FLT-1 is similar to that of VEGF-A while abundant KDR expression is localized to areas of endothelial cells (Ahmed et al., 1995; Clark et al., 1996). These findings suggest that VEGF-A and PI GF may have vital roles in the development of the placental vasculature. At present, data on the expression and function of the other members of the VEGF family in pregnancy and placentation are limited. Recently, it was shown that VEGF-C is expressed in all EVT populations and that VEGF-D is expressed in trophoblasts, decidual stromal cells, endothelial cells and vascular smooth muscle cells of spiral arteries (Schiessl et al., 2009). However, their role in placentation is yet to be determined. Additionally, uterine natural killer cells (NK cells) express high levels of mRNAs for VEGF-C and PI GF while their expression of VEGF-A has been demonstrated to be low (Li et al., 2001; Lash et al., 2006).

Regulation of placental vasculogenesis and angiogenesis

Vasculogenesis and angiogenesis are two processes which are essential in the establishment of the utero-placental circulation. Human placental vascular development begins as early as 21 days post-conception by the formation of haemangioblastic cords and is observed at the stage of early tertiary chorionic villi (Demir et al., 1989). The initial period of vasculogenesis is followed by a phase of branching angiogenesis (Day 32 to week 25 post conception) during which the haemangioblastic cords develop into a richly branched capillary bed (Kaufmann et al., 2004). Placental expressions of VEGF-A, FLT-1 and KDR are intense and the expression of PI GF is moderate during this period (Kaufmann et al., 2004). It has been shown that during the organization of the first vessels, angiogenic factors required for the commencement of angiogenesis are supplied by the cytotrophoblast cells, and that as pregnancy advances and villous maturation occurs, additional VEGF-A is supplied by stromal cells including Hofbauer cells (fetal placental macrophages) (Demir et al., 2004, 2006). In vitro experiments on the chorioallantoic membrane of the chick have shown that binding of VEGF-A to FLT-1 and KDR stimulate branching angiogenesis (Wilting et al., 1996).

From 25 weeks of gestation onwards, angiogenesis switches from branching to non-branching and is accompanied by a decline in VEGF-A and KDR and an increase in the expression of PI GF, FLT-1 and sFLT-1. PI GF, which is expressed in trophoblasts throughout gestation by acting on FLT-1, may, in early gestation, have a supplementary role in vasculogenesis and branching angiogenesis by recruiting macrophages, and from then onwards, it may have a role in the regulation of non-branching angiogenesis which continues to term.

Regulation of trophoblast invasion and spiral artery remodelling

During normal development of the placenta, EVT cells invade the uterine decidua, the inner third of the myometrium (interstitial invasion) and the spiral arteries (endovascular invasion). The process of trophoblast invasion is highly controlled so that the depth of invasion of the uterus is sufficient but not so excessive as to penetrate the myometrium and adjacent organs. For successful invasion, EVT cells need to both increase their motility and secrete specific proteases to break down the extracellular matrix. The urokinase plasminogen activator/plasminogen pathway and the matrix metalloproteinases play a key role in cellular invasion by degrading the extracellular matrix. In addition to its well-researched action of stimulating endothelial cell proliferation and migration, VEGF-A is known to stimulate metalloproteinase activity of endothelial cells (Wang and Keiser, 1998). Most in vitro studies have primarily focused on endothelial cells in examining the biologic actions of the VEGF family. The fact that VEGF-A, PI GF and their receptors are expressed in trophoblast cells, which are non-endothelial cells, raises the possibility that VEGF-A and PI GF may exert similar biological actions on the trophoblast cells.

However, in vitro studies to date report results contrary to this hypothesis. One group has shown that VEGF-A (Athanassiades et al., 1998) and PI GF (Athanassiades and Lala, 1998) promote EVT cell proliferation without influencing their migratory or invasive behaviours, while others have shown that VEGF-A and PI GF do not stimulate EVT proliferation (Lash et al., 1999; Fitzpatrick et al., 2003) or invasive- ness (Lash et al., 1999; Fitzpatrick et al., 2003) but increase motility of trophoblast cells (Lash et al., 1999, 2003; Fitzpatrick et al., 2003). One study has also shown that VEGF-A inhibits the invasion of first trimester trophoblast cells and decreases the cell surface expression of urokinase plasminogen activator, a molecule required for trophoblast invasion (Fitzpatrick et al., 2003). This VEGF-A-induced reduction in invasion is inhibited by the addition of a VEGF-A neutralizing antibody (Fitzpatrick et al., 2003). The results of these in vitro studies could indicate that the increased motility of trophoblast cells in response to VEGF-A may be an initial response to attract trophoblast cells to the decidua, and that VEGF-A may then limit the degree to which the cells invade (Lash et al., 1999).

During endovascular invasion, the endothelium and the underlying vascular smooth muscle cells are replaced by EVT embedded in a fibrinoid rich matrix (Harris, 2011). This spiral artery remodelling results in the conversion of narrow-calibre high-resistance vessels into wide-calibre low-resistance vessels capable of supplying enough maternal blood to the placenta to accommodate the increasing demands of the rapidly growing fetus (Brosens et al., 1967; Pijnenborg et al., 1980; Lyall, 2002). In addition to the trophoblast-mediated spiral artery remodelling, subtle changes in spiral artery structure are observed early in pregnancy in the decidua, and are termed trophoblast-independent or decidua-associated remodelling events (Harris, 2011). Early pregnancy is associated with an influx of leukocytes into the decidua including uterine natural killer (NK) cells and macrophages. Uterine NK cells isolated from first trimester decidua secrete many angiogenic growth factors including VEGF-A, VEGF-C, PI GF and un-remodelled spiral arteries express KDR (Lash et al., 2006). Uterine NK cells are proposed to be a major source of angiogenic growth factors at the fetal–maternal interface responsible for decidua-associated spiral artery remodelling (Hanna et al., 2006). The molecular mechanisms controlling spiral artery remodelling are still not clear, but it is known that during invasion, the cytotrophoblasts lose their ability to divide (Genbacev et al., 1996) and that the cells which interdigitate between maternal endothelial cells lose their epithelial characteristics and acquire an endothelial phenotype in a transition process called pseudovasculogenesis. This includes changes in the expression of cell adhesion molecules. During differentiation, cytotrophoblasts down-regulate adhesion molecules highly characteristic of epithelial cells including integrins αβ1, αβ3, αβ1, vascular endothelial cell adhesion molecule-1 (VCAM-1) and platelet endothelial cell adhesion
mRNA in peripheral blood mononuclear cells (Perelman et al., 1997a, b). These changes in cell adhesion molecules are seen only in the invasive cytotrophoblasts, suggesting that changes in the microenvironment alter gene expression (Iruela-Arispe, 1997). Integrin α,β3 is known to play a key role in angiogenesis (Brooks et al., 1994). VEGF-A induces endothelial cells to express increased levels of mRNA encoding α, and β3 integrin subunits which lead to increased protein expression of α,β3 on the endothelial cell surface. VEGF-A also induces mRNA encoding osteopontin (OPN), an α,β3 ligand. Cell migration assays report that OPN promotes migration of microvascular endothelial cells in a concentration-dependent manner and that continuous VEGF-A stimulation, leading to increased induction of α,β3 expression, results in increased cell migration which is inhibited by an α,β3 neutralizing antibody (Senger et al., 1996). VEGF-A is also known to stimulate the expression of intercellular adhesion molecule-1 and VCAM-1 in Human umbilical vein endothelial cells in vitro via activation of nuclear factor kappa B (Min et al., 2005). These findings suggest that the VEGF family has a role in promoting trophoblast invasion and spiral artery remodelling, but more extensive studies are needed to elucidate the exact role.

**VEGF family gene ablation studies**

Gene ablation studies in mice have demonstrated that VEGF-A, FLT1 and Flk are crucial for embryonic angiogenesis, and homozygous gene mutations in all result in embryonic death (Table I) (Fong et al., 1995; Shalaby et al., 1995; Carmeliet et al., 1996, 2001; Ferrara et al., 1996). However, VEGF-A appears to be the most critical, as embryonic lethality occurs even in the heterozygous state. PlGF does not appear to be essential for embryonic angiogenesis but can be considered a potent stimulator of this process. PlGF stimulates VEGF-A secretion by monocytes (Bottomley et al., 2000), increases VEGF-A mRNA in peripheral blood mononuclear cells (Perelman et al., 2003) and over expression of PlGF in mice results in up-regulation of FLT-1 and Flk transcription (Ondurio et al., 2002). PlGF deficiency impairs endothelial cell response to VEGF-A, which is restored by the administration of exogenous PlGF (Carmeliet et al., 2001). Therefore, although ineffective in being a strong angiogenic stimulator on its own, PlGF appears to amplify the effects mediated via VEGF-A. The aforementioned gene ablation studies in mice have focused on defects in embryonic angiogenesis. At present, there is a paucity of literature on the effects of VEGF family gene ablation on placental vascularization. Such studies may provide new knowledge on the role of these genes in the pathogenesis of pregnancy complications of placental origin.

**Table I The effect of VEGF family gene ablation in mice.**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Gene*+/−</th>
<th>Gene*−/−</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF</td>
<td>Die E11-E12, displaying defects in early vascular development</td>
<td>Die E8-E9 from defects in blood vessel formation</td>
<td>Carmeliet et al. (1996)</td>
</tr>
<tr>
<td>PGF</td>
<td>No apparent vascular defects</td>
<td>Reduced ability to respond to ischaemic damage</td>
<td>Ferrara et al. (1996)</td>
</tr>
<tr>
<td>FLT1</td>
<td>Normal vessels</td>
<td>Formation of endothelial cells not affected, but assembled to form abnormal vessels</td>
<td>Fong et al. (1995)</td>
</tr>
<tr>
<td>Flk</td>
<td>Normal endothelial cells</td>
<td>Endothelial precursors form mature cells do not form</td>
<td>Shalaby et al. (1995)</td>
</tr>
</tbody>
</table>

E, Embryonic day.

