Ingrid Alsos Lian

Mechanisms involved in the pathogenesis of pre-eclampsia and fetal growth restriction

Transcriptional analyses of placental and decidual tissue

Thesis for the degree of Philosophiae Doctor

Trondheim, January 2012

Norwegian University of Science and Technology
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Science and Technology

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ISBN 978-82-471-3303-3 (printed ver.)
ISBN 978-82-471-3304-0 (electronic ver.)
ISSN 1503-8181

Doctoral theses at NTNU, 2012:21

Printed by NTNU-trykk

Mekanismer relatert til utvikling av svangerskapsforgiftning og føtal veksthemming

Genekspresjonsanalyser av placenta og decidua vev

Svangerskapsforgiftning (preeklampsi) og føtal veksthemming er alvorlige svangerskapskomplikasjoner og viktige årsaker til økt sykelighet og død hos gravide kvinner og deres avkom. Selv om forståelsen av hvordan disse tilstandene oppstår har økt de siste årene, er det fortsatt manglende kunnskap rundt mekanismene som bidrar. En rekke observasjoner tyder på at en mangelfull utvikling av morkaken (placenta) kan ligge til grunn. I et normalt svangerskap vil trofoblaster (morkakeceller) invadere og omdanne morkakens tilførende blodkar for å sørge for stabil blodtilførsel til morkaken og fosteret under graviditeten. Ved preeklampsi og føtal veksthemming er denne prosessen ufullstendig, noe som kan resultere i utilstrekkelig tilførsel av blod og næringsstoffer til fosteret, morkaken og det underliggende vevet (decidua). Videre vil en "syk" morkake respondere på redusert blodtilførsel ved å skille ut faktorer til kvinnens sirkulasjon som kan forårsake skade på karendotelet, og føre til høyt blodtrykk og protein i urinen (tegn på preeklampsi) hos affiserte kvinner.

Hensikten med arbeidet som presenteres i denne avhandlingen har vært å kartlegge hvilke molekylære mekanismer som er assosiert med mangelfull morkakedannelse ved preeklampsi og føtal veksthemming. For å gjøre dette, har vi blant annet tatt i bruk genekspresjonsanalyser, hvor det er mulig å måle uttrykket av titalls tusen gener i én analyse.

Resultatene fra disse analysene viste at kvinner med preeklampsi i kombinasjon med føtal veksthemming har økt genuttrykk av faktorer som påvirker kardannelse negativt i morkaken sammenlignet med kvinner med isolert preeklampsi eller føtal veksthemming (**studie I**). Når disse faktorene skilles ut til mors sirkulasjon kan de bidra til utvikling av preeklampsi. Nivået av disse faktorene i morkakevevet ser ut til å ha sammenheng med alvorlighetsgrad av sykdom. Videre fant vi nedsatt gen- og protein uttrykk av matrix metalloproteinase 1 (MMP1) i decidua fra kvinner med preeklampsi og/eller føtal veksthemming (**studie II**). MMP1 er et viktig enzym for nedbrytning av bindevevet i decidua, og lave nivåer av MMP1 kan være en mulig mekanisme bak nedsatt trofoblastinvasjon ved disse tilstandene. I arbeidet som inngår i denne avhandlingen ble også utrykket av alle gener i decidua fra kvinner med preeklampsi og/eller føtal veksthemming sammenlignet med friske gravide. Denne sammenligningen viste at kvinner med preeklampsi og føtal veksthemming hadde forstyrrelser i flere biologiske prosesser som tidligere har vært assosiert med nedsatt oksygentilførsel til vev, som for eksempel endoplasmatisk retikulum (ER) stress, forsvar mot oksidativt stress og fettsyremetabolisme (**studie III**). I videre analyser fant vi at av ER stress responsen var aktivert ved føtal veksthemming, isolert eller i kombinasjon med preeklampsi. Ved isolert preeklampsi så ER stress ut til å være mindre fremtredende (**studie IV**). Dette kan være med på å forklare noen av de kliniske forskjellene som sees ved preeklampsi og føtal veksthemming. Samlet sett har arbeidet i denne avhandlingen bidratt til å kartlegge sentrale mekanismer knyttet til utvikling av preeklampsi og føtal veksthemming, samt skapt noen nye hypoteser som bør undersøkes videre.

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Finansieringskilde: Norges teknisk-naturvitenskapelige universitet

Ovennevnte avhandling er funnet verdig til å forsvares offentlig for graden PhD i molekylærmedisin.
Disputas finner sted i Auditoriet, Medisinsk teknisk forskningssenter, fredag 27. januar 2012, kl. 12.15.

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ACKNOWLEDGMENTS

The work presented in this thesis has been carried out at the Department of Cancer Research and Molecular Medicine, the Norwegian University of Science and Technology (NTNU) from 2004 to 2011, and at the Perinatology Research Branch, National Institute of Child Health and Human Development, Detroit, USA, in spring 2006. The financial support for the work was ensured trough the Medical Student Research Programme at the Faculty of Medicine, NTNU, from 2004-2009 and trough a PhD grant from the Faculty of Medicine for 2009-2011.

First of all, I would like to express my gratitude to Professor Rigmor Austgulen for undertaking the role as my principal supervisor, providing guidance and support throughout the project. I highly appreciate the help from my co-supervisor Mette Langaas, who has been an excellent and patient teacher of statistics and bioinformatics.

I also wish to thank my colleagues and friends at the Medical Student Research Programme, with whom I have shared the frustrations of having 'too much to do and too little time' and the experience of starting research early in medical school. I especially wish to thank Johanne Toft for her enjoyable companionship during our four years together as research partners in the Medical Student Research Programme.

I would like to thank previous and current members of the The Research Group of Human Reproduction; Mona Fenstad, Linda Roten, Mari Løset, Siv Mundal, Line Tangerås, Guro Stødle, Astrid Gundersen, Irina Eide, Åsa Johansson, Guro Olsen, Toai Nguyen, Ann-Charlotte Iversen, Ann-Helen Leknes, and Kristin Rian in the Laboratory Group, for their help, friendship, encouragement and inspiration. There have been many valuable discussions over a cup of coffee.

I am grateful to my international collaborators Adi Tarca, Roberto Romero, Offer Erez, Jimmy Espinoza, Daniel Lott, Matthew Johnson and Eric Moses for their contributions. The experienced help from Toril Rolfseng, Borgny Ytterhus and Unn Granli at the Department of Laboratory Medicine, Children's and Women's Health, NTNU, in immunohistochemical analyses is deeply appreciated. I am also in debt to employees at the Norwegian Microarray consortium, NTNU, for technical assistance in our second microarray experiment. I also wish to thank Kristine Pettersen, Anne Gøril Lundemo and Caroline Pettersen for teaching me how to do Western blot.

I highly appreciate all the mothers that participated in our study, and the doctors that were involved in tissue collection, especially Irina Eide and Line Bjørge.

Finally, I thank my parents Vigdis and Jon-Inge and my sister Ingeborg, for their everlasting love and support.

Ingrid Alsos Lian,
Trondheim, October 2011

ABBREVIATIONS

ANGPTL2	angiopoietin-like 2
ANOVA	analysis of variance
ARL5B	ADP-ribosylation factor-like 5B
ATF6	activating transcription factor 6
BMI	body mass index
CAM	cell adhesion molecule
cDNA	complementary deoxyribonucleic acid
CS	caesarian section
Ct	cycle threshold
CVD	cardiovascular disease
DNA	deoxyribonucleic acid
ECM	extracellular matrix
EIF2α	eukaryotic translation initiation factor 2α
EMT	epithelial to mesenchymal transition
ENG	endoglin
ER	endoplasmic reticulum
ERAP2	endoplasmic reticulum aminopeptidase 2
EVT	extravillous trophoblast
FDR	false discovery rate
FGR	fetal growth restriction
FLT1	fms-related tyrosin kinase 1
FZD4	frizzled family receptor 4
GEE	generalized estimating equations
GST	glutathione s-transferase
HMOX1	heme oxygenase 1
I/R	ischaemia-reperfusion
IDO	indoleamine 2,3-dioxygenase
IPA	ingenuity pathway analysis
IRE1	inositol-requiring enzyme 1
KDR	kinase insert domain receptor
KYNU	kynureninase
MAN1A2	mannosidase α, class 1A, member 2
MCAM	melanoma cell adhesion molecule
MMP	matrix metalloproteinase
mRNA	messenger ribonucleic acid
NRF2	nuclear respiratory factor 2
PE	pre-eclampsia
pEIF2α	phosphorylated eukaryotic translation initiation factor 2α
PERK	PKR-like ER kinase

PLA2G7	phospholipase A2, group VII
PlGF	placental growth factor
qRT-PCR	quantitative real-time polymerase chain reaction
RMA	robust multichip average
RNA	ribonucleic acid
ROAST	rotation gene set tests
ROMER	rotation gene set enrichment analysis
ROS	reactive oxygen species
sENG	soluble endoglin
SEPS1	selenoprotein S
sFLT1	soluble fms-related tyrosin kinase 1
SGA	small for gestational age
SLITRK4	SLIT and NTRK-like family, member 4
SOLAR	sequential oligogenic linkage analysis routines
TGF-β	transforming growth factor β
UPR	unfolded protein response
VEGF	vascular endothelial growth factor
XBP1	x-box binding protein 1
XBP1(S)	x-box binding protein 1 spliced
XBP1(U)	x-box binding protein 1 unspliced
ZEB2	zinc finger E-box binding homeobox 2

LIST OF PAPERS

Paper I Toft JH*, Lian IA*, Tarca AL, Erez O, Espinoza J, Eide IP, Bjørge L, Sun C, Draghici S, Romero R, Austgulen R. **Whole-genome microarray and targeted analysis of angiogenesis-regulating gene expression (ENG, FLT1, VEGF, PlGF) in placentas from pre-eclamptic and small-for-gestational-age pregnancies.** Journal of Maternal-Fetal and Neonatal Medicine 2008;21(4):267-73. **both authors contributed equally*

Paper II Lian IA*, Toft JH*, Olsen GD, Langaas M, Bjørge L, Eide IP, Børdahl PE, Austgulen R. **Matrix metalloproteinase 1 in pre-eclampsia and/or fetal growth restriction: reduced gene expression in decidual tissue and protein expression in extravillous trophoblasts.** Placenta 2010;31(7):615-20. **both authors contributed equally*

Paper III Løset M, Mundal SB, Johnson MP, Fenstad MH, Freed KA, Lian IA, Eide IP, Bjørge L, Blangero J, Moses EK, Austgulen R. **A transcriptional profile of the decidua in preeclampsia**. American Journal of Obstetrics and Gynecology 2011;204(1):84.e1-27.

