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Genetic predisposition to preeclampsia is conferred by fetal DNA variants near *FLT1*, a gene involved in the regulation of angiogenesis

Kathryn J Gray[1], **Richa Saxena**[2], and **S. Ananth Karumanchi**[3]

[1]Brigham and Women's Hospital, Harvard Medical School, Boston, MA

[2]Broad Institute of Harvard and MIT and Massachusetts General Hospital, Harvard Medical School, Boston MA

[3]Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA

Abstract

Preeclampsia risk is influenced by both the mother's genetic background, as well as the genetics of her fetus; however, the specific genes responsible for conferring preeclampsia risk have largely remained elusive. Evidence that preeclampsia has a genetic predisposition was first detailed in the early 1960's, and overall preeclampsia heritability is estimated at ~55%. Many traditional gene discovery approaches have been employed to investigate the specific genes that contribute to preeclampsia risk, but these have largely not been successful or reproducible. Over the past decade, genome-wide association studies (GWAS) have allowed for significant advances in the understanding of the genetic basis of many common diseases. GWAS are predicated on the idea that the genetic basis of many common diseases are complex and polygenic with many variants, each with modest effects that contribute to disease risk. Using this approach in preeclampsia, a large genome-wide association study (GWAS) recently identified and replicated the first robust fetal genomic region associated with excess risk. A screen of >7 million genetic variants in 2,658 offspring from preeclamptic women and 308,292 population controls identified a single association signal close to the *FLT1* (Fms-like tyrosine kinase 1) gene, on chromosome 13. *FLT1* encodes sFLT1, a splice variant of the vascular endothelial growth factor (VEGF) receptor that exerts antiangiogenic activity by inhibiting signaling of proangiogenic factors. The FLT1 pathway is central in preeclampsia pathogenesis, as excess circulating sFLT1 in the maternal plasma leads to the hallmark clinical features of preeclampsia, including hypertension and proteinuria. The success of this landmark fetal preeclampsia GWAS suggests that well-powered, larger maternal and fetal GWAS will be fruitful in identifying additional common variants that implicate causal

Address for Correspondence: S. Ananth Karumanchi, MD, Center for Vascular Biology Research, Beth Israel Deaconess Medical Center, Boston, MA 02215, sananth@bidmc.harvard.edu, Tel: 617-667-1018.

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preeclampsia genes and pathways. Such efforts will rely on the continued development of large preeclampsia consortia focused on preeclampsia genetics in order to obtain adequate sample sizes, detailed clinical phenotyping, and matched maternal-fetal samples. In summary, the fetal preeclampsia GWAS represents an exciting advance in preeclampsia biology, suggesting that dysregulation at the *FLT1* locus in the fetal genome (likely in the placenta) is a fundamental molecular defect in preeclampsia.

Keywords

preeclampsia; heritability; fetal genetics; genome-wide association study (GWAS); FLT1; angiogenic imbalance; hypertension; VEGF

Preeclampsia is observed in approximately 3–5% of pregnancies worldwide. A mother's predisposition to preeclampsia is multifactorial and influenced by a host of factors including her age, race, parity, co-existent medical conditions, and pregnancy and partner characteristics, among others. Interestingly, both a mother's genetic background, as well as the genetics of her fetus, impact risk. To date, the specific genes responsible for conferring preeclampsia risk have largely remained elusive, until recently when the large genome-wide association study (GWAS) titled, "*Variants in the fetal genome near FLT1 locus are associated with risk of preeclampsia*", by McGinnis, Steinthorsdottir, Morgan and colleagues identified and replicated the first robust fetal genomic region associated with excess risk [1]. Here we detail the history of genetic studies of preeclampsia, starting from the studies that initially suggested preeclampsia is heritable to the current findings, and describe the implications of this landmark fetal preeclampsia GWAS.