**Roles of the VEGF family in adverse pregnancy outcomes**

The *VEGF family and pre-eclampsia*

Pre-eclampsia is a multiorgan disorder affecting 2–7% of pregnant women leading to substantial maternal and perinatal morbidity and mortality (Sibai, 2003). Pre-eclampsia is characterized by new onset hypertension and proteinuria after 20 weeks of gestation. In some women, it can progress to HELLP (haemolysis, elevated liver enzymes and low platelets) syndrome and eclampsia (seizures). Pre-eclampsia occurs only in the presence of the placenta (Chun et al., 1964). Therefore, the only successful treatment remains the delivery of the placenta and hence the fetus, which may involve significant morbidity and even death of the baby in the case of early-onset pre-eclampsia.

Early placental defects, including impaired trophoblast invasion and inadequate maternal spiral artery remodelling have been demonstrated in pre-eclampsia (Khong et al., 1986). In vitro and in vivo studies have shown that preeclamptic placentae retain the adhesion molecules α,β4 and E-Cadherin and fail to up-regulate α,β3 and β1. Vascular endothelial cadherin, VCAM-1 and PECAM-1 which are normally expressed by the most differentiated and invasive trophoblasts (Zhou et al., 1993, 1997a, b). Therefore, it is postulated that the failure of cytotrophoblasts to switch to a vascular phenotype could tip the balance of molecules that permit invasion in favour of those that restrain it, leading to a net effect of shallow endovascular invasion (Zhou et al., 1993).

Evidence from many laboratories suggests that the absence of the normal repertoire of VEGF family members at the maternal–fetal interface may result in these deficits in cytotrophoblast differentiation observed in pre-eclampsia. Extensive studies from Susan Fisher’s group have shown that cytotrophoblasts respond to the VEGF ligands they produce, and blocking the ligand binding significantly decreases their expression of integrin α, and increases apoptosis. They have also shown that in severe pre-eclampsia and HELLP syndrome, VEGF-A and FLT-1 expression are decreased (Zhou et al., 2002).

In general, studies on placental VEGF-A protein expression using immunohistochemistry have reported a decrease in VEGF-A immunoreactivity in pre-eclampsia (Lyall et al., 1997; Zhou et al., 2002; Cirpan et al., 2007), severe pre-eclampsia and HELLP syndrome (Zhou et al., 2002) compared with normotensive pregnancies, while some others have reported an increase in VEGF-A protein expression in pre-eclampsia (Simmons et al., 2000; Akerman et al., 2008).

Investigations on placental VEGF-A mRNA have shown that its level is reduced (Cooper et al., 1996; Jarvenpaa et al., 2007) in preeclamptic placentae compared with normal placentae while others have
demonstrated an increase (Chung et al., 2004) or no difference (Ranheim et al., 2001). A recent study found placental VEGF-A mRNA to be higher in gestational hypertension, where hypertension occurs in the absence of proteinuria, but lower in pre-eclampsia with HELLP syndrome, while there was no difference in other pre-eclamptic placentae compared with normal placentae (Sambati et al., 2004). The authors propose that the high VEGFA expression in the gestational hypertension group may be a compensatory mechanism in an attempt to restore placental blood flow to normal and that in more severe states such as in pre-eclampsia and the HELLP syndrome, there is an attempt at compensation with only some components of the placenta being able to produce VEGF-A (Sambati et al., 2004).

Initially, impaired invasion and spiral artery remodelling were thought to lead to defective utero-placental circulation and subsequent placental ischaemia (Brosens et al., 1972). More recently, damage to chorionic villi by the failure of remodelling has been elegantly modelled by Burton et al., and the consequences were described (Burton et al., 2009). True ischaemia probably only occurs in the more advanced stages of the disease. Various stressors, like oxidative stress, inflammation, and possibly even mechanical shear stress are now thought to contribute to the release of soluble factors which enter the maternal circulation inducing endothelial dysfunction leading to the clinical features of pre-eclampsia. There is considerable ongoing research on circulating factors which contribute to this maternal endothelial dysfunction. Many studies have demonstrated that increased placental expression and secretion of sFLT-1 which circulates in maternal plasma antagonises both VEGF-A and PI GF, contributing to endothelial dysfunction (Maynard et al., 2003). Soluble FLT-1 overexpression in rats is known to result in hypertension, proteinuria and glomerular endotheliosis characteristic of pre-eclampsia (Maynard et al., 2003). The anti-angiogenic state induced by excess placental production of sFLT-1 can be ‘rescued’ by administering VEGF-A and PI GF (Maynard et al., 2003). The hypothesis that excess placental sFLT-1 may contribute to the pathogenesis of pre-eclampsia is further supported by the increased incidence of pre-eclampsia in mothers carrying trisomy 13 fetuses (Boyd et al., 1987). The genes for sFLT-1 and FLT-1 are localized to chromosome 13. Therefore, fetuses with an extra copy of this chromosome are likely to produce more of the gene products compared with their normal counterparts (Boyd et al., 1987). It has been shown that the ratio of circulating sFLT-1 to PI GF is significantly increased in women carrying trisomy 13 fetuses, possibly contributing to the increased risk of pre-eclampsia seen in these women (Bdolah et al., 2006). The molecular mechanisms leading to excess placental sFLT-1 in pre-eclampsia and the role of sFLT-1 in placentaion are not yet clear. Till recently, it was believed that hypoxia was the major trigger for the release of sFLT-1 (Gu et al., 2008; Nagamatsu et al., 2004; Shore et al., 1997), however Redman and Sargent recently hypothesized that the primary placental defect that triggers pre-eclampsia is likely to be oxidative stress rather than hypoxia. They propose that this inflammatory stimulus provokes the release of sFLT-1 to a similar or greater extent than hypoxia (Redman and Sargent, 2009). Recently, four splice variants of sFLT-1 were also discovered, of which three are known to be up-regulated in preeclamptic placentae (Heydarian et al., 2009).

The sFLT-14 variant which has anti-angiogenic effects similar to sFLT-1 is primate specific and is known to be up-regulated in syncytial knots in preeclamptic placentae (Sela et al., 2008).

There is consistent evidence that placental expression (Zhou et al., 2002; Maynard et al., 2003; Tsatsaris et al., 2003) and maternal serum (Tsatsaris et al., 2003; Hertig et al., 2004; Levine et al., 2004) sFLT-1 are increased in preeclamptic women compared with normal pregnant women. While the majority of studies have demonstrated that there is no significant difference in maternal serum sFLT-1 levels prior to 20 weeks of gestation in women who develop pre-eclampsia compared with normal pregnancies (McKeeman et al., 2004), a few studies have shown a significant difference prior to 20 weeks (Table II). In women who subsequently develop pre-eclampsia, maternal serum sFLT-1 levels are known to rise at 20 weeks of gestation and the levels are significantly increased 5 weeks prior to the onset of hypertension and proteinuria (Levine et al., 2004). The sFLT-1 level is observed to be directly proportional to the degree of proteinuria (Chaiworapongs et al., 2004) and in preeclamptic women, the concentration is known to be higher in those with earlier onset disease (Chaiworapongs et al., 2004; Levine et al., 2004), or severe disease (Maynard et al., 2003; Chaiworapongs et al., 2004; Levine et al., 2004) and in those who deliver small-for-gestational-age infants (Levine et al., 2004; Shibata et al., 2005).

Maternal serum PI GF is known to be the reciprocal of sFLT-1; the higher the sFLT-1, the lower the PI GF (Levine et al., 2004). During normal pregnancy, there is a steady increase in serum PI GF during the first two trimesters, a peak at 29–32 weeks and a decline thereafter (Taylor et al., 2003; Levine et al., 2004). In women who subsequently develop pre-eclampsia, serum PI GF concentrations are lower as early as 10–13 weeks of gestation (Su et al., 2001; Tidwell et al., 2001; Polliotti et al., 2003; Taylor et al., 2003; Bersinger and Odegard 2004; Krauss et al., 2004; Levine et al., 2004; Thadhani et al., 2004; Akolekar et al., 2008) with a considerable diminution 5 weeks prior to the clinical onset of the disease (Levine et al., 2004). Serum PI GF levels at 21–32 weeks of gestation are known to be lower in early pre-eclampsia (prior to 37 weeks) compared with late onset, severe disease compared with mild disease and in pre-eclampsia associated with a small-for-gestational-age infant compared with an appropriate size for gestational age infant (Levine et al., 2004; Madazli et al., 2005). Urinary PI GF is known to parallel the serum level with a rise in the first two trimesters, reaching a peak at 29–32 weeks and a steady decline thereafter. In preeclamptic women, the pattern is similar but substantially lower (Aggarwal et al., 2006) with the most pronounced difference observed 5 weeks prior to the clinical onset of the disease (Levine et al., 2005). The development of pre-eclampsia, however, is not preceded by altered urinary PI GF in the first trimester of pregnancy (Savvidou et al., 2009).