Paper IV Lian IA, Løset M, Mundal SB, Fenstad MH, Johnson MP, Eide IP, Bjørge L, Freed KA, Moses EK, Austgulen R. **Increased endoplasmic reticulum stress in decidual tissue from pregnancies complicated by fetal growth restriction with and without pre-eclampsia.** Placenta 2011;32(11):823-29.

1. INTRODUCTION

1.1 Definitions, diagnosis and management

Pre-eclampsia (PE) has been recognised as a pregnancy complication since the time of the ancient Greeks [1], and is a leading cause of maternal and perinatal morbidity and mortality worldwide [2-4]. The reported occurrence of PE varies between 2-8%, depending on the use of diagnostic criteria and population under study [5-7]. In Western countries, the prevalence has been reported to increase over the last 30 years [8]. Similarly, an increase in prevalence from 3.3 to 4.5% has been reported in Norway from 1968 to 2002 [9]. The secular increases in PE may have occurred due to changes in maternal characteristics, such as increased maternal body mass index (BMI) at the time of pregnancy [10], and increased prevalence of predisposing disorders such as diabetes and chronic hypertension [11]. PE is characterised by maternal systemic endothelial dysfunction and kidney dysfunction, typically presenting as *de novo* onset of hypertension and proteinuria (cardinal signs) in the latter half of pregnancy. PE may, when severe, rapidly develop into a multisystem syndrome with pulmonary oedema, kidney failure, acute liver injury, coagulopathies, and seizures (eclampsia) [12].

International consensus on how to define and diagnose PE has not yet been reached, probably reflecting that PE is a heterogeneous condition that can vary substantially in its clinical presentation and severity. However, most existing classification systems consider hypertension and proteinuria to be the hallmarks of PE. Once PE is diagnosed or suspected, the condition should be closely monitored and blood pressure controlled with medication if necessary [12]. Conservative treatment of PE gives the fetus time to mature, but inevitably places the mother at risk, as PE usually is a progressive and unpredictable condition. Ultimately, delivery of the placenta remains the only known effective treatment.

The clinical spectrum of PE ranges from mild to severe, and includes both early and late onset of disease (before or after week 34 of gestation, respectively), late onset PE being most frequent (~80%) [13-14]. Most cases of PE are confined to the maternal symptoms, but in approximately one third of all pre-eclamptic pregnancies the fetus is growth restricted [15]. Fetal growth restriction (FGR) occurs more often in early (53%) than late onset (7%) PE [16], and the risk of FGR increases with severity of the pre-eclamptic symptoms [16-17].

FGR has been estimated to affect 3-10% of all pregnancies worldwide [18] and is a leading cause of perinatal morbidity and mortality [19-20]. Normal fetal growth is determined by complex and dynamic interactions between the maternal, placental and fetal environment. Disturbances in any of these factors may lead to altered fetal growth, but placental insufficiency is the most common cause of FGR in developed countries [21-22]. Suboptimal fetal growth is referred to as either FGR or small for gestational age (SGA). These terms are often used interchangeably, but by definition, they are different conditions. The term *fetal growth restriction* indicates that the fetal genetic growth potential has not been reached, and diagnosis should be reserved for fetuses for which there is evidence that growth has faltered [23]. The term *small for gestational age* is used for fetuses born small according to the length of pregnancy, and is commonly used as a proxy for FGR. The World Health Organization defines SGA as a fetus with a birthweight below the 10th percentile of the expected birthweight adjusted for gestational age. Approximately 70% of these infants will actually be constitutionally small and represent the normal end tail in the spectrum of neonatal size [18]. By reducing the cut-off to less than the 5th percentile or even lower, the specificity for true growth restricted infants may be increased, and help identify those at increased risk of adverse outcome [24]. On a

clinical basis, several approaches are used to identify impaired fetal growth, including serial measurements of fundal height [25], serial ultrasound biometry [26], and Doppler ultrasound examination of umbilical arteries [27]. However, as these methods are time consuming, measures of low birthweight (below a given percentile) as indicators of impaired fetal growth are often used for research purposes. No proven preventive or therapeutic strategies exist for FGR, and the primary goals in clinical management remain close surveillance of fetal health and optimising timing of delivery [28]. Induced delivery is often required to avoid additional harm *in utero,* even at the cost of prematurity.

1.2 Risk factors and long term complications

A number of risk factors for developing PE have been identified, such as a family history of PE [29-31], a previous pre-eclamptic pregnancy [29,32-35], nulliparity [32,35], and both high [33-34,36] and low [6,34] maternal age. Risk factors for developing FGR have been less extensively studied, but also include a family history of FGR [37], a previous pregnancy complicated by FGR [34], nulliparity [34,38], maternal smoking [39-40], and high maternal age [39,41].

Epidemiological studies have shown that women experiencing PE and FGR have an unfavourable lipid profile, insulin resistance, increased blood pressure, and higher BMI before and after pregnancy [35,42-47]. They are concurrently at increased risk of developing metabolic syndrome [45] and cardiovascular disease (CVD) [45,48-50] later in life. The similarities in risk factors for PE, FGR and CVD have led to the hypothesis that these syndromes share a common predisposition (genetic or environmental) to vascular and metabolic disease, and merely represent different manifestations of the same underlying pathology. In that regard, pregnancy may represent a ‘stress test’ for later life CVD [51-52], where the increased metabolic and vascular demands of a normal pregnancy may reveal a vulnerable maternal

constitution. It is also possible that having pregnancy complicated by PE or FGR in itself constitutes a strain that ultimately leads to CVD, possibly through persistent subclinical systemic vascular damage [53] or epigenetic changes. These findings have encouraged investigations of possible shared genetic susceptibilities between PE, FGR, and CVD [54].

The concept of shared genetic risk factors between PE and FGR is supported by the fact that having a previous pregnancy complicated by PE or FGR is associated with an increased risk recurrence of *either* PE of FGR. The risk of developing PE after a previous pregnancy with FGR is increased, especially for developing severe and early onset PE [55], and the risk of having an FGR pregnancy after a pregnancy with PE increases with the severity of PE in the previous pregnancy [56-57]. Moreover, risk of developing CVD later in life is higher in women with early onset, severe PE [49] than late onset PE. The risk ratios for CVD appears to be additive for women with multiple pregnancy complications (i.e. having both PE and an infant with low birthweight) [48]. Taken together, this suggests that the severe forms of PE may only differ from FGR in terms of maternal response to the shared placental pathology.

1.3 Pathogenesis of PE and FGR

In 1991, Redman proposed a two-stage model for the development of PE [58], which has been a useful conceptual framework for studies of the pathophysiology of PE (Figure 1). In this model, impaired trophoblast invasion and spiral artery remodelling leading to insufficient blood flow to the placenta is considered stage 1. Stage 2 represents the maternal response to stage 1, where systemic activation of the maternal endothelium is evoked by product(s) released from the suffering placenta.

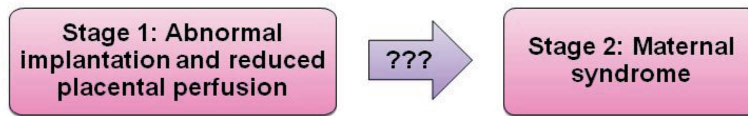

Figure 1. The two-stage model for the development of PE, as proposed by Redman [58].

Several mechanisms have been suggested to link the two stages, predominantly hypoxia or ischaemia-reperfusion (I/R) insults, leading to oxidative stress and placental release of inflammatory cytokines, reactive oxygen species (ROS), anti-angiogenic factors and apoptotic/necrotic trophoblast products [59-60].

Mechanism of impaired placentation

During the first half of human pregnancy, trophoblasts of the anchoring villi differentiate into invasive extravillous trophoblasts (EVTs) that participate in remodelling of the spiral arteries. This process results in dilated, inelastic vessels without maternal vasomotoric control [61], securing adequate blood flow to the placenta and fetus. This process normally extends through the decidua and the adjacent third of the myometrium [62-63]. In contrast, superficial trophoblast invasion and impaired remodelling is observed in PE and FGR [62-67], where the depth of trophoblast invasion as well as the number of vessels invaded by trophoblasts is reduced [62-63]. The subsequent limitation in utero-placental blood supply is presumed to initiate the sequence of events that lead to clinically manifest PE and FGR later in pregnancy.

