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A Systematic Review of Endoglin Gene Expression in Preeclampsia

Mandy J. Bell, BSN, RN¹ and Yvette P. Conley, PhD¹

¹University of Pittsburgh, Pittsburgh, PA, USA

Abstract

Objective—To synthesize scientific literature that addresses the role of endoglin (ENG) gene expression in preeclampsia (PE).

Data sources—A literature search of PubMed and Ovid MEDLINE was conducted using the keywords *endoglin*, *gene*, and *preeclampsia*. Restrictions included English language and humans. Additional articles were identified/selected for evaluation via PubMed e-mail updates (keywords: *endoglin* and *preeclampsia*) and review of article reference lists obtained from the search.

Study selection—The initial 14 abstracts retrieved from the literature search were reviewed and 9 studies were selected for evaluation. Review articles and studies not addressing ENG expression (messenger RNA [mRNA] level) in the context of PE were excluded. An additional six articles were selected from PubMed e-mail updates and reference lists.

Data extraction—Data related to study objective, design, setting, subject information, phenotype, tissue type, data collection method, statistics, and results were extracted.

Data synthesis—Regardless of PE definition, ancestral background, gene expression analysis method, tissue type, and time of specimen collection, endoglin appears to play a role in PE development. Moreover, results suggest that a variety of biological mechanisms have the ability to modulate ENG expression in PE, demonstrating the potential complexity associated with endoglin's role in PE.

Conclusions—This review article is the first to systematically synthesize evidence related to ENG expression in PE. Findings can be utilized to design future studies that (a) address methodological limitations observed in the reviewed studies and (b) specifically examine why ENG expression levels are altered and address mechanisms explaining how these alterations are involved in PE development.

Keywords

preeclampsia; endoglin; gene

Preeclampsia (PE) is a hypertensive, multisystem disorder of pregnancy that significantly impacts maternal and fetal/neonatal health (National Heart, Lung, and Blood Institute [NHLBI] National High Blood Pressure Education Program, 2000). Classified as new-onset hypertension and proteinuria after 20 weeks' gestation in a previously normotensive woman,

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Corresponding Author: Mandy J. Bell, BSN, RN, University of Pittsburgh, 440 Victoria Building, 3500 Victoria Street, Pittsburgh, PA 15261, USA. mjb111@pitt.edu.

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PE complicates 3–5% of pregnancies (Roberts & Cooper, 2001) and is estimated to cost the United States \$7 billion annually (Preeclampsia Foundation, 2000–2010). Although it is believed that PE development involves (a) reduced placental perfusion secondary to abnormal placentation and (b) the maternal syndrome characterized by systemic endothelial dysfunction (Roberts & Hubel, 2009), the factors/mechanisms responsible for these aberrations remain unknown. Several investigations, however, have identified endoglin as a potential factor in the genesis of PE.

Endoglin (ENG) gene, which is expressed on syncytiotrophoblasts and transitioning cytotrophoblast cells of the placenta (St-Jacques, Forte, Lye, & Letarte, 1994), has been shown to participate in the regulation of placental trophoblast differentiation and invasion of the uterus during pregnancy (Caniggia, Taylor, Ritchie, Lye, & Letarte, 1997). In PE, shallow trophoblast invasion of the maternal spiral arteries restricts conversion of these arteries from small muscular vessels to large low-resistance vessels via the replacement of smooth muscle with fibrous tissue within the vessel wall. Without a sufficient physiologic conversion, limited lumen diameter and distensibility of the spiral arteries subsequently leads to the reduction in placental and fetal perfusion that is observed in PE (Brosens, Robertson, & Dixon, 1972; Zhou, Damsky, & Fisher, 1997). Conceptually, this process is referred to as Stage 1 in the two-stage model of PE (Roberts & Hubel, 2009). Therefore, an alteration in ENG function during placental implantation may contribute to PE pathogenesis.