Current evidence suggests that low circulating PI GF and high circulating sFLT-1 early (11–13 weeks) and in mid-pregnancy can distinguish women who subsequently develop pre-eclampsia from those who remain normotensive throughout pregnancy (Table II). It has also been demonstrated that an algorithm that uses PI GF in combination with other markers (Chappell et al., 2002; Espinoza et al., 2007; Stepan et al., 2007; Akolekar et al., 2008; Kusanovic et al., 2009; Poon et al., 2009, 2010; Yu et al., 2010; Akolekar et al., 2011) or the sFLT-1/PI GF ratio (Moore Simas et al., 2007; De Vivo et al., 2008; Diab et al., 2008; Lim et al., 2008) is a better screening tool than either PI GF or sFLT-1 used on their own (Table II).
<table>
<thead>
<tr>
<th>Author</th>
<th>Sample size (n)</th>
<th>Predictive test</th>
<th>GA (weeks)</th>
<th>OR or RR</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>ROC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tidwell et al. (2001)</td>
<td>39</td>
<td>Serum PlGF</td>
<td>5–15</td>
<td>95</td>
<td>90.9</td>
<td>90.5</td>
<td>0.72</td>
</tr>
<tr>
<td>Kusanovic et al. (2009)</td>
<td>1622</td>
<td>PlGF</td>
<td>6–15</td>
<td>62.9, PE; 77.8, EPE</td>
<td>60.4, PE; 69.7, EPE</td>
<td>0.65, PE; 0.74, EPE</td>
<td>0.59, EPE</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PlGF/sFLT-1</td>
<td>6–15</td>
<td>93.5, EPE</td>
<td>22.2, EPE</td>
<td>0.59, EPE</td>
<td>0.65, PE; 0.73, EPE</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PlGF/sEndoglin + sFLT-1</td>
<td>6–15</td>
<td>67.7, PE; 77.8, EPE</td>
<td>53.7, PE; 72.7, EPE</td>
<td>0.65, PE; 0.73, EPE</td>
<td></td>
</tr>
<tr>
<td>Thadhani et al. (2004)</td>
<td>200</td>
<td>Serum PlGF</td>
<td>10</td>
<td>14.7</td>
<td>93.1, EPE</td>
<td>6.0</td>
<td>0.69</td>
</tr>
<tr>
<td>Poon et al. (2009)</td>
<td>7797</td>
<td>Maternal factors, MAP, Uterine PI, PAPP-A, PlGF</td>
<td>11–13</td>
<td>93.1, EPE</td>
<td>88.5, EPE</td>
<td>0.58, EPE</td>
<td>0.66, EPE</td>
</tr>
<tr>
<td>Poon et al. (2010)</td>
<td>402</td>
<td>Maternal factors, MAP, Uterine PI, PlGF</td>
<td>11–13</td>
<td>88.5, EPE</td>
<td>46.7, EPE</td>
<td>0.58, EPE</td>
<td></td>
</tr>
<tr>
<td>Akolekar et al. (2011)</td>
<td>33602</td>
<td>Serum PlGF</td>
<td>11–13</td>
<td>53.5, EPE</td>
<td>27.0, EPE</td>
<td>0.59, EPE</td>
<td></td>
</tr>
<tr>
<td>Akolekar et al. (2008)</td>
<td>824</td>
<td>Maternal factors, Obstetric history, Serum PlGF, Uterine PI</td>
<td>11–14</td>
<td>90.0, EPE</td>
<td>49.0, EPE</td>
<td>0.58, EPE</td>
<td></td>
</tr>
<tr>
<td>Levine et al. (2004)</td>
<td>240</td>
<td>Serum PlGF</td>
<td>13–20</td>
<td>7.4</td>
<td>0.69, EPE</td>
<td>0.58, EPE</td>
<td></td>
</tr>
<tr>
<td>Yu et al. (2010)</td>
<td>124</td>
<td>Doppler PI at 22–24 wks, PlGF, Activin-A</td>
<td>12–16</td>
<td>90.0, EPE</td>
<td>80.8, EPE</td>
<td>0.58, EPE</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Doppler PI at 22–24 wks, PlGF, Activin-A, Inhibin-A</td>
<td>12–16</td>
<td>93.0, EPE</td>
<td>80.8, EPE</td>
<td>0.58, EPE</td>
<td></td>
</tr>
<tr>
<td>Su et al. (2001)</td>
<td>254</td>
<td>Serum PlGF</td>
<td>14–19</td>
<td>2.5</td>
<td>70.0</td>
<td>70.0</td>
<td>0.79</td>
</tr>
<tr>
<td>Lim et al. (2008)</td>
<td>140</td>
<td>sFLT-1</td>
<td>14–21</td>
<td>6.9, PE</td>
<td>0.76, PE</td>
<td>0.58, EPE</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>sFLT-1/PlGF</td>
<td>14–21</td>
<td>38.8, PE</td>
<td>0.85, PE</td>
<td>0.58, EPE</td>
<td></td>
</tr>
<tr>
<td>Polliotti et al. (2003)</td>
<td>80</td>
<td>Serum PlGF</td>
<td>17</td>
<td>4.2</td>
<td>0.79, EPE</td>
<td>0.58, EPE</td>
<td></td>
</tr>
<tr>
<td>Stepan et al. (2007)</td>
<td>63</td>
<td>sFLT-1</td>
<td>19–24</td>
<td>62.6, PE; 67, EPE</td>
<td>7.0, PE; 89.0, EPE</td>
<td>0.65, PE; 0.99, EPE</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>sFLT-1/PlGF</td>
<td>19–24</td>
<td>77.6, PE</td>
<td>62.6, PE</td>
<td>0.65, PE</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Doppler + sFLT-1</td>
<td>19–24</td>
<td>62.6, PE; 67, EPE</td>
<td>51.0, PE; 51.0, EPE</td>
<td>0.65, PE; 0.99, EPE</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Doppler + PlGF</td>
<td>19–24</td>
<td>77.6, PE</td>
<td>73.0, EPE</td>
<td>0.65, PE</td>
<td></td>
</tr>
<tr>
<td>Chappell et al., (2002)</td>
<td>65</td>
<td>Serum PlGF formula</td>
<td>20</td>
<td>0.79</td>
<td>0.65, EPE</td>
<td>0.65, PE</td>
<td></td>
</tr>
<tr>
<td>Kusanovic et al. (2009)</td>
<td></td>
<td>sFLT-1</td>
<td>20–25</td>
<td>51.0, PE; 100.0, EPE</td>
<td>76.4, PE; 95.8, EPE</td>
<td>0.65, PE; 0.99, EPE</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>sFLT-1/PlGF</td>
<td>20–25</td>
<td>66.7, EPE</td>
<td>93.2, EPE</td>
<td>0.65, PE</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>sFLT-1/PlGF/sFLT-1</td>
<td>20–25</td>
<td>40.3, PE; 100.0, EPE</td>
<td>78.5, PE; 89.1, EPE</td>
<td>0.60, PE; 0.99, EPE</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>sFLT-1/PlGF/Inhibin-1</td>
<td>20–25</td>
<td>43.5, PE; 100.0, EPE</td>
<td>77.8, PE; 95.3, EPE</td>
<td>0.63, PE; 0.99, EPE</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>sFLT-1/PlGF/Inhibin-1</td>
<td>20–25</td>
<td>48.4, PE; 100.0, EPE</td>
<td>81.9, PE; 98.0, EPE</td>
<td>0.66, PE; 0.99, EPE</td>
<td></td>
</tr>
<tr>
<td>Madzli et al., (2005)</td>
<td>122</td>
<td>PlGF</td>
<td>21–26</td>
<td>92.9, PE; 100.0, EPE</td>
<td>94.4, PE; 88.1, EPE</td>
<td>0.99, PE</td>
<td></td>
</tr>
<tr>
<td>Espinoza et al. (2007)</td>
<td>3348</td>
<td>PlGF</td>
<td>22–26</td>
<td>9.6, PE; 5.5, EPE</td>
<td>8.0, PE; 8.0, EPE</td>
<td>0.94, PE</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>PlGF and abnormal UADV</td>
<td>22–26</td>
<td>27.3, PE; 64.0, EPE</td>
<td>96.4, PE; 96.5, EPE</td>
<td>0.94, PE</td>
<td></td>
</tr>
<tr>
<td>Diab et al. (2008)</td>
<td>108</td>
<td>sFLT-1</td>
<td>23</td>
<td>96</td>
<td>87</td>
<td>0.94</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>PlGF</td>
<td>23</td>
<td>88</td>
<td>81</td>
<td>0.89</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>PlGF/sFLT-1</td>
<td>23</td>
<td>100</td>
<td>85</td>
<td>0.94</td>
<td></td>
</tr>
<tr>
<td>Chaivorapongsa et al. (2004)</td>
<td>88</td>
<td>Serum sFLT-1</td>
<td>24–28</td>
<td>16.7</td>
<td>97.4</td>
<td>0.94</td>
<td></td>
</tr>
<tr>
<td>De Vivo et al. (2008)</td>
<td>104</td>
<td>sFLT-1</td>
<td>24–28</td>
<td>73.1</td>
<td>80.8</td>
<td>0.87</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>PlGF</td>
<td>24–28</td>
<td>92.3</td>
<td>80.8</td>
<td>0.87</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>PlGF/sFLT-1</td>
<td>24–28</td>
<td>88.5</td>
<td>88.5</td>
<td>0.92</td>
<td></td>
</tr>
<tr>
<td>Hertig et al. (2004)</td>
<td>23</td>
<td>Serum sFLT-1</td>
<td>25–28</td>
<td>80</td>
<td>100</td>
<td>0.94</td>
<td></td>
</tr>
<tr>
<td>Levine et al. (2004)</td>
<td>240</td>
<td>Serum sFLT-1</td>
<td>21–32</td>
<td>5.1</td>
<td>0.94, EPE</td>
<td>0.94, EPE</td>
<td></td>
</tr>
<tr>
<td>Levine et al. (2004)</td>
<td>240</td>
<td>Urinary PlGF</td>
<td>21–32</td>
<td>22.5</td>
<td>0.97, EPE</td>
<td>0.94, EPE</td>
<td></td>
</tr>
<tr>
<td>Moore Simas et al. (2007)</td>
<td>94</td>
<td>sFLT-1</td>
<td>22–36</td>
<td>90.0, EPE</td>
<td>0.94, EPE</td>
<td>0.94, EPE</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>sFLT-1/PlGF</td>
<td>22–36</td>
<td>90.0, EPE</td>
<td>0.94, EPE</td>
<td>0.94, EPE</td>
<td></td>
</tr>
</tbody>
</table>