The mechanisms underlying trophoblast invasion appear to be complex, and are to date not fully understood [68-70]. Normal trophoblast invasion involves differentiation from a proliferative to an invasive phenotype. This includes attachment to and degradation of the extracellular matrix (ECM) and subsequent migration through the decidual stroma. In this process, a complex molecular

dialogue takes place between EVTs, maternal immune cells, decidual cells, and ECM components [68]. Impaired invasion may be a consequence of primary defects in trophoblasts (intrinsic factors), the decidual environment that the trophoblasts are attempting to invade (extrinsic factors), or a combination of these [69]. The trophoblasts' shift to an invasive phenotype involves acquisition of migratory and invasive capacities, changes analogous to epithelial to mesenchymal transition (EMT) and tumour progression [71], including a shift in the cell adhesion molecule (CAM) repertoire. However, in contrast to tumour growth and metastasis, trophoblast invasion is a tightly controlled process. In PE and FGR, invasive trophoblasts show abnormal expression of CAMs such as cadherins and intergrins [72-74], which has been associated with impaired invasiveness. Matrix metalloproteinases (MMPs) play an important role in trophoblast invasion, as the trophoblasts' ability to infiltrate the decidual tissue strongly depends on their ability to secrete MMPs to degrade the ECM [75-76]. In PE and FGR, reduced trophoblast expression of MMP2, -3, -7 and -9 protein has been reported [77-79].

In addition to intrinsic trophoblast defects, impaired invasion also appears to be related to disturbed immunological interactions at the maternal-fetal interface [80-81], where trophoblasts encounter maternal immune cells. Metabolism of tryptophan via catabolism by indoleamine 2,3-dioxygenase (IDO) has been implicated in this process. IDO is expressed by a variety of cells in the placenta [82], and subsequent tryptophan depletion is proposed to suppress cytotoxic T cell responses [83-85]. Decreased placental IDO levels and activity, and elevated maternal serum levels of tryptophan have been reported in PE [82,86-87].

Whatever mechanisms at play, trophoblast invasion is not an all-or-none phenomenon. Instead, there appears to be a gradient of decrease in the percentage of decidual and myometrial arteries invaded from normal to complicated pregnancies

[88]. Failed physiologic transformation of the spiral arteries has been reported in virtually every major obstetrical problem, including PE, FGR, miscarriage, placental abruption, preterm labour, and preterm premature rupture of membranes [89-90]. Impaired remodelling is also present to some degree in normal pregnancies [65-66,88]. As these morphological disturbances manifests in such different ways, it seems likely that the maternal and fetal response to abnormal placentation plays an important part in determining the clinical outcome.

Oxidative stress

Incomplete conversion of the spiral arteries is likely to result in retention of vasoreactivity of the vessels, where subsequent fluctuations in intervillous oxygen supply leads to I/R insults [91-92]. Uterine artery Doppler flow measurements have been shown to correlate with placental bed vascular abnormalities in PE and FGR [67,93], consistent with the idea that impaired remodelling affects utero-placental blood flow. Several lines of evidence suggest that placentas from PE and FGR pregnancies are subject to I/R insults and oxidative stress, including increased levels of ROS, lipid peroxidation products, and other markers of oxidative stress [94-96]. Studies have also shown that decidual tissue represents an important source of oxidative stress [97-100]. In PE and FGR, decidual spiral arteries are characterised by accumulation of lipid-laden macrophages (acute atherosis) [101]. Decidual oxidative stress may arise by peroxidation of these lipid depositions. Correspondingly, Staff et al. reported increased contents of lipid peroxides in decidual tissue from pre-eclamptic pregnancies, and proposed that release of such oxidative lipid derivates could induce endothelial dysfunction when reaching the maternal circulation [98].

Endoplasmic reticulum stress

I/R insults can also induce endoplasmic reticulum (ER) stress [102-103] and activation of the unfolded protein response (UPR). The UPR consists of three signalling pathways, activated by the proximal ER transmembrane sensors inositol-requiring enzyme 1 (IRE1), PKR-like ER kinase (PERK), and activating transcription factor 6 (ATF6) [104], as shown in Figure 2.

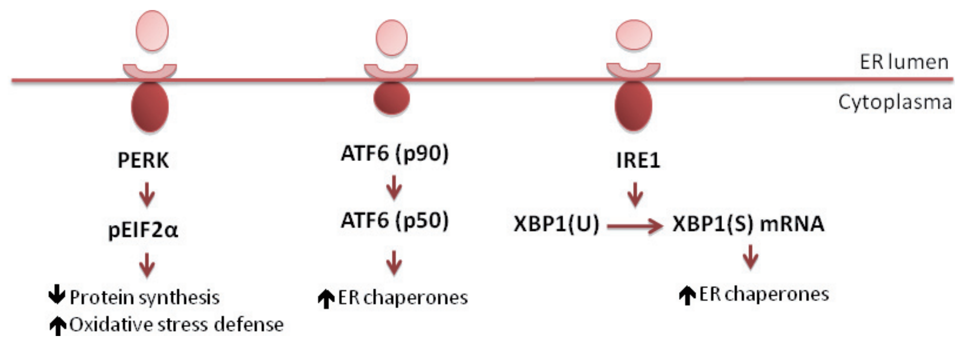

Figure 2. Under ER stress, PERK phosphorylates the eukaryotic translation initiation factor 2α (EIF2α), leading to general attenuation of protein synthesis and upregulation of transcripts involved in oxidative stress defence. IRE1 splices the constitutively expressed X-box binding protein 1 (XBP1(U)) mRNA into XBP1(S), thereby creating a potent transcriptional activator. Similarly, ATF6 is released from the ER membrane and transits to the Golgi for proteolytic cleavage, yielding a cytosolic fragment of 50 kDa that migrates to the nucleus. Together, ATF6 and XBP1 activate transcription of genes that support adaptation to and recovery from ER stress. Figure adapted from Burton et al. [105] with permission.

Depending on the severity and nature of the underlying trigger, ER stress can lead to a diversity of responses, including decreased cellular proliferation [106], oxidative stress [107], activation of inflammatory pathways [108], or apoptosis under severe or prolonged conditions of ER stress [103-104]. Correspondingly, ER stress has been implicated in a wide range of human disorders, including diabetes, hypercholesterolaemia, atherosclerosis, CVD, and inflammatory conditions [109-110]. Recently, ER stress was also implicated in the pathogenesis of PE and FGR [105-106], where increased placental levels of ER stress was associated with

decreased cellular proliferation and apoptosis, and proposed as an important cause for the small placental phenotype observed in these pregnancy complications [105-106]. Furthermore, it was suggested that oxidative stress superimposed on ER stress could cause systemic inflammation and maternal pre-eclamptic symptoms, and account for the different clinical manifestations of PE and FGR [105].

Angiogenic imbalance

Placental vascular development is regulated by numerous placentally-derived angiogenic factors, including vascular endothelial growth factor (VEGF), placental growth factor (PlGF), angiopoietins, and oxygen [111]. VEGF and PlGF signalling is important for normal endothelial function [112-113]. They exert their biological effects mainly through the receptors fms-related tyrosine kinase 1 (FLT1) and kinase insert domain receptor (KDR). A soluble splice variant of the FLT1 receptor, sFLT1, is produced in the placenta [114] and is a potent antagonist of free PlGF and VEGF as it binds them in the circulation and prevents interaction with their endogenous receptors [115].

Mounting evidence suggests that an anti-angiogenic state plays an important role in the pathophysiology of PE and FGR. Exogenously administered sFLT1 was shown to induce a syndrome resembling PE, including hypertension and proteinuria in pregnant rats [116]. This effect was further exaggerated when soluble endoglin (sENG) was co-administered with sFLT1, resulting in severe PE with FGR [117]. Soluble ENG is a truncated form of the transforming growth factor β (TGF-β) co-receptor ENG. Soluble ENG reduces the bioavailability of TGF-β1 and inhibits TGF-β1's role in maintaining endothelial health [117]. Figure 3 shows how sENG and sFLT1 can induce endothelial dysfunction.

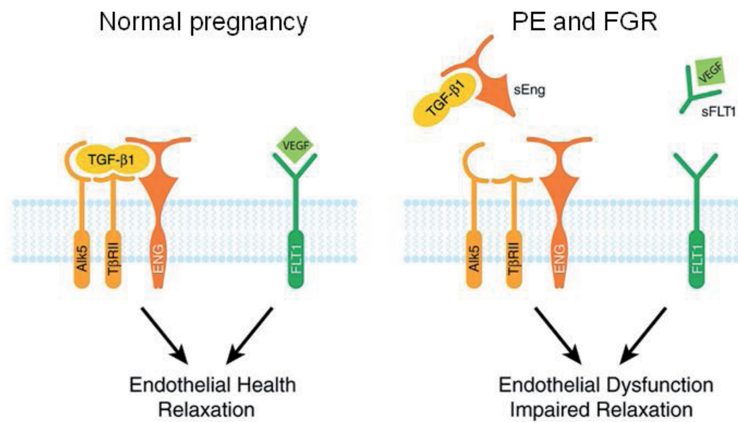

Figure 3. The angiogenic imbalance in PE and FGR. Vascular homeostasis is maintained by physiological levels of VEGF and TGF-β1 signalling during normal pregnancy. In PE and FGR, excess placental secretion of sFLT1 and sENG cause endothelial dysfunction by antagonising these signalling pathways. Reproduced with permission [118].

Numerous papers have reported increased maternal circulating levels of sFLT1, associated with decreased levels of the pro-angiogenic factors VEGF and PlGF in PE and FGR [116,119-123]. Similarly, increased levels of sENG have also been reported in these cases [117,122-125]. The circulating levels of sFLT1 and sENG are correlated with disease severity, being higher in the early onset and severe than late onset and mild subtypes of PE [117,121,126-127]. Studies directly comparing circulating levels of sFLT1 and sENG between PE and FGR show that these changes are more pronounced in PE than FGR [122,125,128-129]. These anti-angiogenic factors are presumably released from the placenta, supported by the fact that the plasma levels sENG and sFLT1 decrease after delivery [116-117]. Correspondingly, placental levels of FLT1, sFLT1, ENG, and sENG are increased in PE [116-117,129], whereas results are conflicting in FGR, as both increased [128,130] and unchanged [119,129] levels of these factors are reported.