Maternal and fetal genomes influence the risk of preeclampsia

Evidence that preeclampsia has a genetic predisposition was first detailed in the early 1960's by Leon Chesley, John Annitto, and Robert Cosgrove, a group of researchers who were working at the Margaret Hague Maternity Hospital in New Jersey, who observed familial aggregation of cases of preeclampsia and eclampsia. By reviewing hospital records of women with eclampsia who had delivered at the hospital, they found an increased rate of preeclampsia and eclampsia in pregnancies of the sisters, daughters, and granddaughters of these women compared to the daughters-in-law of the same women [2, 3]. Preeclampsia heritability from mothers to daughters was then replicated in a much larger group of women in the Swedish Birth Registry [4, 5] and extended to delineate the importance of fetal genetics, as well as maternal genetics, in disease heritability [6–8]. Based on this work, preeclampsia heritability is estimated at ~55%, with both maternal and fetal contributions to risk (estimated at 35% and 20%, respectively) [4–8].

Traditional gene discovery approaches to identify preeclampsia genes

In order to determine the specific genetic markers (i.e., DNA variations) that confer preeclampsia risk, many traditional gene discovery approaches have been employed including linkage studies and candidate gene association studies. In linkage studies, genetic markers of disease (i.e., specific chromosomal regions) are studied in related individuals to

determine which chromosomal regions segregate with disease and positional cloning is then used to identify the specific gene or genetic variant of interest [9]. In candidate gene association studies, the DNA sequence of biologically-plausible genes is determined and allele frequencies (i.e., the specific base at a given position within the gene) are compared between cases and controls [10]. For preeclampsia, linkage approaches have implicated at least eight different chromosomal regions [11–14], and further study of these regions have identified variants within the genes, *ACVR2A* [15] and *ERAP2* [16], that may predispose to preeclampsia. Preeclampsia candidate gene approaches have largely focused on variants within: (a) the Renin–Angiotensin System (RAS); (b) Coagulation Factors; (c) Oxidative Stress pathways; (d) Dyslipidemia; and (e) Immunoregulatory components, in particular within the HLA region. Despite extensive work, these approaches have largely not been successful or reproducible [17]. As for many complex diseases, traditional gene discovery in preeclampsia has suffered from errors of inadequate sample size, inaccurate clinical phenotyping, lack of correction for multiple testing, poorly matched control groups, hidden ethnic bias, failure to replicate in an independent population, positive publication bias, and random error [18].

Genome-wide association studies (GWAS) for unbiased gene discovery

Over the past decade, genome-wide association studies (GWAS) have allowed for dramatic advances in the understanding of the genetic basis of common disease, including type 2 diabetes, obesity, hypertension, autoimmune disease, and psychiatric traits [19]. GWAS is predicated on the idea that the genetic basis of many common diseases are complex and polygenic with many variants, each with modest effects that contribute to disease risk. The unit of genetic variation assessed is the single nucleotide polymorphism (SNP), which is a single-base pair change in the DNA sequence. SNPs are common in the human genome and are used in GWAS as markers of genomic regions. Most SNPs have minimal biologic effects themselves. In a GWAS, genome-wide SNP data is employed to look for SNPs associated with a given disease or trait. The ability of any given GWAS to succeed depends on the sample size, the panel of genome-wide variants genotyped, the genetic architecture (i.e., the effect size and allele frequency of risk variants), how genetic variants contributing to risk segregate in the population, and the heterogeneity of the disease [19]. For example, a heterogeneous disease with many genetic risk loci, each with modest effects on disease, will require a GWAS with a much larger sample size to discover variants with genome-wide significant effects ($p < 5 \times 10^{-8}$, accounting for multiple testing) than a more homogeneous disease with a few genetic risk loci of large effect.

Following initial results of a GWAS for any given trait, several additional steps are necessary. Given the possibility of false discovery when surveying the whole genome, genome-wide significant SNPs must be replicated in an independent population. As SNPs are only markers of a given genomic region, functional follow up of replicated SNPs is required in order to determine the biologic relevance of each genetic locus to disease. Because GWAS test genetic variation across the genome, they are, by nature, unbiased, with the potential to discover novel underlying disease biology. In many cases, GWAS have uncovered genes distinct from those previously chosen in hypothesis-driven candidate gene studies, highlighting the importance of the unbiased approach.