PE, pre-eclampsia; SPE, severe pre-eclampsia; EPE, early pre-eclampsia; LPE, late pre-eclampsia; MAP, mean arterial pressure; Uterine PI, uterine artery pulsatility index; Uterine RI, uterine artery resistance index; UADV, uterine artery Doppler velocimetry; PAPP-A, pregnancy-associated plasma protein-A.
Although VEGF-A plays an important role in normal pregnancy and in the pathogenesis of pre-eclampsia, maternal serum VEGF-A has a limited clinical role in the prediction of pre-eclampsia. Soluble FLT-1 binds VEGF-A with a higher affinity than PlGF, resulting in extremely low circulating levels of free VEGF-A. A few studies have reported reduced serum VEGF-A in pre-eclampsia (Reuvenkamp et al., 1999) and have proposed VEGF-A as a promising marker for prediction of severe, early-onset pre-eclampsia (Palliotti et al., 2003), but others have failed to observe this due to the concentration being below the detection level of currently available ELISA kits (Taylor et al., 2003; Thadhani et al., 2004; Akolekar et al., 2010). Contrary to the theory of reduced serum VEGF-A in pre-eclampsia, several investigators have shown that maternal serum VEGF-A is increased in preeclamptic pregnancies compared with normotensive pregnancies (Baker et al., 1995; Kupferminc et al., 1997; Hunter et al., 2000; Bosio et al., 2001). This discrepancy is believed to be due to the type of VEGF-A measured. It is proposed that total serum VEGF-A may be increased in preeclamptic pregnancies and that the free level is reduced due to binding to sFLT-1. VEGF-A is produced by many cells at the maternal–fetal interface. A recent study demonstrated that the proportions of VEGF-A expressing peripheral blood T and NK cells were markedly decreased in preeclamptic women compared with healthy pregnancies, suggesting that the reduced maternal serum VEGF-A is not only the result of antagonism by sFLT-1 (Molvarec et al., 2010).

The strong evidence supporting the use of PlGF and sFLT-1 as predictive screening tests for pre-eclampsia has led to these biomarkers being validated for routine clinical use in some countries. However, extensive studies have demonstrated that these biomarkers are more predictive of early-onset pre-eclampsia and severe pre-eclampsia, and that the highest predictive values are demonstrated during second trimester screening (Table II). Therefore, although maternal serum and urinary angiogenic proteins provide a strong predictive tool for a subset of preeclamptic women, they have a limited role in prediction of intermediate and late pre-eclampsia. Furthermore, for intervention to be successful, an early screening tool would be of more utility than a second trimester test.

Recently, the potential use of the VEGF family in the treatment of pre-eclampsia has also been explored. Li and colleagues describe a pre-eclampsia model in pregnant rats induced by adenoviral overexpression of sFLT-1 (Adv-sFLT-1). Infection with Adv-sFLT-1 in rats resulted in hypertension and proteinuria. Histologically, the kidneys from these rats showed glomerular endotheliosis, reminiscent of the renal lesions associated with pre-eclampsia in pregnant women. Administration of recombinant VEGF-A121 resulted in a reduction in systolic blood pressure and proteinuria and an improvement in glomerular endotheliosis (Li et al., 2007). Similar findings have been reported in other animal models (Gilbert et al., 2010; Mateus et al., 2011; Woods et al., 2011), suggesting that VEGF-A121 may have a therapeutic potential in the management of pre-eclampsia. In a mouse model of pre-eclampsia, treatment with either VEGF-A164 or PlGF-2 was shown to reduce the blood pressure, but proteinuria was unaffected by either treatment (Suzuki et al., 2009). These findings provide interesting insights into the potential role of VEGF-A and PlGF in the management of pre-eclampsia. Future studies focusing on the possible adverse effects of VEGF therapy on the fetus and the placenta will be beneficial.

The VEGF family and SGA neonates

Being born SGA significantly increases the risk for neonatal complications and is a leading cause of perinatal death (de Courcy-Wheeler et al., 1995; Tenovuo, 1988). SGA is also associated with impaired neural development and cognition demonstrated by lower intelligence, poor academic performance and behavioural abnormalities in childhood (Pryor et al., 1995; McCormick et al., 1996; Grantham-McGregor, 1998), as well as a lower academic achievement in adulthood (Strauss, 2000). A consistent association has also been demonstrated between SGA and adult onset diseases including increased risk for developing coronary artery disease, stroke, hypertension and type 2 diabetes mellitus (Barker et al., 1989, 1990; McKeigue et al., 1998; McMillen and Robinson, 2005). Those born with low birthweight (LBW) are known to develop endothelial dysfunction, which is an early event in atherosclerosis, which becomes apparent in childhood (Leeson et al., 1997) and persists into adulthood when additional cardiovascular risk factors start to play a role in vascular pathology (Leeson et al., 2001).

The majority of SGA babies are born to normotensive mothers (81.7%), but some are born to women with pre-eclampsia (10.7%) or gestational hypertension (7.6%) (Groom et al., 2007). Pregnancies complicated by SGA caused by placental vasculopathy share many pathogenic abnormalities with pre-eclampsia. In addition, SGA pregnancies also demonstrate maternal endothelial cell dysfunction (Friedman et al., 1994; Bretelle et al., 2001; Johnson et al., 2002) and leukocyte activation (Sabatier et al., 2000).

Some studies report that maternal serum PlGF levels are lower in normotensive women with SGA infants as early as first trimester of pregnancy compared with gestational age-matched controls (Thadhani et al., 2004; Erez et al., 2008; Poon et al., 2008; Romero et al., 2008; Karagiannis et al., 2010). Others, however, report no significant difference between SGA and controls early in gestation but significant different levels later in gestation. A longitudinal study examining PlGF concentration in pregnant women at <14, 15–19, 21–25, 27–30, 35–38 weeks gestation has shown a lower level of PlGF approaching significance at 27–30 weeks in normotensive women delivering an SGA infant compared with normotensive controls, but did not observe any difference earlier in gestation (Taylor et al., 2003). Another study reported that maternal serum PlGF levels in normotensive women with SGA infants were significantly lower than gestational age- matched controls at 33 weeks but not at 17 or 25 weeks (Bersinger and Odegard, 2004). Urinary PlGF during mid-pregnancy has not been shown to be affected by the presence of an SGA infant (Levine et al., 2005).

As with PlGF, several studies have investigated sFLT-1 in normotensive pregnancies with SGA infants compared with matched controls. Some report maternal serum sFLT-1 to be increased in normotensive women who deliver a SGA infant at term (Tsatsaris et al., 2003; Wallner et al., 2007) but others report no difference at term (Shibata et al., 2005) or in the second (Wathen et al., 2006) or first trimester (Thadhani et al., 2004). A longitudinal study evaluating sFLT-1 at 4 week intervals from the first antenatal clinic visit until delivery has shown no difference in maternal serum sFLT-1 levels in normotensive women destined to deliver a SGA infant compared with controls (Romero et al., 2008).

A recent large study of 1536 SGA and 3134 non-SGA pregnancies reported that using a combination of maternal characteristics with
mean arterial pressure, uterine artery pulsatility index, fetal nuchal translucency thickness, pregnancy-associated plasma protein-A (PAPP-A), free β-hCG, PI GF, placental protein-13 (PP13) and a disintegrin and metalloprotease can detect SGA in the absence of pre-eclampsia at 11–13 weeks. The detection rates in this study were 73% for SGA requiring delivery before 37 weeks and 46% for those delivering at term (Karagiannis et al., 2010).

Drawing overall conclusions on the predictive value of VEGF family on SGA pregnancies is hampered by the widespread practice of using different definitions to diagnose SGA, as well as the use of the terms SGA and IUGR (intrauterine growth restriction) synonymously.

The VEGF family and IUGR

IUGR remains a major challenge in pregnancy but lacks a precise definition. IUGR results from many causes including congenital abnormalities, infections and substance abuse. However, most cases of IUGR are associated with placental insufficiency (Lackman et al., 2001). Umbilical, and to a lesser extent uterine, artery Doppler are used as surrogate markers to evaluate placental function and to diagnose IUGR.