Placental release of anti-angiogenic factors was initially proposed to result from placental malperfusion. However, as changes in circulating levels of these factors

occur before the spiral artery remodelling has been completed [122,131-132], the angiogenic imbalance may not necessarily be secondary to vascular malperfusion, but could also be associated with disturbed placental development. Placentas from pregnancies complicated by FGR and PE in combination with FGR (PE+FGR) are characterised by abnormal villous and vascular morphology in terms of decreased surface area and volume of villi and villous capillaries, whereas placental morphology in PE is comparable to that of normal pregnancies [133-135]. These morphological differences have been proposed to result from different profiles of angiogenic factors [136].

A step-wise model for the development of PE and FGR

In the recent years, the prevailing two-stage model [58] has been modified to encompass a broader spectre of pregnancy disorders, including both PE and FGR, and a continuum theory has emerged [92,105,137]. Alterations in factors associated with the pathophysiology of PE and FGR, such as impaired spiral artery remodelling [88], oxidative stress [138], ER stress [105-106], inflammation [139], and anti-angiogenic markers [123] are also present in normal pregnancies, but of a greater magnitude in PE and FGR. In other words, these conditions merely represent more or less extreme ends of changes caused by pregnancy itself.

Spiral artery remodelling is impaired in both PE and FGR, but apparently to a larger degree in PE+FGR and PE than FGR [63-64,67]. Also, levels of oxidative stress, the maternal inflammatory response and endothelial dysfunction in FGR appear to be intermediate between that of normal pregnancies and PE [138,140], whereas it is greater in PE+FGR than in isolated PE [95-96,141-142]. In addition, the anti-angiogenic state appears to be higher in PE than FGR [122,125,128-129], and most pronounced in the severe types of PE [117,121,126-127] (see Table 1).

Table 1. Pathohysiological aberrations in PE and/or FGR.

	FGR	PE	PE+FGR	References
Spiral artery remodelling	↓↓	↓↓↓	↓↓↓	Khong et al. [63], Brosens et al. [64], Madazli et al. [67]
Placental weight and morphology	↓↓	↓	↓↓↓	Teasdale et al. [133], Egbor et al. [134], Mayhew et al. [135]
Oxidative stress	↑	↑↑	↑↑↑	Takagi et al. [95], Fujimaki et al. [96], Burton et al.[138], Wiktor et al. [141]
ER stress	↑		↑↑	Burton et al. [105], Yung et al. [106]
Anti-angiogenic factors	↑	↑↑	↑↑↑	Venkatesha et al. [117], Wikström et al. [121], Romero et al. [122], Stepan et al. [125], Chaiworapongsa et al. [126], Tsatsaris et al. [128], Jebalayan et al. [129]
Inflammation	↑	↑↑	↑↑↑	Ness et al. [140], Laskowska et al. [142]

If there is a continuum of placental and maternal vascular changes from normal to complicated pregnancies, how do these changes account for the differences in clinical outcome? Roberts and Hubel [137] proposed that placental malperfusion could initiate a distress signal from the feto-placental unit, aiming to compensate for reduced blood supply by modifying the maternal metabolism and vasculature to increase nutrient delivery and blood pressure. This is an extension of the genetic conflict theory posed by Haig in 1993, suggesting that over evolution, fetal genes have been selected to increase nutrient transfer to the fetus, and maternal genes have been selected to restrict transfer exceeding the maternal optimum [143].

Different scenarios may emerge as a result of the woman's ability to respond to and tolerate these distress signals, as illustrated in Figure 4. In other words, there may be varying contributions of maternal and placental factors in the different pregnancy outcomes. Some women may fail to adapt but have a high tolerance to the placental

distress signals, and deliver a growth restricted infant (FGR) without any maternal symptoms. Others may appropriately adapt to but have a low tolerance to these signals, developing pre-eclamptic symptoms but deliver a normally grown infant (PE). Alternatively, PE may be the result of a placental adaptive 'overshoot'. In the worst case scenario, the woman fails to respond and adapt to the placental signals while having a low tolerance to these, in which PE+FGR is the result. A low maternal liability threshold to placental signals could be associated with increased susceptibility to vascular and metabolic stressors, and be modified by pre-existing medical conditions such as diabetes, hypertension, or obesity.

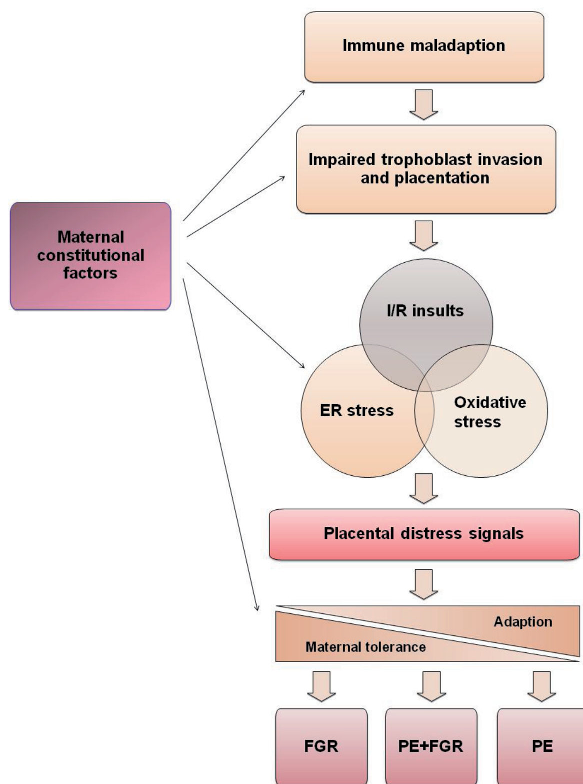

Figure 4. An extended step-wise model for the development of PE and/or FGR. Disturbed immunological interactions between maternal and fetal cells lead to impaired spiral artery remodelling and subsequent I/R insults, oxidative stress, and ER stress. Distress signals from the suffering placenta may or may not be sufficient to overcome impaired oxygen and nutrient supply, and the mother may or may not tolerate these signals. Maternal constitutional factors influence the maternal tolerance to placental distress signals.

However, the continuum theory does not explain all pathological changes that are observed in and between PE and FGR, but merely represents a framework to understand how the different clinical scenarios may emerge. In this regard, it is important to keep in mind that the diagnosis of PE and FGR, pertaining to continuous biological variables such as birthweight, blood pressure and proteinuria, are based on arbitrarily set diagnostic cut-offs.

1.4 Transcriptional analyses of PE and FGR

Microarray-based transcriptional profiling is frequently used to analyse and explore gene expression patterns to increase the understanding of molecular mechanisms of complex diseases. This technology has proven a powerful strategy for identification of novel biomarkers and for developing new hypotheses of disease-related processes [116-117,144]. In addition, gene expression data may be combined with previous knowledge of susceptibility genes and used to assist prioritisation of genes for sequencing after genome-wide association studies [145].

Placental tissue is a convenient source for transcriptional analyses as it is relatively easy to obtain. The placenta is central in the pathogenesis of PE and FGR, and represents a relevant site to investigate processes behind placental dysfunction and mechanisms of release of placental factors that cause maternal pre-eclamptic symptoms. In the recent years, a large number of transcriptional studies of *placental* tissue from PE and FGR pregnancies have been performed [146-149]. A variety of differentially expressed transcripts have been identified, including the potential biomarkers sFLT1 [116] and sENG [117], and transcripts associated with immunoregulation [150-152], inflammation [148,150-151], hypoxia [153], oxidative stress [150], and angiogenesis [154].

The maternal-fetal interface (decidua) is a relevant site for the study of mechanisms leading to impaired spiral artery remodelling, such as immune maladaption and impaired trophoblast invasion. However, only a few microarray analyses of decidual tissue have been published [145,155-157]. These have shown altered expression of immune components [155] and members of the renin-angiotensinogen system [156] in association to PE. Moses et al. focused on genes residing within their region of significant genetic linkage to PE on chromosome 2q in their analysis of decidual tissue, and found that 17 transcripts within this region were differentially expressed in PE [145]. Winn et al. identified 55 differentially expressed transcripts comparing pre-eclamptic and normal pregnancies, including both novel (e.g. sialic acid binding Ig-like lectin 6) and previously PE-associated transcripts (e.g. FLT1, ENG, and leptin) [157].

The results of these transcriptional analyses have been inconsistent, which may simply reflect the mixed aetiology and complex nature of disease processes in these pregnancy complications. However, upregulation of some transcripts (FLT1, ENG, and leptin) has been successfully replicated in many of these studies [147-150,152,154,158-159], indicating that these could represent more robust disease related factors. The association between polymorphisms in FLT1 [160] and leptin [161-162] and risk for developing PE indicates that genetic variations in FLT1 and leptin may contribute to their altered expression in PE.

2. AIMS OF THE STUDIES

Overall aim

PE and FGR are presumed to occur as consequences of impaired placentation, but the molecular mechanisms leading to clinically manifest disease are not completely understood. How differences in pathophysiology between PE and FGR account for the diversity in clinical outcome is also under debate. The overall aim of this work was to elucidate such mechanisms by comparing transcriptional profiles of placental and decidual tissue from women with impaired placentation (PE and/or FGR) and normal pregnancies. More specifically, the main aims of the papers included in this thesis were:

Paper I

- To investigate possible divergent mechanisms underlying *placental* disease in PE and FGR by performing a whole-genome transcriptional profiling of placental tissue from women with PE and/or FGR pregnancies.
- Further, disturbed angiogenesis appears to be involved in the placental disease of both PE and FGR, but may differ between these phenotypes as a consequence of an angiogenic imbalance. To determine if such differences existed, a targeted comparison of placental levels of key angiogenic and anti-angiogenic factors between these study groups was performed.

Paper II

- To elucidate possible molecular mechanisms underlying impaired trophoblast invasion and the development of disease in PE and FGR, we performed a focused comparison of transcriptional profiles of *decidual* tissue from women with pregnancies complicated by PE and/or FGR and healthy pregnant women.