ENG is also expressed on vascular endothelial cells (Gougos & Letarte, 1990) and is involved in the maintenance of vascular tone through the regulation of nitric oxide–dependent vasodilation (Jerkic et al., 2004; Toporsian et al., 2005). In PE, in addition to abnormal implantation, vascular endothelial function, including vasorelaxation and cell membrane permeability, is disrupted (American College of Obstetricians and Gynecologists [ACOG], 2002). Conceptually, this is referred to as Stage 2 in the two-stage model of PE and is associated with reduced organ perfusion, hypertension, proteinuria, and intravascular fluid loss (Roberts & Gammill, 2005; Roberts & Hubel, 2009). Therefore, an alteration in ENG function in the vasculature may contribute to PE pathogenesis.

Given the biologic plausibility of a role for ENG in PE pathogenesis, a plethora of research reports addressing ENG's involvement in the disorder has recently inundated the scientific literature. Despite the sizable amount of evidence being generated, which has primarily targeted ENG protein levels in PE, critical review and synthesis of the scientific literature addressing ENG messenger RNA (mRNA) expression in PE is lacking. We thus conducted this systematic review to critique/synthesize scientific literature that addresses the role of ENG expression in PE.

Data Collection Method

We used the PubMed and Ovid (MEDLINE) databases to identify articles addressing the role of ENG in PE from a human gene expression standpoint. The keywords we used were *preeclampsia*, *endoglin*, and *gene*. In PubMed, we combined all three keywords with the AND Boolean operator. Due to differences in MeSH terms across the databases, we used the following combination of keywords to retrieve articles in OVID (Medline): *endoglin* AND (*gene* OR *genes*) AND (*preeclampsia* OR *pre-eclampsia*). We limited the literature search, which covered literature through January 2011, to the English language and articles involving human research.

After completing the literature search, we reviewed abstracts of retrieved articles for relevance, excluding review articles, duplicate articles, and articles not addressing ENG expression in the context of PE. We also reviewed weekly PubMed e-mail updates related to endoglin and PE, along with reference lists of selected articles, to identify additional articles.

After independent review, we met to discuss findings and synthesize results. We extracted from each study data related to study objective, design/approach, setting, subject information, phenotype, tissue type, data collection methods, statistics, and results and summarized them in tabular format.

Results

We selected 9 of the 14 articles we identified from the initial literature search for inclusion. Of the excluded articles, 1 was a review article, 1 addressed ENG expression at the DNA level in PE, and 3 addressed ENG expression outside the context of PE. We also selected for inclusion of six additional articles that we identified from reference lists or PubMed e-mail updates. Table 1 includes a summary of the results and characteristics of the articles comparing ENG expression in human subjects with PE to a control group/groups. Table 2 includes a summary of the results and characteristics of the articles utilizing gene expression methods to investigate mechanisms that may explain the role of ENG in PE.

Discussion

Establishing a Role for ENG in PE via Gene Expression Studies

We conducted this systematic review in order to critique and synthesize scientific literature that addresses the role of ENG in PE from a human gene expression standpoint. In reviewing the 11 studies that compared gene expression levels of ENG between women with and without PE, we consistently found ENG expression to be significantly elevated in women with PE regardless of definition of PE, ancestral background, methods for gene expression analysis, tissue type, or time of specimen collection (Table 1). Four of these studies (Purwosunu et al., 2008; Purwosunu, Sekizawa, Okazaki, et al., 2009; Purwosunu, Sekizawa, Yoshimura, et al., 2009; Sekizawa et al., 2010), however, may represent one parent study instead of independent replicates. Although the number of independent studies would be reduced to eight, the support for ENG's involvement in PE remains strong.

Moreover, ENG expression was elevated throughout all three trimesters of pregnancy in women who developed PE, suggesting that ENG's role in PE is initiated early in pregnancy and sustained through delivery. Both first- and third-trimester placental samples of women who developed PE had significantly elevated levels of ENG expression (Farina et al., 2008; Nishizawa et al., 2007; Sitras et al., 2009; Toft et al., 2008; Venkatesha et al., 2006). Furthermore, the cellular and cellular-free (plasma) components of blood in women who developed PE had significantly elevated levels of ENG expression near the end of the first trimester and in the second and third trimesters (Farina et al., 2010; Purwosunu et al., 2008; Purwosunu, Sekizawa, Okazaki, et al., 2009; Purwosunu, Sekizawa, Yoshimura, et al., 2009; Sekizawa et al., 2010).