Studies using umbilical or uterine artery Doppler in identifying IUGR in the absence of maternal hypertensive disease show that maternal serum sFLT-1 is increased in these pregnancies compared with pregnancies of normotensive women delivering average for gestational age infants (Stepan et al., 2004, 2007; Crispi et al., 2006; Savvidou et al., 2006; Wallner et al., 2007; Chaiworapongsa et al., 2008a, b). A recent study reported that there was no difference in mean maternal plasma sFLT-1 between normotensive women who delivered an SGA infant compared with matched controls, but that a significant increase was only seen in a subset of women who had an abnormal uterine artery Doppler and delivered an SGA infant (Chaiworapongsa et al., 2008a, b). This study also demonstrated that the magnitude of the increase of sFLT-1 was related to the Doppler abnormalities in the maternal and fetal vasculatures, suggesting that oxidative stress and shear stress may contribute to the increase in sFLT-1 in the maternal circulation (Burton and Jauniaux, 2004; Chaiworapongsa et al., 2008a, b). A study that measured maternal plasma PI GF and sFLT-1 in the second trimester in a subgroup of women with abnormal uterine per-fusion revealed that concurrent measurement of uterine perfusion with angiogenic proteins allows effective prediction of early onset IUGR. However, a strong predictive value was not detected for overall IUGR (Table III) (Stepan et al., 2007).

From the above findings, it appears that studies of predictive markers for IUGR pregnancies have demonstrated more consistent results compared with those for SGA pregnancies. However, at present, there are insufficient data to recommend the VEGF family angiogenic growth factors as reliable biomarkers for the prediction of SGA or IUGR pregnancies.

Soluble KDR in pre-eclampsia and SGA

The soluble form of the KDR receptor (sKDR) has been detected in human plasma (Ebos et al., 2004) and the recombinant form of this protein has been shown to have anti-angiogenic activity (Agostini et el., 2005). Maternal plasma sKDR is known to be reduced in pre-eclampsia (Kim et al., 2005) and in growth-restricted pregnancies (Wallner et al., 2007). In a cross-sectional study, plasma sKDR was analysed in four groups; non-pregnant women, women with an uncomplicated pregnancy, preeclamptic women and non-preeclamptic women who delivered an SGA infant. There was no significant difference in mean plasma sKDR between non-pregnant women and women with uncomplicated pregnancies. Preeclamptic women, as well as non-preeclamptic women with SGA infants, were shown to have lower mean plasma sKDR compared with women with uncomplicated pregnancies. There was no significant difference in mean plasma sKDR between preeclamptic women and non-preeclamptic women with SGA infants (Chaiworapongsa et al., 2008a, b). Interestingly, no significant difference was observed in plasma sKDR concentration between SGA with and without abnormal uterine artery Doppler, indicating that utero-placental ischaemia may not be a major determinant of plasma sKDR (Chaiworapongsa et al., 2008a,b). In contrast to the above study, one study reported that there was no difference in maternal plasma sKDR levels in preeclamptic women compared with women with uncomplicated pregnancies, however, the small sample size in this study may have yielded insufficient statistical power to detect a difference (Masuyama et al., 2006).

VEGF-A is known to mediate most of its actions via the membrane-bound KDR receptor. The role of sKDR in VEGF-A signalling is yet to be determined. However, sKDR is known to have anti-angiogenic properties. The administration of adenovirus encoding murine sKDR to non-pregnant rats induces hypertension and proteinuria, but this effect is not seen in pregnant rats. The high levels of unopposed PI GF produced by the placenta in the pregnant rat may explain these findings (Maynard et al., 2003). Reduced maternal plasma sKDR in pre-eclampsia and SGA compared with uncomplicated pregnancies is unexpected, given that pre-eclampsia and, to a lesser extent, SGA are considered anti-angiogenic conditions. While it is still not clear what biological factors contribute to the expression of sKDR, the expression of the membranous isoform of KDR is stimulated by VEGF-A (Shen et al., 1998). Therefore, it was proposed that the low sKDR in pre-eclampsia and SGA could possibly result from low availability of free VEGF-A to stimulate KDR on endothelial cells (Reuevkamp et al., 1999; Maynard et al., 2003; Chaiworapongsa et al., 2008a, b). At present, very few studies have evaluated the role of sKDR in normal and complicated pregnancies and future research is needed to validate the results.

Soluble endoglin in pre-eclampsia

Although not a member of the VEGF family, soluble endoglin (sEng), in combination with PI GF and sFLT-1, may be useful in prediction of pre-eclampsia. Endoglin (CD 105) is a transmembrane glycoprotein predominantly expressed on endothelial cells. It is a co-receptor for TGF-β1 and TGF-β3 (Chefitzet al., 1992). Endoglin modulates signalling of TGF-β by interacting with TGF-β receptors I and II (Fonsatti and Maio, 2004). Endoglin regulates nitric oxide-dependent vasodilatation (Jerck et al., 2004) and its expression is up-regulated in tissues undergoing angiogenesis (Fonsatti and Maio, 2004). Endoglin gene mutations are associated with hereditary haemorrhagic telangiectasia type I, a vascular disorder characterized by focal telangiectases and arteriovenous malformations (Lebrin and Mummary, 2008). These suggest the involvement of endoglin in angiogenesis, vascular development and in maintaining vessel wall integrity (Fonsatti and Maio, 2004). Proteolytic processing of the membrane-bound endoglin results in sEng, an N-terminal cleavage product of the full-length endoglin (Venkatesha et al., 2006). In vitro studies have demonstrated that sEng inhibits TGF-β signalling,
blocks TGF-β-mediated vasodilatation and interferes with endothelial proliferation and capillary formation (Venkatesha et al., 2006). Endoglin deficiency does not affect vasculogenesis but results in poor vascular smooth muscle development and arrested endothelial remodelling, and endoglin is shown to be essential for angiogenesis (Li et al., 1999). sEng is also implicated in the pathophysiology of pre-eclampsia.

Placental expression of sEng is up-regulated in pre-eclampsia. It is proposed that sEng enters the maternal circulation and inhibits TGF-β signalling resulting in endothelial dysfunction (Venkatesha et al., 2006). Over-expression of sEng in rodents leads to increased vascular permeability and hypertension without proteinuria, and over-expression of both sEng and sFLT-1 results in severe vascular damage, nephrotic-range proteinuria, severe hypertension, a syndrome similar to HELLP syndrome and IUGR (Venkatesha et al., 2006). Maternal serum sEng concentrations increase during the last 2 months of normal pregnancy and this rise is higher and occurs earlier in women who develop pre-eclampsia. The increase in sEng is associated with an increase in the ratio of sFLT-1:PIGF and a composite measure incorporating all three molecules, the ratio of (sFLT-1+sEng):PIGF, is considered a predictive biomarker for pre-eclampsia (Levine et al., 2006).

The VEGF family and preterm birth

Preterm deliveries are those that occur before the completion of 37 weeks of gestation. In developed countries, the rate of preterm birth is 5–9% and as much as 12% in the USA (Goldenberg et al., 2002, 2003). In the group with preterm labour with intact membranes as well as in those with PPROMs (Kim et al., 2002, 2003). In the group with preterm labour with intact membranes, the mean percentage of spiral arteries with failure of physiological transformation was reported in patients with preterm labour and intact membranes as well as in those with PPROMs (Kim et al., 2002, 2003). The mechanisms responsible for this failure of physiological transformation have not yet been determined. A possible mechanism is the inability of trophoblasts to down-regulate cell adhesion molecules characteristic of epithelial cells and to up-regulate those characteristic of endothelial cells (Zhou et al., 1997a,b).

At present, data from clinical studies on the role of VEGF family in preterm birth are limited. One large study of 292 women with spontaneous preterm birth and 937 controls analysed PIGF and sFLT-1 in maternal serum collected between 10 and 14 weeks of gestation. This study did not find any association between early PIGF levels and subsequent preterm birth. However, women with higher circulating sFLT-1 at 10–14 weeks of gestation were at a lower risk of spontaneous preterm birth (Smith et al., 2007). The biological basis of this finding is yet to be determined. It has been shown that VEGF-A is

### Table III Angiogenic biomarkers used for detection/prediction of SGA and IUGR.

<table>
<thead>
<tr>
<th>Author</th>
<th>Sample size (n)</th>
<th>Predictive test</th>
<th>GA (weeks)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stepan et al.</td>
<td>63 women with abnormal uterine perfusion</td>
<td>sFLT-1</td>
<td>19–24</td>
<td>64,IUGR; 8,early</td>
<td>54,IUGR; 94,early</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PIGF</td>
<td>19–24</td>
<td>36,IUGR; 4,early</td>
<td>84,IUGR; 84,early</td>
</tr>
<tr>
<td></td>
<td></td>
<td>sFLT-1/PIGF</td>
<td>19–24</td>
<td>53,IUGR; 6,early</td>
<td>57, IUGR; 100,early</td>
</tr>
<tr>
<td></td>
<td></td>
<td>sFLT-1 + PIGF</td>
<td>19–24</td>
<td>64,IUGR; 8,early</td>
<td>78,IUGR; 100,early</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Doppler + sFLT-1</td>
<td>19–24</td>
<td>64,IUGR; 8,early</td>
<td>70,IUGR; 100,early</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Doppler + PIGF</td>
<td>19–24</td>
<td>64,IUGR; 8,early</td>
<td>73,IUGR; 76,early</td>
</tr>
<tr>
<td>Karagiannis et al.</td>
<td>1536 SGA</td>
<td>Combination of maternal characteristics</td>
<td>19–24</td>
<td>73, SGA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3134 non-SGA</td>
<td>biophysical and biochemical markers including PIGF</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SGA, small-for-gestational-age infant; IUGR, intrauterine growth restriction; GA, gestational age.
expressed in the myometrium of the pregnant human uterus (Oh et al., 2006). In pregnant rats, VEGF-A, FLT-1 and Flk are expressed in the cervix and VEGF-A expression is known to peak in association with ripening of the cervix (Mlowa et al., 2004). Therefore, it is proposed that low sFLT-1 resulting in increased free VEGF-A may promote preterm birth (Smith et al., 2007). Hence, it is plausible that high sFLT-1 could have a protective role in preterm birth. However, a recent study revealed that in the setting of inflammation (elevated high sensitivity C-reactive protein), women with low serum PI GF levels at the time of onset of spontaneous preterm labour had a 6.8-fold increased risk of preterm birth (Bastek et al., 2010). These findings suggest an interaction between the angiogenic and inflammatory pathways which merits future research.