Despite the success of GWAS for understanding many complex diseases, GWAS of obstetric traits have lagged behind. Several challenges in pregnancy-related genetic research (including heterogeneous phenotypes, lack of large cohorts with detailed pregnancy information, exclusion of pregnant women from research studies, and the involvement of at least two genetically-distinct individuals (mother and fetus(es)) in each obstetric outcome) have made it difficult to obtain the sample sizes needed for well-powered GWAS of obstetric traits. In preeclampsia, three recent small-scale maternal preeclampsia GWAS have been reported [20–22], but the DNA variants identified have not been replicated.

Discovery of the first robust preeclampsia genetic association: FLT1 (fms-like tyrosine kinase 1) fetal DNA variants

The recently published fetal GWAS, "Variants in the fetal genome near *FLT1* are associated with risk of preeclampsia", by McGinnis, Steinthorsdottir, Morgan and colleagues is the first preeclampsia GWAS to identify a genetic risk variant with genome-wide significance with convincing replication in an independent cohort [1]. This GWAS finding provides compelling evidence that alterations near the *FLT1* locus in the human fetal genome are causal in the development of preeclampsia. It is striking that this first well-powered unbiased fetal GWAS homes in on the *FLT1* genomic region, given the body of literature devoted to the role of the FLT1 pathway in preeclampsia pathogenesis (see below).

In the discovery phase of this fetal preeclampsia GWAS, >7 million genetic variants were assessed in 2,658 offspring from preeclamptic women and 308,292 population controls of European descent (from Iceland and the UK). McGinnis and Steinthorsdottir et al. identified a single association signal with genome-wide significance close to the *FLT1* gene, on chromosome 13 (SNP rs4769613, risk allele C, $p = 3.2 \times 10^{-8}$; see Figure 1), with the C allele (present at 53% frequency) conferring increased risk of preeclampsia (OR=1.22 95% CI (1.14–1.31) per allele). The finding was replicated in an independent European case-control cohort from Norway and Finland (n =1,722 cases, 1,946 controls), and a meta-analysis of the subjects in the discovery and replication populations demonstrated an even stronger genome-wide association ($p = 5.4 \times 10^{-11}$) with a similar allelic effect (OR=1.21 95% CI (1.14–1.28)), increasing confidence of the role of this genetic locus in preeclampsia risk. The investigators then examined other SNPs in the region of the *FLT1* locus in further detail and found two additional independent association signals with preeclampsia (represented by SNPs rs12050029 (14% frequency) and rs149427560 (6% frequency)) suggesting that multiple causal variants at the fetal FLT1 locus contribute to disease risk.

A series of analyses were then performed to further investigate the lead risk SNP, rs4769613[C]. Specifically, by joint modeling of association in mothers, offspring and controls in each of the three cohorts, the authors tested if the variant exerted effects through the maternal and/or fetal genomes and determined that the effect was primarily through the fetal genome (R_1 and R_2 see Figure 2a). Additionally, there was no difference in the preeclampsia risk conferred based on whether the risk allele was given to the fetus from the paternal vs. the maternal genome. Thus, these results suggest that increased preeclampsia

risk due to variation at this genetic locus is mediated by fetal gene expression (perhaps within the placenta), and this risk can be conferred by either parent.