Further research is needed to understand why expression levels are altered and how these alterations are involved in the development of PE. Two approaches that may provide insight into why ENG expression is altered in PE are evaluating ENG at the molecular level (DNA) and exploring it from an epigenetic point of view. In the only study like it to date, Srinivas, Morrison, Andrela, and Elovitz (2010) examined the association between PE and allelic variation in an angiogenic pathway among Black ($N = 184$ cases and $N = 305$ controls) and White subjects ($N = 32$ cases and $N = 85$ controls) separately. Using the previously developed ITMAT-Broad-CARE, version 2 (IBCv2) array, they evaluated 124 tagging single nucleotide polymorphisms (SNPs) across the six candidate genes (vascular endothelial growth factor A, B, and C; fms-like tyrosine kinase 1 and 4; endoglin). Investigators failed to demonstrate a significant association between variation in ENG and PE; however, it is

unclear if they fully evaluated the entire ENG. Further research examining the association between PE and allelic variation across the entire ENG in larger samples is needed.

Mechanisms Explaining the Role of ENG via Gene Expression Studies

Using gene expression as a tool, research has identified potential mechanisms that may help to explain ENG's role in PE (Table 2). In the reviewed studies investigating mechanisms that may explain ENG's role in PE, investigators identified the liver X receptor and the STOX1 transcription factor as potential regulators of ENG expression. Given that liver X receptors and ENG have been shown to be involved in placental implantation (Caniggia et al., 1997; Pavan et al., 2004), it is possible that the abnormal implantation observed in PE could be attributed to altered regulation of ENG by liver X receptor. STOX1 may also be involved in PE despite inconsistent results (Rigourd et al., 2008). Given that ENG expression is elevated in PE (Table 1) and that overexpression of STOX1 in a choriocarcinoma cell line (Table 2) leads to the induction of ENG expression (Rigourd et al.), it is plausible that STOX1 is involved in the development of PE and may epistatically contribute to ENG's role in PE.

The two remaining studies investigated the effect of hypoxia on ENG expression. Although one group of investigators did not find hypoxia to modulate ENG expression in first-trimester placental villous explants after 48 hr of incubation (Munaut et al., 2008), another group found hypoxia to significantly increase ENG expression in BeWo cells (choriocarcinoma cell line) after 4 and 6 hr of incubation (Fujita et al., 2010). These disparate results may be due to the different types of cells used to assess hypoxia's impact on ENG. Furthermore, results by Fujita et al. (2010) suggest that 3-kinase-AKT-MTOR-HIF-1 α and ERK-HIF-1 α signaling pathways influence ENG expression under hypoxic conditions.

Limitations of Studies Comparing ENG Expression in Subjects With PE Versus Control Group/Groups

Despite consistent findings, we did note limitations across studies and within individual studies that may impact the validity and overall interpretation of the gene expression results. One limitation that we found in all 11 studies is that they were cross sectional in nature. Although the studies demonstrated that ENG expression levels were elevated in all three trimesters of pregnancy cross-sectionally, studies utilizing a prospective, longitudinal approach have the ability to observe changes in gene expression across pregnancy in the same subjects. Ultimately, such information could provide further insight into ENG's role in PE throughout pregnancy. However, one must consider that, although the longitudinal assessment of gene expression from the blood is feasible, longitudinal assessment of placental gene expression is neither feasible nor ethical (e.g., second-trimester biopsies of pregnant women).

The variability in PE phenotype along with the variability in inclusion/exclusion criteria utilized to classify cases and controls among the reviewed studies represents another limitation that impacts the ability to compare results across studies. Such a limitation further hampers the ability to combine studies for the purpose of conducting a meta-analysis, which can be employed to estimate effect sizes.