The VEGF family and recurrent pregnancy loss
Spontaneous pregnancy loss affects 12–15% of couples (Stirrat, 1990) and 0.5–3% experience recurrent pregnancy loss defined as the occurrence of three or more consecutive pregnancy losses (Li et al., 2002). There is increasing evidence that there is no significant difference in the possible causes between those who experience two compared with three miscarriages (Habayeb and Konje, 2004) and it is recommended that couples should be investigated after two miscarriages (Quenby and Farquharson, 1993; Stephenson et al., 1998). Parental chromosomal abnormalities (Houwert-de Jong et al., 1989), uterine defects (Houwert-de Jong et al., 1989), thrombophilias (Habayeb and Konje, 2004; Kist et al., 2008), anti-phospholipid syndrome (Habayeb and Konje, 2004), endocrinological disorders, infections and nutritional and environmental factors (Li et al., 2002) have all been documented as possible causes for recurrent miscarriage. However, in ~50% of recurrent pregnancy loss, the cause remains unknown (Li et al., 2002; Habayeb and Konje, 2004).

The majority of spontaneous miscarriage occurs during the first trimester of pregnancy. Blighted ovum, the most common type of first trimester miscarriage, is mostly accompanied by aneuploidy. With regard to defective placentaion as a cause of early miscarriage, it should be noted that remodelling of spiral arteries in the decidual segments is well established by 8 weeks of gestation and is completed by the end of the first trimester (Pijnenborg et al., 1980). However, failed trophoblast invasion and spiral artery remodelling are not demonstrated in early miscarriage (Ball et al., 2006a, b). In contrast to this, late sporadic miscarriage which occurs after 12 weeks of gestation and complicates ~1–2% of pregnancies (Regan and Rai, 2000) does exhibit abnormalities in spiral artery transformation and trophoblast invasion (Ball et al., 2006a, b).

VEGF-A, PI GF and the main receptors KDR and FLT-1 are known to regulate decidal vascularization in early human pregnancy (Piaisier et al., 2007). Decidual vascularization is shown to be different in first trimester spontaneous miscarriages compared with matched controls. Decidual samples from miscarriage show fewer vessels with larger circumference in both decidua basalis and decidua parietalis compared with those obtained from first trimester terminations and these vascular differences correlate with increased expression of KDR and FLT-1 (Piaisier et al., 2009). In contrast, a higher vessel density in the decidua parietalis (Vailhe et al., 1999) and decreased VEGF-A and FLT-1 are reported in missed abortions (Vuorela et al., 2000).

In addition to post-implantation pregnancy loss, ~30% of spontaneously conceived embryos are lost prior to implantation and ~50% of embryos conceived through IVF fail to implant (Boomsma et al., 2009). In humans, the ability of the conceptus to implant in the endometrium is restricted to a few days in the menstrual cycle. This window of implantation minimises the risk of late implantation of compromised embryos (Teklenburg et al., 2010). Differentiation of endometrial stromal cells into specialized decidual cells, termed decidualization, is critical for the endometrium to achieve the characteristics essential to recognize, respond to and eliminate compromised implanting embryos (Teklenburg et al., 2010). A recent paper by Dr Jan Brosen’s group demonstrates that impaired cyclic decidualization of the endometrium facilitates implantation yet predisposes to subsequent pregnancy loss by disabling natural embryo selection and by disrupting the maternal response to embryonic signals (Salker et al., 2010). Intrauterine VEGF-A levels are known to be cycle dependent with increasing levels during the late-secretory phase shown to be correlated with decidualization (Licht et al., 2003). A positive correlation is also shown between intrauterine VEGF-A levels and the decidualization marker IGF-binding protein (IGFBP)-1 (Licht et al., 2003). There is a sharp rise in intrauterine IGFBP-1 levels coinciding with the time of the closing of the implantation window, suggesting that intrauterine IGFBP-1 may be involved in the mechanisms restricting endometrial receptivity (Licht et al., 2002). Although correlations do not necessarily reflect causality, it could be hypothesized that low intrauterine VEGF-A levels may have a role in impaired decidualization and prolonged endometrial receptivity in recurrent pregnancy loss.

VEGF family gene polymorphisms and adverse pregnancy outcomes
The VEGF family plays a pivotal role in normal pregnancy, and consistent evidence exists for its causal role in pregnancy complications. Therefore, genetic variations in the genes encoding these angiogenic proteins have been good candidates to study in pregnancy complications (Table IV).

VEGF family polymorphisms in pre-eclampsia
Several groups have studied functional VEGF-A polymorphisms in pre-eclampsia. The VEGF-A +936C/T polymorphism is in the 3′ untranslated region (3′UTR) of the VEGF gene and the T allele is associated with lower plasma VEGF-A compared with the C allele (Renner et al., 2000). Two groups report the association of the VEGF-A+ 936C/T polymorphism with pre-eclampsia. A Korean study reports the association of the maternal VEGF +936T allele with pre-eclampsia (Shim et al., 2007), while a Greek study reports no association of the maternal polymorphism with pre-eclampsia, but found an association of the T allele with severe pre-eclampsia. However, their study sample was relatively small and the severe pre-eclamptic group comprised only 20 women (Papazoglou et al., 2004b).

The VEGF-A+405G/C is a promoter polymorphism and the CC genotype is associated with the highest plasma VEGF-A levels (Watson et al., 2000; Stevens et al., 2003). The G allele of this polymorphism is shown to be associated with a reduced risk for severe pre-eclampsia (Banyasz et al., 2006a, b) while the CC genotype is shown to be associated with an increased risk for HELLP syndrome (Nagy et al., 2008). The VEGF-A −460C/T promoter polymorphism is in linkage disequilibrium with the +405G/C polymorphism and
<table>
<thead>
<tr>
<th>Pregnancy complication and author</th>
<th>Cases (n)</th>
<th>Controls (n)</th>
<th>Ethnicity</th>
<th>Polymorphism</th>
<th>rs number</th>
<th>Function of polymorphism</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pre-eclampsia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Papazoglou et al. (2004b)</td>
<td>42</td>
<td>73</td>
<td>Greek</td>
<td>VEGF-A – 2578C/A</td>
<td>rs699947</td>
<td>C allele—higher plasma VEGF-A</td>
<td>No association</td>
</tr>
<tr>
<td>Banyasz et al. (2006a, b)</td>
<td>84(^a)</td>
<td>96</td>
<td>Hungarian</td>
<td>VEGF-A – 2578C/A</td>
<td>rs699947</td>
<td>C allele—higher plasma VEGF-A</td>
<td>No association</td>
</tr>
<tr>
<td>Nagy et al. (2008)</td>
<td>71(^b)</td>
<td>93</td>
<td>Hungarian</td>
<td>VEGF-A – 2578C/A</td>
<td>rs699947</td>
<td>C allele—higher plasma VEGF-A</td>
<td>No association</td>
</tr>
<tr>
<td>Sandrim et al. (2009)</td>
<td>94</td>
<td>108</td>
<td>White and non white</td>
<td>VEGF-A – 2578C/A</td>
<td>rs699947</td>
<td>C allele—higher plasma VEGF-A</td>
<td>No association</td>
</tr>
<tr>
<td>Papazoglou et al. (2004b)</td>
<td>42</td>
<td>73</td>
<td>Greek</td>
<td>VEGF-A + 936C/T</td>
<td>rs3025039</td>
<td>T allele—lower plasma VEGF-A</td>
<td>2.7 (1.1 – 6.6), SPE</td>
</tr>
<tr>
<td>Shim et al. (2007)</td>
<td>110</td>
<td>209</td>
<td>Korean</td>
<td>VEGF-A + 936C/T</td>
<td>rs3025039</td>
<td>T allele—lower plasma VEGF-A</td>
<td>2.2 (1.5 – 3.4) for T allele</td>
</tr>
<tr>
<td>Banyasz et al. (2006a, b)</td>
<td>84</td>
<td>96</td>
<td>Hungarian</td>
<td>VEGF-A + 405G/C</td>
<td>rs2010963</td>
<td>G allele—higher plasma VEGF-A</td>
<td>0.3 (0.1 – 0.9) for G allele</td>
</tr>
<tr>
<td>Nagy et al. (2008)</td>
<td>71(^b)</td>
<td>93</td>
<td>Hungarian</td>
<td>VEGF-A + 405G/C</td>
<td>rs2010963</td>
<td>G allele—higher plasma VEGF-A</td>
<td>3.7 (1.1 – 12.8) for CC genotype</td>
</tr>
<tr>
<td>Sandrim et al. (2009)</td>
<td>94</td>
<td>108</td>
<td>White and non-white</td>
<td>VEGF-A – 1154G/A</td>
<td>rs1570360</td>
<td>G allele—higher plasma VEGF-A</td>
<td>No association</td>
</tr>
<tr>
<td>Papazoglou et al. (2004b)</td>
<td>42</td>
<td>73</td>
<td>Greek</td>
<td>VEGF-A – 634G/C</td>
<td>rs2010963</td>
<td>C allele—higher plasma VEGF-A</td>
<td>No association</td>
</tr>
<tr>
<td>Srinivas et al. (2010)</td>
<td>184</td>
<td>305</td>
<td>Black</td>
<td>VEGF-C – 1830A/C</td>
<td>rs1485766</td>
<td>tagSNP</td>
<td>1.6 (1.1 – 2.3)</td>
</tr>
<tr>
<td></td>
<td>184</td>
<td>305</td>
<td>Black</td>
<td>VEGF-C – 2698B/G</td>
<td>rs6838834</td>
<td>tagSNP</td>
<td>1.6 (1.1 – 2.4)</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>35</td>
<td>White</td>
<td>VEGF-C – 33G/A</td>
<td>rs7664413</td>
<td>tagSNP</td>
<td>2.0 (0.99 – 4.7)</td>
</tr>
<tr>
<td></td>
<td>184</td>
<td>305</td>
<td>Black</td>
<td>FLT-1 – 523G/C</td>
<td>rs12584067</td>
<td>tagSNP</td>
<td>1.6 (1.0 – 2.4)</td>
</tr>
<tr>
<td></td>
<td>184</td>
<td>305</td>
<td>Black</td>
<td>FLT-1 – 4471C/T</td>
<td>rs7335588</td>
<td>tagSNP</td>
<td>1.6 (1.1 – 2.4)</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>35</td>
<td>White</td>
<td>FLT-1 + 4244G/A</td>
<td>rs722503</td>
<td>tagSNP</td>
<td>2.1 (1.1 – 4.2)</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>35</td>
<td>White</td>
<td>FLT-4 + 1480A/G</td>
<td>rs307826</td>
<td>tagSNP</td>
<td>3.1 (1.2 – 7.9)</td>
</tr>
<tr>
<td>Kim et al. (2008)</td>
<td>170</td>
<td>201</td>
<td>Korean</td>
<td>FLT-1 repeat</td>
<td></td>
<td>may affect signal transduction</td>
<td>No association</td>
</tr>
<tr>
<td>Andraweera et al. (2012)</td>
<td>71</td>
<td>404</td>
<td>Caucasian</td>
<td>KDR – 604C/T</td>
<td>rs2071559</td>
<td>C allele—lower KDR transcription</td>
<td>1.9 (1.0 – 3.5) for paternal SNP</td>
</tr>
<tr>
<td></td>
<td>71</td>
<td>404</td>
<td>Caucasian</td>
<td>KDR – 604C/T</td>
<td>rs2071559</td>
<td>C allele—lower KDR transcription</td>
<td>2.2 (1.1 – 4.4) for neonatal SNP</td>
</tr>
<tr>
<td>SGA pregnancies</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Banyasz et al. (2006a, b)</td>
<td>128(^d)</td>
<td>200</td>
<td>Greek</td>
<td>VEGF-A + 405G/C</td>
<td>rs2010963</td>
<td>G allele—higher plasma VEGF-A</td>
<td>1.3 (1.0 – 1.6) for C allele</td>
</tr>
<tr>
<td></td>
<td>128(^d)</td>
<td>200</td>
<td>Greek</td>
<td>VEGF-A – 2578C/A</td>
<td>rs699947</td>
<td>C allele—higher plasma VEGF-A</td>
<td>No association</td>
</tr>
</tbody>
</table>
Andraweera et al. (2011) 157\textsuperscript{c} 958\textsuperscript{c} Caucasian VEGF-A + 936C/T rs3025039 T allele—lower plasma VEGF-A 1.4 (1.0–2.1) for neonatal SNP