To determine if this lead genetic locus had differential effects by preeclampsia subtype, the investigators explored the association of the rs4769613 genotype with early- vs. late-onset preeclampsia and pregnancies with small-for-gestational age (SGA) infants vs. non-SGA infants. Interestingly, the rs4769613[C] risk allele occurred more commonly in pregnancies with late-onset preeclampsia and non-SGA infants. When examined together, the risk effect was strongest in the subgroup with late-onset preeclampsia and non-SGA infants, and weakest in the subgroup with early-onset preeclampsia and SGA infants (see Figure 2b). While this result may initially seem contrary to what one might predict, it can likely be attributed to reproductive fitness. As reproductive selection is a very powerful evolutionary force, any genetic variants that remain in a sizeable fraction of the population are, by nature, likely to have mild effects. This is consistent with the observed increased effects of the rs4769613[C] risk allele in women with late onset preeclampsia and non-SGA infants. This does not exclude the possibility of discovering more deleterious genetic variants within the same genomic region upon sequencing of additional fetal cases, but highly deleterious mutations at this locus are expected to be rare, as these are likely rapidly removed from the population due to negative selection. Overall, these differential effects suggest that mechanistic understanding of how genetic variation in the *FLT1* locus increases preeclampsia risk will provide insights into disease heterogeneity.

Both the lead risk SNP, rs4769613, and rs12050029 are located in placental enhancer regions near *FLT1*, suggesting that these variants may influence *FLT1* expression by exerting effects on gene transcription. To explore this possibility, the investigators assessed the relationship of fetal rs4769613 genotype to levels of FLT1 and sFLT1 in placentas from women with preeclampsia vs. control placentas; no association of the genotype with protein level was seen. The relationship of fetal rs4769613 genotype to first and third trimester maternal serum sFLT1 levels were also assessed and demonstrated an association of third trimester sFLT1 levels with the risk genotype in normal pregnancies, but not in preeclamptic pregnancies where all sFLT1 levels were increased regardless of genotype. The authors suggest that the effects of the risk allele on sFLT1 levels were masked in cases by the already elevated sFLT1 levels.

As with other GWAS for complex traits, the genetic variants identified in the fetal preeclampsia GWAS are non-coding, lying outside of the *FLT1* coding region. As mentioned above, both rs4769613 and rs12050029 are located in placenta enhancer regions and, thus, could influence *FLT1* transcription. Functional follow-up to this fetal GWAS will be essential for pinpointing the causal variants and verifying the culprit gene(s) implicated by this GWAS.

Previous studies of FLT1 pathway in preeclampsia point to a key placental role

To date, elucidation of the role of the FLT1 pathway in the maternal manifestations of preeclampsia has been the most impactful both in understanding disease pathophysiology

and directing predictive and therapeutic efforts [23]. Initial evidence of the central role of the FLT1 pathway in preeclampsia was reported in 2003 when gene expression profiling of preeclamptic placentas revealed upregulated *sFLT1* mRNA [24]. Because sFLT1 is a splice variant of the vascular endothelial growth factor (VEGF) receptor FLT1 and exerts its antiangiogenic activity by inhibiting signaling of proangiogenic factors (i.e., VEGF, placental growth factor (PlGF)) in the vasculature, it was hypothesized that excess circulating sFLT1 in the maternal plasma led to the hallmark clinical features of preeclampsia, hypertension and proteinuria. Evidence was quickly generated supporting this hypothesis [25–35] (see Figure 3). In the past decade, an extensive body of literature on the role of angiogenic imbalance in preeclampsia has developed, both mechanistic and clinical (recently reviewed in [36]). While the specific pathways that mediate sFLT1-induced hypertension are still under investigation, potential mediators include decreased endothelial nitric oxide production, increased endothelin secretion, as well as alterations in prostacyclins and hydrogen sulfide[37]. In the clinic, recent evidence supports the utility of the sFLT1 and PlGF for predicting adverse maternal and perinatal outcomes for preterm patients, for ruling out preeclampsia in patients with suspected disease, and for predicting which high-risk patients are at low risk for severe adverse outcomes [38–50].