Other noted limitations across studies were related to methods used to evaluate gene expression. Authors frequently failed to report the following steps involved in gene expression analysis: (a) performance of RNA quality/quantity control checks on extracted RNA prior to gene expression analysis, (b) use of an RNA stabilizer to prevent RNA degradation in the tissue until extraction and gene expression analysis, and (c) use of an

endogenous control when conducting real-time quantitative reverse transcriptase–polymerase chain reaction (qRT-PCR). Although these limitations may reflect editing to meet journal page constraints, they may also represent methodological flaws that could cause the validity of the findings to be in question.

Like the limitations noted across studies, limitations unique to several individual studies may also impact validity and generalization of results. In the study by Sitras et al. (2009), we noted two limitations that have the potential to impact placental gene expression. First, gestational age at delivery (sample collection) was significantly earlier in cases compared to controls. Tsai et al. (2011) also noted this limitation; however, more in-depth analysis by this group indicated that estimated gestational age had minimal independent contribution to their gene expression data. Ultimately, the magnitude of observed differences in ENG mRNA expression of the placenta may have been impacted by differences related to developmental stage of pregnancy. Although this issue may be mitigated through the matching of healthy controls to cases for gestational age at delivery, one must consider if a control who delivers preterm truly represents a “healthy” control. Second, the study sample included subjects who delivered via cesarean section prior to labor onset and those who delivered vaginally. Because placental gene expression profiles may differ between laboring and nonlaboring women, differences related to labor may influence study results. Such limitations deserve consideration when researchers are designing studies that evaluate relationships between gene expression and disease/health outcomes.

In the study by Toft et al. (2008), the absence of a normotensive control group and the use of a small sample size to conduct targeted gene analysis via qRT-PCR represent potential limitations. If the investigators had included a normotensive control group, it would be possible to compare differences in ENG expression between those with PE and those with an uncomplicated pregnancy within the study and across similar studies. As for the issue related to small sample size, significant results could ultimately indicate false-positive findings as opposed to large effect sizes.

Limitations of Studies Investigating Mechanisms That May Explain the Role of ENG in PE

We also noted several limitations in the studies investigating mechanisms that may explain the role of ENG in PE. In the study by Munaut et al. (2008), hypoxia did not modulate ENG expression in first-trimester placental villi culture explants. However, unlike the other candidate genes under study, authors did not report the effects of hypoxia on ENG expression in human umbilical vein endothelial cells and immortalized first-trimester extravillous trophoblast. It is unclear if the authors simply omitted these results from the report or if they did not study the effects of hypoxia on ENG expression in these cell types. Because ENG is expressed on trophoblast and vascular endothelial cells, study of its expression in these cell types would have provided additional insight into hypoxia’s effect on ENG’s role in PE.

The use of cell lines to conduct research concerning biological processes in humans represents an additional limitation of the mechanistic studies reviewed. Although the choriocarcinoma cell lines utilized by Fujita et al. (2010), Henry-Berger et al. (2008), and Rigourd et al. (2008) were human in origin, the representativeness of an immortal cell line as “normal” decreases and the risk of genetic abnormalities increases with each cell passage. As a result, study findings may not accurately represent biological activities that are occurring in vivo.

Conclusion

PE represents a multisystem, hypertensive disorder of pregnancy that significantly contributes to maternal and fetal/ neonatal morbidity and mortality worldwide. At present, the etiology of PE remains unknown, but gene expression studies included in this systematic review support ENG's involvement in the development of PE. Despite the methodological limitations in these studies, ENG expression was consistently elevated in women with PE. In addition, these studies showed that ENG's role in PE may be explained by several mechanisms that may represent a variety of biological functions.