Andraweera et al. (2012) 101\textsuperscript{d} 401\textsuperscript{d} Caucasian KDR-604C/T rs2071559 C allele—lower KDR transcription 2.1 (1.3–3.5) for paternal SNP

Spontaneous preterm labour

Papazoglou et al. (2004a) 54 79 Greek VEGF-A 2634G/C rs2010963 C allele—higher plasma VEGF-A 2.05 (1.4–3.1) for T allele

Papazoglou et al. (2004a) 54 79 Greek VEGF-A + 936C/T rs3025039 T allele—lower plasma VEGF-A 2.05 (1.4–3.1) for T allele

Recurrent miscarriage

Papazoglou et al. (2005) 52 82 Greek VEGF-A + 2578C/A rs699947 C allele—higher plasma VEGF-A 1.9 (1.2–3.2) for A allele

Coulam and Jeyendran (2008) 152 65 A variety of ethnics VEGF-A + 2578C/A rs699947 C allele—higher plasma VEGF-A 2.7 (1–7.4) for A allele

Lee et al. (2010) 215 113 Korean VEGF-A + 2578C/A rs699947 C allele—higher plasma VEGF-A 2 (1.2–3.5) for GA + AA

Eller et al. (2011a, b) 99 181 Caucasian and Hispanic VEGF-A + 2578C/A rs699947 C allele—higher plasma VEGF-A No association

Su et al. (2011b) 115 170 Taiwanese VEGF-A + 1154G/A rs1570360 G allele—higher plasma VEGF-A 1.9 (1.2–3.2) for A allele

Xing et al. (2011) 339 291 Chinese Han VEGF-A + 1154G/A rs1570360 G allele—higher plasma VEGF-A No association

Papazoglou et al. (2005) 52 82 Greek VEGF-A + 2578C/A rs699947 C allele—higher plasma VEGF-A No association

Lee et al. (2010) 215 113 Korean VEGF-A + 2578C/A rs699947 C allele—higher plasma VEGF-A No association

Eller et al. (2011a, b) 99 181 Caucasian and Hispanic VEGF-A + 2578C/A rs699947 C allele—higher plasma VEGF-A No association

Papazoglou et al. (2005) 52 82 Greek VEGF-A + 2578C/A rs699947 C allele—higher plasma VEGF-A No association

Lee et al. (2010) 215 113 Korean VEGF-A + 2578C/A rs699947 C allele—higher plasma VEGF-A No association

Eller et al. (2011a, b) 99 181 Caucasian and Hispanic VEGF-A + 2578C/A rs699947 C allele—higher plasma VEGF-A No association

Traina et al. (2011) 89 191 Brazilian VEGF-A + 2578C/A rs699947 C allele—higher plasma VEGF-A 0.7 (0.5–0.9) for A allele

Eller et al. (2011a, b) 99 181 Caucasian and Hispanic VEGF-A + 2578C/A rs699947 C allele—higher plasma VEGF-A No association

Papazoglou et al. (2005) 52 82 Greek VEGF-A + 2578C/A rs699947 C allele—higher plasma VEGF-A No association

Lee et al. (2010) 215 113 Korean VEGF-A + 2578C/A rs699947 C allele—higher plasma VEGF-A No association

Eller et al. (2011a, b) 99 181 Caucasian and Hispanic VEGF-A + 2578C/A rs699947 C allele—higher plasma VEGF-A No association

Papazoglou et al. (2005) 52 82 Greek VEGF-A + 2578C/A rs699947 C allele—higher plasma VEGF-A No association

Lee et al. (2010) 215 113 Korean VEGF-A + 2578C/A rs699947 C allele—higher plasma VEGF-A No association

Eller et al. (2011a, b) 99 181 Caucasian and Hispanic VEGF-A + 2578C/A rs699947 C allele—higher plasma VEGF-A No association

Traina et al. (2011) 89 191 Brazilian VEGF-A + 2578C/A rs699947 C allele—higher plasma VEGF-A No association

Eller et al. (2011a, b) 99 181 Caucasian and Hispanic VEGF-A + 2578C/A rs699947 C allele—higher plasma VEGF-A No association

Su et al. (2011b) 115 170 Taiwanese KDR + 116A/G rs6838752 tagSNP 0.4 (0.3–0.8)

KDR + 116A/T rs1870377 tagSNP 0.5 (0.3–0.9)

\(\text{a}\)Severe preeclamptic women, SPE, severe pre-eclampsia.

\(\text{b}\)HELLP syndrome.

\(\text{c}\)Parent–infant trios.

\(\text{d}\)Infants with a birthweight \(\leq 1500\) g.
the TT genotype is shown to be associated with an increased risk for the HELLP syndrome (Nagy et al., 2008).

The VEGF-A −2578C/A is a promoter polymorphism with the CC genotype having the highest transcriptional activity and VEGF-A protein production by leukocytes (Shahbazi et al., 2002). Four groups have studied this polymorphism and report no association with pre-eclampsia (Papazoglou et al., 2004b; Sandrim et al., 2009), severe pre-eclampsia (Banyasz et al., 2006a, b) or the HELLP syndrome (Nagy et al., 2008). However, Banyasz reported that hypertension and proteinuria were detected earlier in carriers of the VEGF-A −2578C/A A allele in their population of severe preeclamptic women (Banyasz et al., 2006a, b). Sandrim et al. (2009) also report that when stratified by ethnicity, the AA genotype of the VEGF-A −2578C/A polymorphism was less common in preeclamptic white women compared with healthy pregnant controls.

The VEGF-A −634G/C and −1154G/A polymorphisms are located in the 5′ untranslated region (5′UTR) of the VEGF-A gene and the −634C and −1154G alleles are associated with higher VEGF-A production (Awata et al., 2002; Shahbazi et al., 2002). Both polymorphisms have been studied in pre-eclampsia and no association is reported (Papazoglou et al., 2004b; Sandrim et al., 2009). However, in the study by Sandrim et al., the GG genotype of the VEGF-A −634G/C polymorphism is reported to be significantly less common in the white preeclamptic women compared with controls.