Paper III

- To comprehensively identify perturbed mechanisms and biological pathways associated with PE by comparing the whole-genome transcriptional profile of *decidual* tissue from women with PE and PE+FGR to that of healthy pregnant women.

Paper IV

- ER stress has been implicated in the pathogenesis of both PE and FGR, but whether the degree of ER stress differs between these pregnancy complications is unknown. The aim of this study was to investigate whether PE and FGR are characterised by differences in the degree of ER stress or differential activation of the ER stress pathways. This was performed by comparing gene expression and protein levels of key mediators of each branch of the ER stress response between pregnancies complicated by PE and/or FGR, as well as to that of healthy pregnant women.

3. MATERIAL

3.1 Study population

The study population for the work presented in this thesis included women with singleton pregnancies delivering at St. Olavs University Hospital (Trondheim, Norway) and Haukeland University Hospital (Bergen, Norway) from 2002 to 2006. Women with pregnancies complicated by PE and/or FGR were consecutively enrolled as cases, as well as women with healthy pregnancies, who served as controls. PE was defined as persistent hypertension (systolic blood pressure of >140 mmHg or diastolic >90 mmHg), plus proteinuria (≥0.3 g in a 24 hour urine collection or ≥1+ according to a dipstick test), developing after 20 weeks of gestation [163]. FGR was defined by birthweight ≤2 standard deviations below the expected birthweight according to gestational age and sex, corresponding to the 2.5th percentile in a Scandinavian normogram [164]. In addition, restricted fetal growth was confirmed by at least one of the following additional criteria; 1) reduced fundal height in serial measurements, 2) serial ultrasound biometry identifying failure to grow along a consistent percentile, or 3) abnormal umbilical artery wave form. Pregnancies with chromosomal aberrations, fetal and placental structural abnormalities, or suspected perinatal infections were excluded from all study groups. Due to tissue sampling procedures, decidual and placental tissue was collected during caesarean section (CS) in women without labour activity. Cases had CS performed due to clinical indications, whereas controls were undergoing elective CS irrelevant to the aim of the studies, (i.e. breech presentation, previous CS or maternal request).

In **paper I**, small infants were defined as SGA, whereas in **papers II-IV**, they were defined as FGR. This discrepancy is due to the fact that we did not have access to Doppler ultrasound biometry data at the time when **paper I** was prepared,

preventing us from confirming faltered fetal growth. Retrospectively, it was shown that all SGA infants in **paper I** had true FGR, and will henceforth be referred to as FGR. For the purpose of **paper IV**, cases with debut of pre-eclamptic symptoms or FGR before gestational week 34 were classified as early onset. PE was considered severe in accordance with the classification recommended by Sibai et al. [165]; including severe hypertension (>160 mmHg systolic and/or 110 mmHg diastolic) associated with proteinuria, or severe proteinuria (≥5g per day) associated with hypertension and/or multiorgan involvement (pulmonary oedema, seizures, oliguria, thrombocytopenia, abnormal liver enzymes etc.). Severe FGR was defined as birthweight <1.7 percentile [19].

The study group composition in the respective studies included in this thesis (see Table 2) varies for a number of economical, methodological, and practical reasons. In **paper I**, only cases matched for gestational age were included, and some samples were excluded due to low ribonucleic acid (RNA) quality. The microarray experiment in **paper II** was conducted at a time when the recruitment of cases and controls was still ongoing, and our biobank was of limited size. Subsequent enrolment of cases and controls allowed for a larger sample size in the quantitative real-time polymerase chain reaction (qRT-PCR)- and immunohistochemical experiments performed at a later time. In **paper III**, all available cases with PE and PE+FGR and controls with sufficient RNA quality were included in the microarray analyses, and all samples except two (excluded due to insufficient RNA material) were included in the RT-qPCR experiments. In **paper IV**, all available cases (PE, FGR and PE+FGR) and controls with sufficient RNA quality were included in the microarray analyses. For the Western blot experiments, a subset of cases with clinical characteristics suggesting vascular malperfusion (i.e. abnormal uterine artery Doppler and/or early onset, severe PE [120] and FGR [166]) were included.

Table 2. Overview of the study population in the respective studies presented in this thesis.

Paper	Microarray (cases + controls)	qRT-PCR (cases + controls)	Immunohistochemistry/Western blot (cases + controls)
I	28 (28 + 0)	28 (28 + 0)	
II	35 (18 + 17)	50 (31 + 19)	76 (56 + 20)
III	95 (37 + 58)	93	
IV	104 (46 + 58)		16 (12 + 4)/30 (22 + 8)

3.2 Tissue sampling and preparation

<u>Placental tissue</u>

Placental biopsies were obtained immediately after delivery. Multiple biopsies from various locations of the placenta were collected, but only a single biopsy, cut from the central region of the maternal side of the placenta, close to the insertion of the umbilical cord, was stored for subsequent RNA isolation. Areas with infarctions and haematomas were not included. All collected placentas were examined by a pathologist in accordance with established routines at the University Hospitals in Trondheim and Bergen.

<u>Decidual tissue</u>

Decidual tissue was obtained during CS after delivery of the placenta, as described in detail elsewhere [98,167]. Briefly, the placenta was located by manual palpation after delivery, and allowed to separate spontaneously from the uterine wall. Decidual tissue from the identified placental bed was collected by vacuum suction and immediately flushed with sterile saline solution to remove excess blood.

Tissue preparation

Decidual and placental tissue for RNA analyses (microarray and RT-qPCR) was immediately submerged in RNAlater solution (Ambion, Huntington, UK) to prevent RNA degradation and stored at –80°C. Decidual tissue for immunohistochemical analyses was fixed in 10% neutral-buffered formalin, paraffin embedded and cut into 4 m sections. Decidual tissue for Western blot analyses was snap frozen in liquid nitrogen and stored at –80°C.

3.3 Ethical considerations

The studies included in this work were approved by the Regional Committee for Medical Research Ethics, REK Central Norway (REK no. 054.02). Written informed consent was obtained from all study participants.

4. METHODS

4.1 Microarray gene expression analyses

Microarray technology has become a powerful research tool for studying gene expression patterns associated with a broad assortment of biological outcomes. Microarrays consist of small, solid surfaces onto which short oligonucleotide sequences (probes) are attached at fixed locations, each probe representing a messenger ribonucleic acid (mRNA) transcript. In a microarray experiment, the relative abundance of tens of thousands of mRNA transcripts can be measured simultaneously (Figure 5).

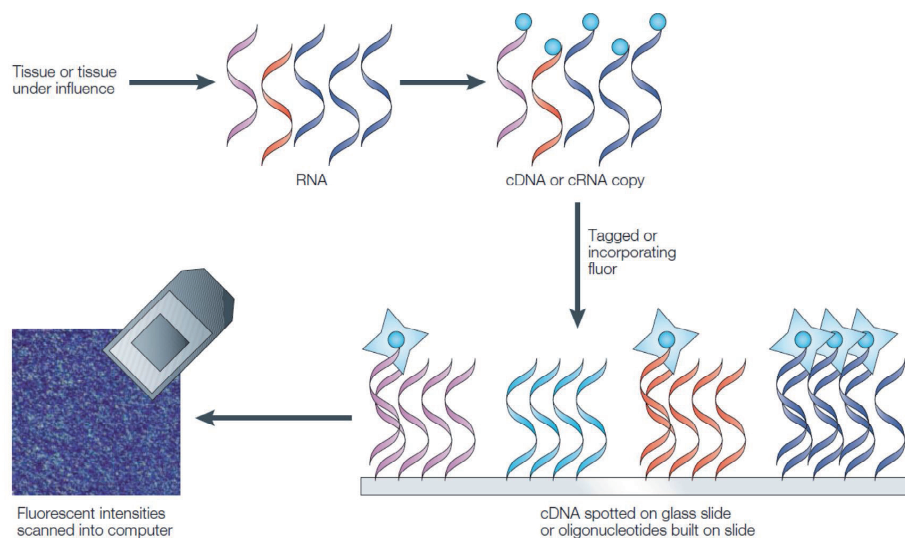

Figure 5. In microarray experiments, isolated RNA is reverse transcribed into complementary deoxyribonucleic acid (cDNA), labelled with fluorescence and applied to an array. The cDNA molecules will hybridise to probes if there is sequence complementarity, and the bound cDNA is quantified by scanning and measuring the intensity of emitted light from the various probe locations. The fluorescence intensity of each spot on the array is proportional to the level of expression of the gene represented by that spot. Reproduced with permission [168].

Different microarray manufacturers utilise different methods for probe selection and design. Affymetrix uses multiple 25mer probes, where each transcript is measured

by multiple probes representing different parts of an mRNA sequence [169], whereas Illumina uses multiple copies of a single 50mer probe attached to microbeads to interrogate an mRNA sequence [170]. Both platforms have been reported to yield good intra-platform reliability [171-172], inter-platform consistency [171,173], and display a high degree of correlation with alternative gene expression measurement such as qRT-PCR analysis [171].

In the work included in this thesis, both Affymetrix and Illumina microarray platforms were used for transcriptional analyses. In **paper I**, the placental transcriptome was analysed using the Affymetrix U133 plus 2.0 arrays (Affymetrix, Santa Clara, CA, USA), interrogating over 47.000 transcripts. In **paper II**, the decidual transcriptome was analysed on Affymetrix HG Focus array (Affymetrix), interrogating ~8500 transcripts. Finally, in **paper III**, Illumina HumanWG-6 v2 Expression BeadChips (Illumina Inc., San Diego, CA, USA) were used, interrogating over 48.000 transcripts. The selection of platforms and microarrays differed between these studies due to economical and practical reasons. The transcriptional analyses included in **papers I-III** were performed at three different institutions (**I**: Perinatology Research Branch, National Institute of Child Health and Human Development, Detroit, MI, USA; **II**: Norwegian Microarray Consortium, NTNU, Trondheim, Norway; **III**: Texas Biomedical Research Institute, San Antonio, TX, USA), each with their specific platform installed. We would have preferred to use a whole-genome array for the analyses included in **paper II**, but due to limited financial resources, the less comprehensive and less costly Affymetrix HG Focus array was chosen.