Investigators can utilize the findings of this review to design future studies examining ENG's role in PE. First, research addressing methodological limitations found in the gene expression studies is needed to validate previous findings. Steps to mitigate such limitations include conducting and reporting RNA quality/quantity control checks, using RNA stabilizers to optimize RNA integrity of samples, using and reporting endogenous controls when appropriate, and collecting tissues of interest at comparable times (e.g., similar gestational age) between groups. Second, research that examines why ENG expression levels are altered and how these alterations are involved in PE development is needed. Ultimately, such studies have the potential to increase overall understanding of PE and to solve PE's etiologic puzzle, which may include ENG as one of its pieces.

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Table 1

Cross-Sectional Studies Comparing Endoglin (ENG) Gene Expression in Subjects With Preeclampsia (PE) Versus Control Group/Groups.

Citation (Population)	Design	Expression Evaluation	Phenotype	Tissue	Endoglin Results
Farina et al., 2008 (Italian)	Prospective case-control Matching (1:5): GA and fetal sex $N = 30$ (5 cases and 25 controls)	Candidate gene analysis via qRT-PCR	Case: PE; severe PE Control: Pregnant women without PE	Placenta: CVS at 11 weeks' gestation	ENG mRNA levels significantly higher in 11-week CVS in cases ($p < .001$)
Farina et al., 2010 (Italian)	Case-control Matching (1:8): GA and fetal sex $N = 99$ (11 cases and 88 controls)	Candidate gene analysis via qRT-PCR	Case: PE; severe PE Control: Pregnant women without PE	Blood: Cellular component at 10–14 weeks' gestation	ENG expression significantly higher in cellular component of blood in cases ($p < .001$)
Nishizawa et al., 2007 (Japanese)	Case-control + within-case subanalysis Matching: Maternal age, GA, and prepregnancy BMI N (microarray analysis): 10 cases and 4 controls	Nonparametric whole-genome analysis via microarray with qRT-PCR validation	Case: Severe PE Control: Normotensive subjects	Placenta: C-section prior to labor onset	ENG expression significantly upregulated in cases ($p = .000275$) ENG not differentially expressed between early-onset (<31 weeks) and late-onset PE placentas at delivery
Purvosunu et al., 2008 (Indonesian)	Case-control $N = 84$ (43 cases and 41 controls)	Candidate gene analysis via qRT-PCR	Case: PE; severe PE; HELLP Control: Healthy pregnant women	Blood: Cellular-free (plasma) component at 35–41 weeks' gestation	ENG expression significantly higher in cases ($p < .001$)
Purvosunu, Sekizawa, Okazaki, et al., 2009 (Indonesian)	Prospective cohort + subsequent case-control analysis Matching (1:5): GA at sample collection, maternal weight, and fetal gender $N = 372$ (62 cases and 310 controls)	Candidate gene analysis via qRT-PCR	Case: PE; severe PE; HELLP Control: Healthy pregnant women	Blood: Cellular-free component at 15–20 weeks' gestation	ENG expression significantly higher in plasma component of blood in cases ($p < .001$)
Purvosunu, Sekizawa, Yoshimura, et al., 2009 (Indonesian)	Case-control $N = 48$ (24 cases and 24 controls)	Candidate gene analysis via qRT-PCR	Case: PE; severe PE; HELLP Control: Healthy pregnant women	Blood: Cellular component at 35–41 weeks' gestation	ENG expression significantly higher in the cellular component of blood in cases ($p < .001$) In PE, ENG expression in cellular component of blood significantly correlated with SBP ($p < .001$, $R^2 = .631$) and proteinuria ($p < .001$, $R^2 = .671$)
Sekizawa et al., 2010 (Indonesian)	Prospective cohort + subsequent case-control analysis Matching (1:5): GA at sample collection, maternal weight, and fetal gender $N = 372$ (62 cases and 310 controls)	Candidate gene analysis via qRT-PCR	Case: PE; severe PE; HELLP Control: Healthy pregnant women	Blood: Cellular component at 15–20 weeks' gestation	ENG expression significantly higher in cellular component of blood in cases (statistics not provided)
Sitras et al., 2009 (Norwegian)	Case-control + within-case subanalysis	Nonparametric whole-genome analysis	Case: Severe PE; HELLP	Placenta: Delivery (C-section before	ENG expression significantly upregulated (fourfold; $p = .01$) in cases