The placenta is necessary for preeclampsia, and delivery of the placenta remains the only cure. Both the fetal GWAS and prior data suggest that alterations in the placental FLT1 pathway may explain the essential role of the placenta in disease. Epidemiologic studies suggest that increased circulating placental sFLT1 explains several risk factors for preeclampsia, including multiple gestation (increased placental dosage), trisomy 13 (*FLT1* encoded on chromosome 13), nulliparity (increased sFlt1 compared to multiparas), anti-phospholipid antibody syndrome, pre-existing diabetes, and molar pregnancies (increased chromosomal dosage) [36, 51–58]. The excess placental sFLT1 is primarily the sFLT e15a isoform and arises from degenerating placental syncytiotrophoblast syncytial knots [59–61] (see Figure 4). Exposure of the maternal vasculature to excess sFLT1 and other anti-angiogenic factors may even be responsible for effecting long-term changes that lead to the increased risk of cardiovascular disease in later life [62, 63].

Biological and clinical implications of the fetal preeclampsia GWAS

The fetal preeclampsia GWAS now provides an opportunity to investigate how specific dysregulation at the FLT1 locus leads to preeclampsia at the level of the gene, cell, placenta, and fetal-maternal interface. The significant *FLT1* genetic variants highlight genomic loci involved in the function and/or regulation of *FLT1*. Interrogation of these loci will allow for elucidation of the origins of excess placental sFLT1 (i.e., assessing the impact of hypoxia, immunologic factors, inflammatory processes, and oxidative stress on the FLT1 locus), leading to improved mechanistic understanding of placental dysfunction and maternal disease. Therapeutics directed aimed at reducing sFLT1 levels by therapeutic apheresis, binding of excess sFlt1 with recombinant VEGF or PlGF ligands, and small molecules that upregulate proangiogenic factors are already under development. These efforts will be greatly enhanced by improved biologic understanding of the placental FLT1 locus.

In the development of novel preeclampsia therapeutics, understanding the underlying genetics has the potential to greatly increase the likelihood of success and for this reason, the identification of the fetal variant, rs4769613, near *FLT1* is a particularly important and impactful finding. As genetic approaches are less prone to bias and confounding than other epidemiologic methods, novel therapeutic agents with targets selected on the basis of genetic evidence may be twice as likely to succeed in clinical development [64]. For example, discovery of rare variants of strong effect in the lipid gene, *PCSK9*, led to its clinical translation into an extraordinarily effective target for LDL cholesterol-lowering therapeutics [65]. For metabolic syndrome, the central role of the nuclear hormone receptor peroxisome proliferator-activated receptor gamma (*PPAR-γ*) in adipogenesis, lipid metabolism, and metabolic homeostasis was highlighted by patients with dominant negative mutations in this gene [66]; the highly effective class of oral medications, thiazolidinediones, for type 2 diabetes acts via *PPAR-γ*.

Implications of this finding on future genetic studies of preeclampsia

Similar to other complex diseases, preeclampsia heritability is estimated to be polygenic, and, as such, unbiased gene discovery approaches are needed to elucidate influential genetic variants contributing to disease. The sample size needed to demonstrate significance for any given genetic variant is dependent on the frequency of the risk variant and the effect that that variant has on disease. The fetal preeclampsia GWAS [1] is the first preeclampsia GWAS with adequate power to detect a common genetic variant (frequency >5%) with genome-wide significance. This finding suggests that well-powered, larger maternal and fetal GWAS will be fruitful in identifying additional common variants implicating genes and pathways underlying preeclampsia.

As preeclampsia is a trait with severe maternal and fetal morbidity and mortality, deleterious genetic variants that affect reproductive fitness by contributing to preeclampsia risk are expected to be under strong negative selection in the population [67]. Thus, in addition to common variants, low frequency (0.01–5%) and rare (<0.01%) coding variants of large effect likely contribute considerably to preeclampsia risk. Focused sequencing of the FLT1 region in fetal cases may identify partial loss of function coding variants that protect from preeclampsia. To detect low frequency and rare variants that contribute to disease, future preeclampsia genetic studies will need to employ larger case-control cohorts, whole exome or genome sequencing strategies to enable an unbiased search, and novel analytic approaches including both the maternal and fetal genomes.