4.2 Quantitative RT-PCR analyses

Quantitative RT-PCR is based on the polymerase chain reaction, which is a primer-mediated enzymatic amplification of specific deoxyribonucleic acid (DNA)

sequences used to quantify mRNA transcript abundance. A qRT-PCR experiment consists of two principal steps; 1) reverse transcription of RNA into cDNA and 2) denaturation of cDNA strands, primer annealing to a specific cDNA sequence and cDNA amplification. The accumulation of cDNA of interest is exponential and monitored by emittence of fluorescence [174]. Two common methods for detection of products in qRT-PCR are; 1) non-specific fluorescent dyes that intercalate with any double-stranded DNA (such as SYBR Green) and 2) sequence specific DNA probes labelled with a fluorescent reporter which permits detection only after hybridisation with its complementary DNA target (such as TaqMan). The qRT-PCR analyses were performed using preoptimised TaqMan gene expression assays (Applied Biosystems, Foster City, CA, USA) for the transcripts ENG, FLT1, VEGF and PlGF (**paper I**); phospholipase A2, group VII (PLA2G7), angiopoietin-like 2 (ANGPTL2), mannosidase α, class 1A, member 2 (MAN1A2), SLIT and NTRK-like family, member 4 (SLITRK4), frizzled family receptor 4 (FZD4), and ADP-ribosylation factor-like 5B (ARL5B) (**paper III**); whereas MMP1 (**paper II**) was analysed using SYBR Green (Bio-Rad Laboratories, Hercules, USA). The selection of qRT-PCR methods differed between these studies due to practical reasons, as the experiments were performed at different institutions with differing preferential choice of technology.

4.3 Immunohistochemical analyses

Immunohistochemistry is a method for localising proteins in tissue or cells using the principle of an antigen-antibody reaction. The reaction can be visualised by immunoenzymatic staining, utilising antibodies conjugated to an enzyme that can generate a coloured product [175]. Alternatively, in immunofluorescent techniques, the antibody can be labelled with a fluorophore, such as fluorescein, to indicate the occurrence of a specific antigen-antibody reaction [176]. Immunofluorecence

analyses were used to detect and quantify MMP1 in EVTs (**paper II**), and XBP1 and ATF6 in EVTs, decidual cells, and macrophages (**paper IV**) in decidua basalis tissue.

4.4 Western blot analyses

Western blot is used to detect and quantify proteins in a given tissue or cell sample. Denatured proteins are separated by their polypeptide length on a gel by electrophoresis. The proteins are then transferred to a membrane where they are detected and visualised using antibodies specific to the target protein [177-178]. The principle for visualising the antigen-antibody reaction is similar to that in immunohistochemical methods. Western blot was used to detect and quantify protein levels of pEIF2α, EIF2α ATF6, XBP1(U), and XBP1(S) in decidua basalis tissue (**paper IV**), using Odyssey Infrared Imaging System (Li-Cor Biosciences, Cambridge, UK).

4.5 Statistical analyses

Clinical variables, results from the cell counting in immunohistochemical analyses, and Western blot analyses were analysed using the Students *t*-test if normally distributed, whereas Mann-Whitney U test was used for non-normally distributed data. Fisher's exact test and Pearson's χ^2 test were used for categorical data, and Spearman's rank correlation was used for correlation analyses. The significance threshold was set to 0.05. All analyses were performed using SPSS v15 (SPSS, Chicago, IL, USA).

4.5.1 Microarray data analyses

For Affymetrix gene expression measurements, preprocessing was performed using the Robust Multichip Average (RMA) method [179], using a global background correction and a quantile normalisation method. The expression values (one for each probeset) were produced with the aid of a linear model. Differential mRNA

expression was tested using a moderated *t*-statistic [180], and the raw *p*-values were corrected for multiple testing using the Benjamini-Hochberg method using the false discovery rate (FDR) [181]. The RMA method, the moderated *t*-test, and the FDR procedure are available via the Bioconductor Project (www.bioconductor.org).

For Illumina gene expression measurements, Illumina's BeadStudio Gene Expression software module v3.2.7 (Illumina Inc.) was used to subtract background and generate an output file for statistical analyses. Transcript data was preprocessed and analysed using Sequential Oligogenic Linkage Analysis Routines (SOLAR) [182], as described by Göring et al. [144]. To evaluate the magnitude of differential gene expression, the displacement of each detected transcript's mean expression value was measured between study groups, and a standard regression analysis was used to test whether the mean transcription levels differed between study groups.

4.5.2 Bioinformatic pathway analyses

The goal of microarray analyses is often to identify differentially expressed transcripts between e.g. diseased and healthy tissues. These can be selected based on *p*-values and/or magnitude of difference in expression levels (fold change). When such transcripts have been identified, the challenge remains in elucidating which biological functions the transcripts are involved in. Pathway analyses offer the advantage of evaluating expression *patterns* and identifying which pathways are over-represented in a given set of transcripts (either the total dataset or the list of differentially expressed transcripts). In the work included in this thesis, two different approaches were used to identify over-represented biological pathways involved in the pathogenesis of PE and FGR.

Ingenuity pathway analysis (IPA) v7.5 (Ingenuity Systems, Redwood City, CA, USA) was used to determine whether any pathways were over-represented among the lists

of differentially expressed transcripts (**papers II** and **III**). The significance of the association between the pathway and the dataset was calculated using Fisher's exact test. In **paper IV**, a more targeted pathway analysis approach was used, testing the involvement of a group of ER stress-related transcripts in the *total* dataset, using Rotation Gene Set Tests (ROAST) [183] and Rotation Gene Set Enrichment Analysis using rotation (ROMER) [184]. ROAST was used to test whether any of the transcripts in the ER stress pathway were differentially expressed, whereas ROMER was used to test whether the subset of transcripts in the pathway was more differentially expressed than any other subset of transcripts in the total dataset. For both methods, transcripts were ranked by moderated *t*-statistics, and a rotation test [185], which is a smoothed version of permutation suitable for linear models, was used to assess significance. Both methods are available via the Bioconductor Project.

4.5.3 Quantitative RT-PCR data analysis

For **paper I**, relative quantification of mRNA expression was calculated using the Generalized Estimating Equations (GEE) approach [186]. Differences in average cycle threshold values (ΔCt) between target and reference transcripts over technical triplicates for each sample were calculated. Statistical significance of differences between study groups was determined using an analysis of variance (ANOVA)-like model. The ANOVA method for GEE models is available via the Bioconductor Project. For **papers II** and **III**, relative quantification of mRNA expression levels was calculated using the comparative Ct method [187]. For each target transcript, the average Ct values over technical triplicates were calculated for each sample. Differences in ΔCt between the target and the reference transcript for each sample were used for statistical analyses.

5. MAIN FINDINGS

Paper I: "Whole-genome microarray and targeted analysis of angiogenesis-regulating gene expression (ENG, FLT1, VEGF, PlGF) in placentas from pre-eclamptic and small-for-gestational-age pregnancies"

Whole-genome microarray analysis of placental tissue did not reveal any significant differences in the transcriptome between pregnancies complicated by PE, FGR or PE+FGR, after a correction for multiple comparisons was performed. However, a targeted expression analysis of the angiogenesis-regulating factors ENG, FLT1, VEGF and PlGF by qRT-PCR demonstrated significantly higher placental expression of the anti-angiogenic factors ENG and FLT1 in PE+FGR compared to isolated PE or FGR. The expression of VEGF and PlGF did not differ between the case groups. In addition, a positive correlation of fold change values for ENG, FLT1, VEGF and PlGF obtained by microarray and qRT-PCR analysis was observed.

Paper II: "Matrix metalloproteinase 1 in pre-eclampsia and fetal growth restriction: reduced gene expression in decidual tissue and protein expression in extravillous trophoblasts"

Microarray analysis of decidual tissue revealed that 200 transcripts were differentially expressed between pregnancies complicated by PE and/or FGR and normal pregnancies. Altered expression of several transcripts involved in trophoblast differentiation and invasion was observed, including decreased expression of melanoma cell adhesion molecule (MCAM), integrin α5, zinc finger E-box binding homeobox 2 (ZEB2), MMP1, -7 and -12. Reduced decidual MMP1 mRNA expression was confirmed in all case groups by qRT-PCR analysis. Furthermore, the proportion of MMP1 positive EVTs was reduced in all case groups compared to controls.

Paper III: "A transcriptional profile of the decidua in preeclampsia"

Whole-genome microarray analysis of decidual tissue revealed that 455 transcripts were differentially expressed between pregnancies complicated by PE and PE+FGR and normal pregnancies. Pathway analysis of the differentially expressed transcripts identified seven significantly overrepresented pathways, including tryptophan metabolism, notch signalling, endoplasmic reticulum stress, nuclear respiratory factor 2 (NRF2) mediated oxidative stress response and pathways related to metabolism of fatty acids (linoleic acid metabolism, fatty acid metabolism and arachidonic acid metabolism).

Paper IV: "Increased endoplasmic reticulum stress in decidual tissue from pregnancies complicated by fetal growth restriction with and without pre-eclampsia"

Using pathway analyses on decidual tissue transcriptional profiles, we found an upregulation of the ER stress pathway in pregnancies complicated by FGR and PE+FGR, whereas in PE, only a trend towards upregulation was observed. Targeted transcriptional and protein analyses of the three ER stress signalling branches demonstrated an upregulation of the PERK-pEIF2α and ATF6 branches in FGR and PE+FGR. In PE, none of these branches were significantly altered, however a marked increase in XBP1(U) was observed. Both nuclear and cytoplasmic immunoreactivity for XBP1 and ATF6 was observed in most (>80%) EVTs, decidual cells and macrophages. No differences in the proportion of XBP1 or ATF6 immunopositive cells or staining pattern were observed between any of the study groups.