At present, genetic association studies on the above polymorphisms are limited by the sample size. A meta-analysis was deemed inappropriate due to the phenotypic differences in the preeclamptic study populations.

A recent large study evaluated 112 tagging polymorphisms in VEGF-A, VEGF-B, VEGF-C, FLT1 and FLT4 in white and black preeclamptic women with matched controls. This study reported the association of polymorphisms in VEGF-C and FLT1 genes in either white and black preeclamptic women, but did not find the same polymorphism to be associated with pre-eclampsia in both ethnic groups. This may be partially explained by ethnic differences in the distribution of these polymorphisms and the relatively small group of white preeclamptic women in the study (Srinivas et al., 2010).

A dinucleotide repeat polymorphism in the 3′ non-coding region of the FLT1 gene has also been studied in preeclamptic Korean women, with no association reported (Kim et al., 2008).

In a preliminary study, we recently demonstrated that homozygosity for the C allele of the KDR −604C/T polymorphism in both the father and the infant was associated with pre-eclampsia. KDR −604T/C is a promoter polymorphism with the C allele exhibiting 68% lower transcriptional activity compared with the T allele and the CC homozygotes having significantly lower KDR levels compared with TT homozygotes (Wang et al., 2007). The KDR −604T/C polymorphism has previously been shown to be associated with coronary artery disease, hence the association with pre-eclampsia is interesting, but needs to be replicated in a larger cohort (Andraweera et al., 2012).

VEGF family polymorphisms in SGA infants

At present, literature on the role of VEGF family polymorphisms in SGA is limited. VEGF-A +405G/C and −2578C/A polymorphisms have been studied in a group of LBW infants compared with term healthy controls. The C allele of the +405G/C polymorphism is associated with an increased risk for LBW infants (Banyasz et al., 2006a, b).

VEGF-A −2578C/A polymorphism was not associated with LBW but the A allele was associated with increased risk for necrotizing enterocolitis and a decreased risk for acute renal failure during the perinatal period.

In our preliminary study mentioned above, we also demonstrated that the KDR +604C/T polymorphism in both the father and the infant was associated with SGA as well as SGA with an abnormal Doppler (Andraweera et al., 2012). In addition, our group recently demonstrated that the VEGF-A +936C/T polymorphism in the infant was associated with SGA as well as SGA with an abnormal Doppler. We also found that the polymorphism in both the mother and the infant was associated with uterine and umbilical artery Doppler abnormalities and that the polymorphism in the first trimester placenta was associated with reduced first trimester placental VEGF-A mRNA expression, suggesting a potential role of this SNP in the pathogenesis of SGA (Andraweera et al., 2011).

VEGF family polymorphisms in preterm birth

Only one study has so far looked at VEGF SNPs in spontaneous preterm birth; it reported an increased frequency of the VEGF-A +936C/T T allele in women who experienced spontaneous preterm birth compared with controls, but no association for the VEGF-A −634G/C polymorphism (Papazoglou et al., 2004a).

VEGF family polymorphisms in recurrent pregnancy loss

The VEGF-A polymorphisms, −1154G/A, −2578C/A, +936C/T and −634G/C have been studied in women who have experienced recurrent pregnancy loss in a few ethnic groups.

The VEGF-A −1154G/A polymorphism has been investigated in six studies of which three reported the association of the A allele with an increased risk for recurrent miscarriage (Papazoglou et al., 2005; Coulam and Jeyendran, 2008; Lee et al., 2010) and the other three reported no association (Eller et al., 2011a, b; Su et al., 2011a, b; Xing et al., 2011).

The VEGF-A 2578C/A, +936C/T and −634G/C polymorphisms have not been associated with recurrent pregnancy loss (Papazoglou et al., 2005; Lee et al., 2010; Traina et al., 2011), except in a recent study which reported a decreased frequency of the VEGF-A −2578A allele and an increased frequency of the VEGF-A −634C allele in recurrent pregnancy loss (Eller et al., 2011b).

Of these polymorphisms, a recent meta-analysis demonstrated that the VEGF-A −1154G/A polymorphism was significantly associated with recurrent pregnancy loss (odds ratio (OR), 1.5; 95% confidence interval, 1.1–2.0) (Su et al., 2011a).

Conclusions regarding VEGF family polymorphisms

Overall, these candidate gene association studies demonstrate that polymorphisms in the VEGF family of angiogenic growth factors appear to be implicated in pregnancy complications. However, the effect of these polymorphisms on adverse pregnancy outcomes appears to be small as evidenced by the reported ORs. The repeatability of the results is also poor across different ethnic groups. Therefore, polymorphisms in the VEGF family genes explored so far do not appear to have a role in the clinical prediction of adverse pregnancy outcomes. Considering the important environmental and lifestyle modifiable risk factors for pregnancy complications, it will be
interesting to investigate potential gene–gene and gene-environment interactions in the VEGF family genes.

**Discussion**

Recent years have seen considerable advances in the role of the VEGF family in pregnancy complications, mainly in pre-eclampsia. Extensive studies by Ananth Karumanchi’s group have clearly demonstrated that the anti-angiogenic factors sFLT-1 and sEng play important roles in the pathophysiology of pre-eclampsia. These findings have been replicated in later years by other groups showing consistent evidence. Several prospective cohort studies have demonstrated that serum/plasma levels of PI GF, sFLT-1, sEng and the sFLT-1/PI GF ratio may be useful biomarkers in the prediction of pre-eclampsia, although they may not be useful sufficiently early in gestation to permit intervention. The reviewed literature also demonstrates that these biomarkers in combination with other clinical and biochemical investigations are better at predicting pre-eclampsia than when used alone. The deviation of the plasma/serum levels of these biomarkers from normal reference ranges are more pronounced in women with earlier onset pre-eclampsia, while the deviation is minimal in those who develop late-onset pre-eclampsia. Therefore, the clinical utility of these biomarkers is limited to a subset of women. Ohkuchi et al. (2011) recently proposed that these findings suggest the existence of thresholds for the onset of pre-eclampsia and set out to determine the thresholds for plasma concentrations of angiogenic (PI GF) and anti-angiogenic (sFLT-1 and sEng) factors at the onset of pre-eclampsia. The authors report that these thresholds exist for sFLT-1 and sFLT-1/PI GF ratio, and the threshold for sFLT-1/PI GF ratio measured between 26 and 31 weeks of gestation may be useful for detecting pre-eclampsia with onset at <36 weeks. Consistent with previous studies, the authors also report that consideration of established maternal risk factors in the algorithm increases the likelihood ratios of the prediction model. The onset threshold levels of sFLT-1 and sFLT-1/PI GF ratio at an earlier gestational age were very high and deviated markedly from the reference ranges, suggesting a possible reason for the lower incidence of early-onset pre-eclampsia compared with late onset disease.

Endothelial dysfunction has long been recognized to play a key role in the pathophysiology of pre-eclampsia. However, most studies on prediction of pre-eclampsia have not incorporated measures of endothelial function into the predictive algorithms. Maternal endothelial function is shown to be markedly altered before the onset of clinical features of pre-eclampsia (Khan et al., 2005). Therefore, inclusion of non-invasive measures of endothelial function may improve the predictive value of these models (Banek and Gilbert, 2011).

Maternal endothelial dysfunction in pre-eclampsia is mainly attributed to defective placentation. However, there is increasing evidence to suggest that abnormal placentation may not be the sole reason for altered endothelial function in preeclamptic women (Chambers et al., 2001; Kaaja and Greer, 2005). A history of pre-eclampsia, as well as delivery of a growth-restricted baby, is associated with increased long-term risk for vascular diseases including coronary artery disease and stroke (McDonald et al., 2008). It is debatable as to whether endothelial dysfunction manifests as a consequence of these pregnancy complications and persists throughout life or whether women with risk factors for endothelial dysfunction manifest these vascular diseases at different stages. The shared risk factors for both pre-eclampsia and later-life vascular diseases, as well as the familial segregation of all these disorders suggest that these diseases share a common genetic predisposition that interacts with the environment and may predispose individuals to vascular disorders which manifest at different time points throughout the life course.

Prospective cohort studies with the aim of collecting data on relevant clinical, environmental and lifestyle risk factors coupled with longitudinal measurement of angiogenic factors, endothelial function and genetic variations in candidate gene pathways will be beneficial in establishing interactions which predispose to pregnancy complications.

**Authors’ roles**

P.H.A. reviewed the literature and wrote the manuscript. G.A.D. and C.T.R. critically reviewed the manuscript for important intellectual content. All authors edited the manuscript to produce the final draft.

**Funding**

This study was funded by the National Health and Medical Research Council of Australia and the Channel 7 Children’s Research Foundation. P.H.A. holds and Australian Leadership Award funded by the Australian government. The study sponsors had no role in study design, data analysis and interpretation or writing this report.

**Conflict of interest**

None of the authors have any conflicts of interest to declare.

**References**


Baker PN, Krasnow J, Roberts JM, Yeo KT. Elevated serum levels of vascular endothelial growth factor receptor-1 concentration is elevated in SGA and the magnitude of the increase relates to Doppler abnormalities in the maternal and fetal circulation. J Matern Fetal Neonatal Med 2008a;21:25–40.


He Y, Smith SK, Day KA, Clark DE, Licence DR, Charnock-Jones DS. Alternative splicing of vascular endothelial growth factor (VEGF)-R1 (FLT-1) pre-mRNA is important for the regulation of VEGF activity. Mol Endocrinol 1999;13:537 –545.