Finally, the success of the first fetal preeclampsia GWAS highlights the need for continued development of large preeclampsia consortia focused on preeclampsia genetics. These consortia will enable the collection of cohorts with: (a.) adequate sample size to have power for detection of loci with genome-wide significance; (b.) detailed clinical phenotyping allowing for appropriate classification of preeclampsia subtypes; and (c.) matched maternal and fetal samples to understand genomic interactions that increase preeclampsia risk. In other disorders, common variants identified by GWAS studies typically explain less than half of the apparent heritability and more recently researchers have focused on identifying rare variants to explain missing heritability [68]. We remain optimistic that with improvements

in exome and whole genome sequencing methods, rare variant studies using large sample sizes (typically >25,000 cases) will be complementary to GWAS to identify other causal pathways that explain the heritability of preeclampsia.

In summary, the fetal preeclampsia GWAS represents an exciting advance in preeclampsia biology, suggesting that dysregulation at the *FLT1* locus in the fetal genome (likely in the placenta) is a fundamental molecular defect in preeclampsia.

Glossary

Gene	A gene is the basic physical and functional unit of heredity. Genes, which are made up of DNA, act as instructions to make molecules called proteins.
rs number	A **r**eference SNP ID number, or “rs” ID, is an identification tag assigned by NCBI to a group (or cluster) of SNPs that map to an identical location.
Locus	A locus (plural **loci**) in genetics is the physical position on a chromosome. Can either refer to a large region such as a complete gene or a very specific region, like a particular base pair position.
Allele	A variant of the similar DNA sequence located at a given locus is called an allele. For example, at a given position along a chromosome, most people might have the DNA base “A”. A few might have an alternative sequence. Each defined type is an allele.
Gene mapping	Describes the methods used to identify the locus of a gene and the distances between genes.
GWAS	Genome-wide association study or GWAS is a study of the markers (usually SNPs) across the entire genome to see which ones are statistically more or less common in one group of people (often patients with a specific disease) compared to another group of people (typically people unaffected by that disease).
Manhattan plot	A Manhattan plot is a type of scatter plot used in genome-wide association studies (GWAS) in which genomic coordinates are displayed along the x-axis, with the negative logarithm of the association ($-\log_{10}$) *p*-value for each single nucleotide polymorphism (SNP) displayed on the y-axis. The term Manhattan plot is used because significant results, such as those for *FLT1*, have the appearance of skyscrapers like the Manhattan skyline. Results are displayed as $-\log_{10}$ (*p*-value) for ease of

visualization. Because the strongest associations have the smallest *p*-values, their negative logarithms are the greatest.

Heritability	The percentage of a trait estimated to be due to the variations you've inherited, as opposed to the contribution of non-genetic influences such as your environment or diet.
Autosomes	Chromosomes 1, 2, … 22. Autosomal DNA does not include the sex-determining chromosomes (X and Y) or mtDNA (mitochondrial DNA).
Phenotype	The traits or conditions you can see, measure, or diagnose, like eye color or breast cancer.
Genotype	The two alleles inherited at a given SNP position, one inherited from father, one inherited from mother. Example: rs16260 (A:C) is how we indicate someone has a (A:C) genotype at SNP rs16260.
Odds ratio (OR)	The ratio of the odds within one group compared to the odds within another group, for the association between an allele or genotype with a phenotype (usually a trait or a disease). Typically, carriers of a less common allele or genotype are being compared to people with two copies of the most common allele. An OR of 1.0 means that the DNA variant has no effect on the odds of having the disease, while values above 1.0 indicate a statistical association between that variant and having the disease. OR values below 1 indicate a lower association (risk).