6. DISCUSSION

6.1 Methodological considerations

6.1.1 Diagnostic criteria and phenotype

Following hospital routines, proteinuria in the women enrolled with PE was diagnosed by a dipstick test or spot morning urine protein-to-creatinine ratio. This may have less diagnostic accuracy than measuring protein in a 24 hour sample, which is considered the gold standard for diagnosing proteinuria [188], but considerably time consuming and cumbersome. However, all of the women with pregnancy complications that were enrolled in our studies were hospitalised and went through a thorough monitoring by an obstetrician, including multiple blood pressure and proteinuria measurements, prenatal ultrasound measurements and fetal birthweight estimations. In addition, these women were delivered by CS due to maternal and/or fetal medical indications (indicating severe manifestation of disease), which clearly decreases the chances of false positive diagnosis. This also means that the enrolled women with PE and/or FGR would tend to have more severe disease than the overall group of patients delivering at the University Hospitals in Trondheim and Bergen. Thus, it may be argued that our data cannot be generalised to the more common, milder forms of PE and impaired fetal growth. However, as these severely affected patients represents the most clinically challenging group regarding both maternal and fetal health, research on these patients is encouraged. Also, when pregnancy complications are seen as a continuum ranging from normal pregnancy to severe disease, studies in the more extreme tails of the distribution might help clarify disease mechanisms, while still being representative for the whole population [189].

Both PE and FGR are heterogeneous pregnancy complications with a multitude of possible causal factors. It has been hypothesised that partly different pathogenic mechanisms may underlie the diverse clinical presentations, such as early versus late onset of PE, and PE with or without FGR [14,105]. Therefore, it has been suggested that these cases should be studied in phenotypically homogenous subgroups. However, with regards to transcriptional studies, this has to be weighted against the loss of power in statistical analyses that occurs when one compares relatively small study groups. In this work, various strategies for subgrouping has been chosen, which has to be taken into considerations when interpreting results. In **paper II**, cases with PE and/or FGR were analysed together, as the available sample size was too small for subgrouping. This was also the case in **paper III**, where cases with PE and PE+FGR were analysed together to provide more robustness against corrections for multiple testing. We are currently enrolling more women into our biobank to increase numbers and open for subgroup analyses in future studies.

6.1.2 Tissue sampling

Placental tissue

The collection of tissue samples for our biobank was originally designed for immunohistochemical studies, and a number of samples were collected from various locations of the placenta. Only one sample was prepared in accordance with protocols for RNA analysis (i.e. submerged in RNAlater), and the transcriptional analysis of the placenta was therefore restricted to a single placental biopsy. It can be discussed whether one piece of tissue is representative for the entire placenta. In retrospect, a more systematic collection and analysis of tissue from the entire placenta would have been preferred. However, the placental biopsy was collected from the same site for all study participants, and sampling from a standardised location reduces potential bias related to differences in gene expression depending on placental sampling site [190-191].

Decidual tissue

Decidua basalis tissue was collected by vacuum suction of the placental bed during CS [98,167]. This procedure ensures collection of decidual tissue from the entire placental bed, and the samples are more representative for the placental bed as a whole than the traditional placental bed biopsies [167].

6.1.3 Gestational age

From apparent reasons, sampling of decidual and placental tissue can only be performed at delivery. As women with pregnancies complicated by PE and FGR often have delivery induced before term due to maternal and/or fetal medical indications, studies comparing these to women with normal pregnancies are often biased by differences in gestational age. It has been shown that gene expression in utero-placental tissue is influenced by the length of gestation [192-193], which should be taken into consideration when interpreting results. With reference to these problems, some have chosen to use women with preterm labour as controls [152-153,157-158]. However, preterm labour may have a variety of causes, such as infections, placental ischeamia or vascular lesions [194], which could significantly influence gene expression and hamper the interpretation of results.

In **paper I**, the placental transcriptome was compared between cases with PE, FGR and PE+FGR. As no normal pregnancies (controls) were included, we were able to match these study groups for gestational age. In **papers II-IV**, we chose to use normal pregnancies with delivery at term as controls in the decidual transcriptional analyses, taking the existing alternatives and associated problems into consideration. Gestational age was therefore lower in cases than controls. Winn et al. compared gene expression in basal plate biopsies from normal pregnancies at mid-gestation (week 14–24) and term (week 37-40) [192], and found that 418 transcripts (of 39,000 transcripts examined) changed expression. This dataset has provided a useful

reference set to compare our results to, and we found that only a minimal number of transcripts were potentially affected by gestational age. However, the comparison is not straight forward, as the tissues used differ somewhat (basal plate biopsy versus decidual tissue). In addition, we have compared gene expression between early third trimester and term, and it is not known whether differences in gene expression in this period are comparable to those observed between mid-gestation and term. Given the nature of the problem at hand, we have tried our best to take gestational age into account when interpreting results.

Furthermore, PE and FGR represent the clinical endpoint of pathophysiological events probably initiated as early as in the first trimester. Due to apparent reasons, decidual and placental tissue cannot be sampled until delivery, and a considerable time lapse thus exists between implantation and tissue collection. It is therefore difficult to distinguish whether the observed changes in PE and/or FGR represent causes or consequences of these events.

6.1.4 Some problems related to transcriptional analyses

For PE and FGR, transcriptional analyses of placental and decidual tissue have identified dysregulation of a number of biological processes. However, the results of these studies have been inconsistent [146], perhaps reflecting that PE and FGR are heterogeneous conditions. In addition, there are several obvious differences between these previous studies. These include biological factors, such as differences in study group selection, sample size, inclusion of women of different ethnicities, and the use of tissue collected after both vaginal and CS delivery. It has previously been shown that labour affects placental gene expression [195-196], especially with regard to oxidative stress and inflammation [195]. In addition, cellular composition of tissue used for transcriptional analyses may vary according to sampling site, and influence gene expression [190-191]. Methodological factors, such as the use of different types

of microarray platforms and methods for data analysis, may also contribute to the diverging results. Depending on the method used, analysis of the same dataset may come out with different results. Millenaar et al. concluded that comparing six methods for microarray data analysis, only 27-36% overlap was observed in lists of differentially expressed genes generated by the alternative methods [197]. In our transcriptional analyses (**papers I-III**), two different methods for data analyses (RMA vs. SOLAR) were used. To make sure that these methods provided similar results, we reanalysed the dataset from **paper III** (analysed using SOLAR) with the RMA method used in **papers I** and **II**. This reanalysis demonstrated 84% overlap in the list of differentially expressed transcripts. This can be seen as a validation of the statistical methods used and increases our confidence in interpretation of the results.

When comparing expression of thousands of transcripts, a correction for multiple testing is necessary to control false positive results. However, such methods may prove too stringent and result in over-correction [198]. This was partly demonstrated in **paper I**, where no significant differences in placenta gene expression were detected by microarray analysis after FDR correction. Transcriptional analysis has been used to identify individual candidate transcripts, where an FDR significance threshold of 0.05 is commonly used [198]. This implies an expected false positive rate of 5% among the significant findings. However, it may be advantageous to focus on pathways and networks instead of single transcripts when the aim is to obtain insight in the pathophysiology of complex diseases such as PE and FGR. These methods offer the advantage of identifying *groups* of transcripts involved in a biological process associated with disease. If several transcripts in a biological pathway are co-regulated, this decreases the likelihood that they are false positives and a more relaxed FDR (for example 0.1) may be used to include a larger number of transcripts. This increases the chance to discover important pathways and networks in a dataset [199]. From a statistical

point if view, analysing groups of transcripts also increase power and reduce the dimensionality of the underlying statistical problem [199-200].

6.1.5 Global versus targeted analyses of gene expression

In **paper I**, we assumed that differences in gene expression between cases with PE, FGR and PE+FGR existed, although the microarray results suggested that this was not the case. We therefore extended our microarray analysis with qRT-PCR analysis on a selection of angiogenesis-regulating transcripts that were expected to be differentially regulated between these case groups based on previous findings [119,125,128]. Interestingly, transcripts that did not differ significantly between case groups in the microarray analysis did so in the qRT-PCR analysis. In addition, we observed a positive correlation of fold change values obtained by microarray and qRT-PCR, in accordance with previous reports [171,201-202], although fold change values were compressed in microarray compared to qRT-PCR results. This suggest that both technologies accurately predict the direction of change of mRNA expression (i.e. either up- or downregulation), but provide results that differ in magnitude. Based on this, we conclude that microarray analysis represents a good tool for exploring gene expression patterns and developing new hypotheses. However, when it comes to *targeted* comparison between expression levels of selected transcripts, qRT-PCR may be a more appropriate choice of method, as the need for correction for multiple testing is reduced.

6.2 Biological considerations – discussion of main findings

6.2.1 Transcriptional analysis of placental tissue

No differences in the placental transcriptome were observed between PE, FGR and PE+FGR in **paper I.** This was unexpected, considering that differences between these case groups with regards to placental morphology [133-135] and levels of oxidative stress and inflammation [95-96,141-142] has been reported. Changes in the placental transcriptome have previously been reported in PE or FGR compared to controls [146-148]. However, to the best of our knowledge, no direct global transcriptional comparisons have previously been performed *between* these case groups, which limit our basis for comparisons. Differences in gene expression between cases and healthy controls are probably more pronounced than changes between case groups, and thus more robust towards statistical correction for multiple testing, which may have caused negative results in our analysis (discussed in 6.1.4).