Citation (Population)	Design	Expression Evaluation	Phenotype	Tissue	Endoglin Results
Toft et al., 2008 (Norwegian)	Matching (1:1): Parity N = 71 (21 cases and 50 controls; analysis conducted on 16 cases and 21 random controls) Three-group comparative Matching: GA at delivery N = 28 (10 PE; 8 SGA; and 10 PE + SGA)	via microarray with qRT-PCR validation Nonparametric whole genome analysis via microarray Candidate gene analysis via qRT-PCR	Control: Healthy women with uncomplicated pregnancies Group 1: PE Group 2: SGA Group 3: PE + SGA	labor and vaginal delivery) Placenta: C-section prior to labor onset	ENG not a differentially expressed gene between early-onset (<34 weeks) and late-onset PE at delivery Microarray: No significant placental gene expression differences between study groups after correction for multiple comparisons ($p > .05$) Real-time qRT-PCR: ENG placental expression significantly higher in the PE + SGA group compared to PE and SGA groups ($p = .033$) ENG expression significantly upregulated in cases ($p = 1.5054 \times 10^{-06}$)
Tsai et al., 2011 (American)	Case-control N = 60 (23 cases and 37 controls)	Nonparametric whole-genome analysis via microarray with qRT-PCR validation	Case: PE Control: Pregnant women without PE	Placenta: Within 1 hr of delivery	ENG expression significantly upregulated (fourfold ↑) in cases (no p value reported)
Venkatesha et al., 2006 (American)	Case-control Matching: GA N (30 normal-term; 8 normal-preterm; 11 mild PE; 17 severe PE without HELLP; and 11 severe PE with HELLP)	Nonparametric whole-genome analysis via microarray with Northern blot confirmation	Case: PE; severe PE; HELLP Control: Healthy pregnant women	Placenta: Immediately following delivery	ENG expression upregulated (fourfold ↑) in cases (no p value reported)

Note. BMI = body mass index; C-section = cesarean section; CVS = chorionic villous sampling; DBP = diastolic blood pressure; GA = gestational age; HELLP = hemolysis, elevated liver enzymes, low platelets; mRNA = messenger RNA; qRT-PCR = quantitative reverse transcriptase-polymerase chain reaction; SBP = systolic blood pressure; SGA = small for gestational age.

Table 2

Studies Investigating Mechanisms That May Explain the Role of Endoglin Gene (ENG) in Preeclampsia.

Citation	Design	Expression Evaluation	Tissue	Endoglin Results
Fujita et al., 2010	Mechanistic/experimental	Candidate gene analysis via qRT-PCR	BeWo choriocarcinoma cell line	ENG mRNA levels significantly ↑ in BeWo cells incubated under hypoxia compared to normoxia at 4 and 6 hr Treatment with kinase inhibitors for AKT and ERK attenuated hypoxia-induced ENG mRNA expression HIF-1α silencing significantly ↓ hypoxia-induced ENG mRNA expression
Henry-Berger et al., 2008	Mechanistic/experimental	Candidate gene analysis via Northern blot	Human choriocarcinoma JAR cell line	JAR cells treated with a synthetic liver X receptor agonist led to significant ↑ in ENG mRNA levels
Munaut et al., 2008	Mechanistic/experimental	Candidate gene analysis via qRT-PCR	First-trimester placental villous extracts (N = 30; 8–14 weeks' gestation)	ENG mRNA levels not modulated under hypoxia compared to normoxia after 48 hr
Rigourd et al., 2008	Mechanistic/experimental	Nonparametric whole genome analysis via microarray with qRT-PCR validation	JEG-3 choriocarcinoma cell line	Overexpression of STOX1 in JEG-3 choriocarcinoma cell line resulted in 2.23 fold induction of ENG expression

Note. qRT-PCR = quantitative reverse transcriptase-polymerase chain reaction; mRNA = messenger RNA.