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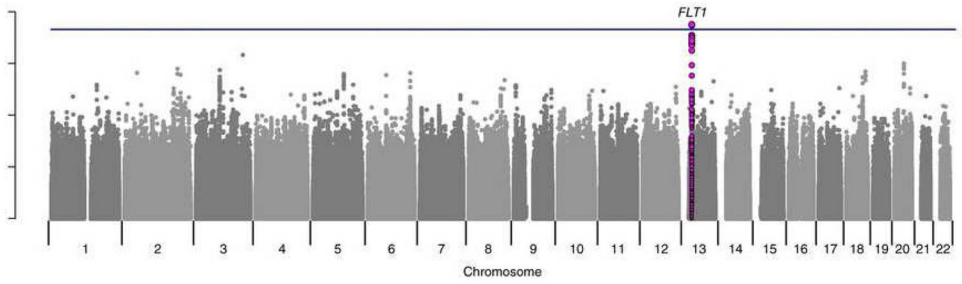

Figure 1.

Manhattan plots showing GWAS results for 2,658 cases and 308,292 controls across all autosomes. The Manhattan plot shows the strength of association of each tested SNP (shown as individual dots) with preeclampsia in GWAS meta-analysis, plotted as $-\log_{10}$ (p-value) on the y-axis against corresponding variant position on the x-axis. A single peak, whose apex is the sentinel SNP rs4769613, near *FLT1* on chromosome 13, crosses the blue line denoting genome-wide significance ($p < 5 \times 10^{-8}$). Variants within 100 kb (the region of linkage disequilibrium (LD; the region in which nearby genetic variants are correlated within a given population)) of rs4769613 are colored purple. Figure reproduced with permission from Mcginnis et al [1].

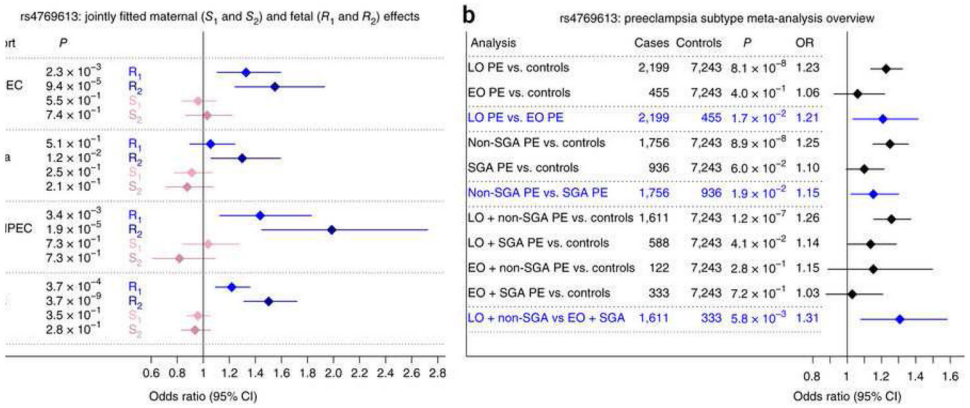

Figure 2.

Key observations about rs4769613 in relation to preeclampsia. **(A)** Forest plots showing the odds ratios (ORs) and 95% confidence intervals (95% Cis) calculated for preeclampsia risk conferred by one or two copies of rs4769613 [C] risk allele carried by the fetus (R_1, R_2) or mother (S_1, S_2). Individual data sets (GOPEC, MoBa and FINNPEC) and meta-analysis across the data sets (Meta) show that OR is increased by each fetal copy of the C risk allele: R_1, P=3.7 × 10^{-4}; R_2, P= 3.7 × 10^{-9}). By contrast, the ORs for maternal copies (S_1, S_2) are not significantly different from 1, implying that, after accounting for fetal copies, maternal copies of the C allele confer no additional increased risk of preeclampsia. **(B)** Forest plots showing the preeclampsia (PE) subtypes, defined by early and late onset (EO and LO) and by birth weight that is small for gestational age (SGA) or not (non-SGA). Case-case comparisons, in blue font, show that the C risk allele is more significantly associated with LO preeclampsia and non-SGA preeclampsia than with EO preeclampsia and SGA preeclampsia. Dividing cases into four possible subcategories showed that the C allele confers the greatest risk of preeclampsia to LO + non-SGA cases and the least risk of preeclampsia to EO + SGA cases. Figure reproduced with permission from Mcginnis et al [1].

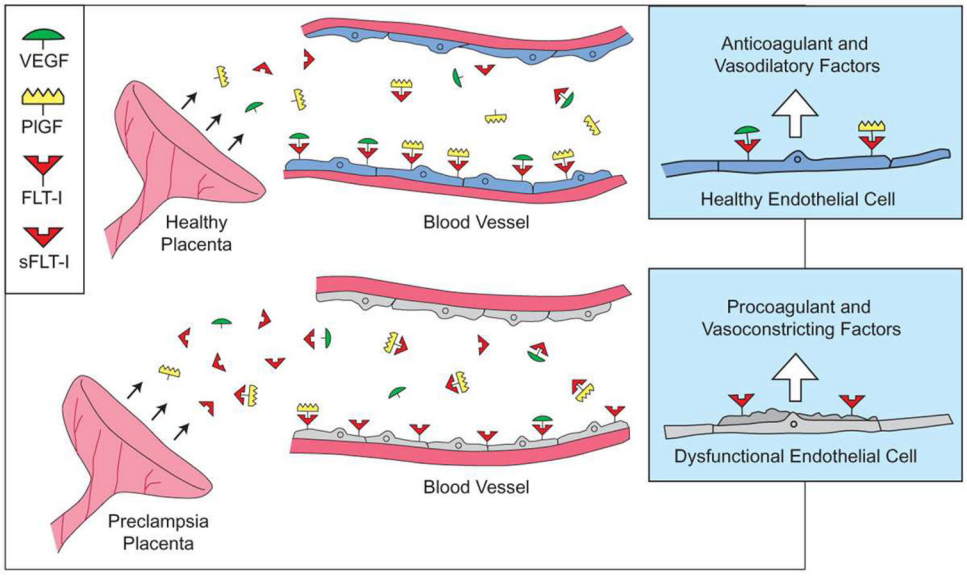

Figure 3.

sFLT1 causes endothelial dysfunction by antagonizing VEGF and PlGF. There is mounting evidence that VEGF, and PlGF are required to maintain endothelial health in several tissues including the kidney and perhaps the placenta. In normal pregnancy, the placenta produces modest concentrations of VEGF, PlGF, and sFlt1. In preeclampsia, excess placental sFLT1 binds circulating VEGF and PlGF and prevents their interaction with endothelial cell-surface receptors. This results in endothelial cell dysfunction, including decreased prostacyclin, nitric production and release of procoagulant proteins such as von Willebrand factor, endothelin, and thrombomodulin. Endothelial cell dysfunction is thought to be directly responsible for the hallmark clinical features of preeclampsia, including hypertension and proteinuria. Figure reproduced with permission from Karumanchi et al [69].

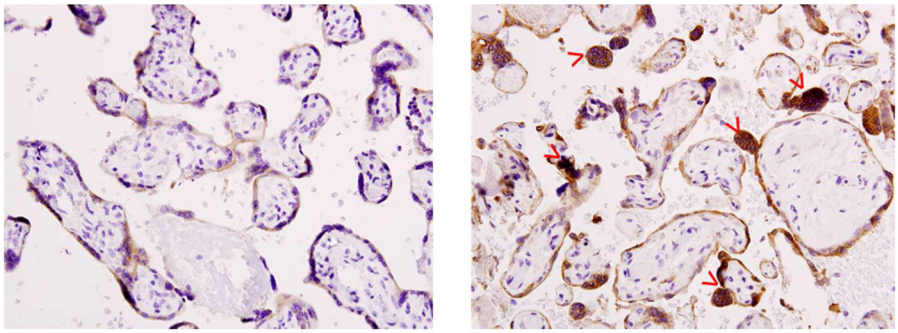

Figure 4.

Immunohistochemistry for sFLT1 expression in normal and preeclamptic placentae. A representative staining of normal (left) and preeclamptic placenta (right), is depicted. The red arrowheads represent syncytial knots. Magnification 400X. Figure reproduced with permission from Rajakumar et al [59].