6.2.2 Anti-angiogenic factors in placental tissue

Using a targeted approach (qRT-PCR) for gene expression measurements, we found that cases with PE, FGR and PE+FGR differed with regard to placental production of anti-angiogenic factors (**paper I**). The placental expression of ENG and FLT1 was significantly higher in PE+FGR than in isolated PE or FGR (with a trend towards being higher in PE than FGR), whereas no differences for VEGF and PlGF were detected between these case groups. Similar to our findings, Jebalayan et al. reported that placental levels of ENG were slightly higher in PE+FGR than in isolated PE, and both these case groups had higher levels of ENG compared to FGR [129]. Increased placental levels of FLT were reported in PE compared to FGR [119,128], but in these studies, no discrimination between PE and PE+FGR was made. Our findings also correlate with maternal circulating levels of anti-angiogenic factors, reported as higher in PE than FGR [122,125,128-129], and highest in the most severe forms of

PE [117,121,126-127]. A similar gradient in severity of spiral artery impairment [63-64,67] and oxidative stress [96,138,141] has been observed between these case groups, PE+FGR being most and FGR least affected, with PE as an intermediate phenotype. This indicates that the biological phenomenona underlying isolated PE and FGR may be similar, but less pronounced when these pregnancy complications occur separately than in combination. However, the angiogenic imbalance does not appear to correlate with placental morphological alterations pertaining to impaired villous and capillary surface area, which have been reported as altered in PE+FGR and FGR, but not in isolated PE [133-135].

6.2.3 Trophoblast differentiation and invasion

In **paper II**, we demonstrated altered decidual expression of several transcripts involved in trophoblast differentiation and invasion in PE and/or FGR, including decreased expression of MCAM and integrin $\alpha5$. This is in accordance with previous findings of failed upregulation of MCAM [203] and integrin $\alpha5/\beta3$ [73] in trophoblasts from pre-eclamptic pregnancies. A decreased expression of ZEB2, a transcriptional repressor of E-cadherin [204] was also observed. E-cadherin is normally downregulated as trophoblasts differentiate from an invasive to a proliferative phenotype, but is retained in trophoblasts from pre-eclamptic pregnancies [73]. Moreover, ZEB2 promotes EMT, a process analogous to trophoblast differentiation [205]. Decreased expression of ZEB2 may therefore interfere with the invasive capacity of EVTs, suggesting a potential role for ZEB2 in the pathogenesis of PE and FGR. In addition, the ZEB2 gene is located at a previously identified PE susceptibility locus, 2q22.3 [145], and may be a candidate gene for PE, associated with increased susceptibility to impaired trophoblast invasiveness.

Previous studies have shown that insufficient trophoblast invasion in PE and FGR is linked to reduced MMP production in EVTs [77-79]. Correspondingly, we found decreased MMP1 levels in EVTs from pregnancies with PE and/or FGR. Decreased levels of MMP1 has previously been reported in decidual endothelial cells [78,206] and umbilical cord blood [207] from pre-eclamptic pregnancies, but to our knowledge, our study is the first to demonstrate decreased MMP1 in EVTs from these cases. MMP1 is detected in trophoblasts throughout gestation in normal pregnancy. However, expression of MMP1 increases in the deeper invasive stages when the EVTs face the decidual tissue [208]. Here, it becomes more functionally relevant, as interstitial collagen (the main substrate for MMP1) is abundant in decidual tissue [208]. Recently, reduced placental MMP1 expression was reported in a rat model of placental insufficiency [209], and it has been shown that MMP1 expression is crucial for the migratory capacity of mesenchymal stem cells [210]. We also observed a reduced decidual gene expression of MMP12, in accordance with a previous study demonstrating reduced expression of MMP12 in first trimester chorionic villi from women who developed PE later in pregnancy [151]. Moreover, Harris et al. demonstrated that first trimester EVTs express MMP12, and inhibition of this enzyme reduced the proteolytic capacity of these cells [211]. Taken together, these results support previous findings of an association between impaired trophoblast invasion and altered CAM and MMP expression.

6.2.4 Altered biological pathways in the pathogenesis of PE

In **paper III**, we found that perturbations of seven biological pathways were associated with PE. One of these, tryptophan metabolism, is important for promoting immune tolerance at the maternal-fetal interface. IDO, a tryptophan catabolising enzyme, has been shown to prevent maternal anti-fetal T cell responses in a murine model [212]. This is probably mediated via tryptophan depletion, as decreased tryptophan levels lead to suppression of cytotoxic T cell responses and

maternal-fetal immunological tolerance [83-85,212]. An altered tryptophan metabolism has previously been implicated in the pathogenesis of PE, where decreased levels and activity of IDO and increased levels of tryptophan has been observed [82,86-87]. Other tryptophan catabolites, such as L-kynurenine, are also important for suppressing immune cells [213]. Correspondingly, decreased levels of L-kynurenine have been observed in PE [87], and we observed an increased expression of kynureninase (KYNU), an enzyme catabolising L-kynurenine. Our findings support the role of altered tryptophan metabolism in PE, and we propose that increased levels of KYNU at the maternal-fetal interface could contribute to decreased levels of L-kynurenine, which may impair the necessary immune modulation during pregnancy in PE.

The second most significant pathway in **paper III** was ER stress, which included upregulation of key mediators from each of the UPR branches; ATF6, XBP1 and PERK. Increased placental ER stress has previously been demonstrated in pregnancies complicated by PE+FGR and FGR [106], but the findings are novel with regard to decidual tissue in these pregnancy complications. Polymorphisms in the genes coding for the ER-resident proteins selenoprotein S (SEPS1) and endoplasmic reticulum aminopeptidase 2 (ERAP2), involved in processing and folding of misfolded proteins, have been associated with increased susceptibility to for PE in a Norwegian population [214-215].

The pathway analysis in **paper III** also showed over-representation of ER stress-associated pathways, including NRF2-mediated oxidative stress response and three pathways related to lipid metabolism. The transcription factor NFR2 can be activated downstream to PERK under ER stress [216], and plays an essential role in oxidative stress defence by initiating transcription of antioxidant enzymes like glutathione s-transferase (GST) and heme oxygenase 1 (HMOX1) [217]. The overall

observed upregulation of transcripts within the NRF2 pathway (including GSTA3 and HMOX1) indicates a defensive response to oxidative stress in decidual tissue. Increased decidual oxidative stress has been demonstrated in PE [97-99], and may lead to peroxidation of the atherotic lesions (characterised by lipid depositions) observed within the uterine spiral arteries in PE [101]. Correspondingly, increased decidual levels of lipid peroxides have been observed in PE [98]. Moreover, ER stress has been identified as a key mechanism in progression of atherosclerosis [110] and may also promote acute atherosis, which show similar morphological features to that of atherosclerosis [218]. In addition, the observed alterations of three lipid metabolism pathways in our dataset are consistent with previous findings of increased decidual contents of phospholipids and cholesterol [98] in PE.

The relationship between ER stress and oxidative stress is intricate, as I/R can induce both [91,103], and ER stress can lead to increased oxidative stress and vice versa [107]. A complex relationship also exists between ER stress and altered lipid/cholesterol metabolism, as ER stress can be activated by increased cellular cholesterol load [219], and ER stress can activate expression of genes involved in cholesterol biosynthesis [220]. Thus, it is difficult to separate cause and effect with regard to ER stress, oxidative stress and altered lipid metabolism. The UPR signalling branches intersect with a variety of inflammatory and oxidative stress signalling networks, all of which can influence metabolism [221]. Not surprisingly, these mechanisms are associated with conditions with a vascular or inflammatory character such as obesity, diabetes, and atheroclerosis [222]. The pathway analyses of our microarray dataset suggest an integrated role of decidual ER stress, oxidative stress, and altered lipid metabolism in the pathogenesis of PE.

6.2.5 ER stress in PE and FGR

In **paper IV**, we elaborated the findings on ER stress' involvement in PE from **paper III**, and showed that the ER stress pathway was upregulated in PE+FGR and FGR, but not in isolated PE. These findings were further supported by protein analyses of factors from each of the three UPR branches, where increased levels of ATF6 and pEIF2α were found in these case groups, but not in isolated PE. Similar to our results, Yung et al. demonstrated increased placental levels of pEIF2α in PE+FGR and FGR [106], and proposed ER stress as a mechanism causing the small placental phenotype observed in PE+FGR and FGR [105-106]. Our data support their hypothesis, as we observed a negative correlation between placental weight and protein levels of ER stress markers from the PERK-pEIF2α and ATF6 signalling branches of the UPR.

In PE, a marked increase of XBP1(U) was observed. It was recently proposed that XBP1(U) functions as a negative regulator of ER stress, by inhibiting XBP1(S) and mediating degradation of ATF6 [223-225]. It has been shown that PERK-pEIF2α and IRE1-XBP1 signalling may have opposite effects on cell viability *in vitro*, as PERK-pEIF2α signalling leads to decreased cellular proliferation and apoptosis, but sustained IRE1-XBP1 signalling enhances cellular proliferation without promoting cell death [226]. In PE, the increased levels of XBP1(U) may protect against or decrease the harmful effects of ER stress, which could be reflected by the normal placental morphology that has been reported in these cases [133-135]. Taken together, the results from **paper IV** thus indicate divergent activation of UPR signalling branches in FGR and PE+FGR compared to isolated PE, and that ER stress is greater in PE+FGR and FGR than in isolated PE.

Immunohistochemical analyses of ER stress markers in EVTs, decidual cells, and macrophages demonstrated no differences in the proportion of XBP1 or ATF6

immunopositive cells or staining pattern between the study groups. Most cells in the decidual tissue, even in that from normal pregnancies, showed nuclear localisation of ATF6 and XBP1, indicating activation of ER stress. This may not be that surprising, considering that ER stress is a general cellular homeostatic response, and that I/R insults and oxidative stress also occurs during normal pregnancy [138], probably caused by spontaneous uterine contractions occurring in third trimester (Braxton Hicks contractions) [227].

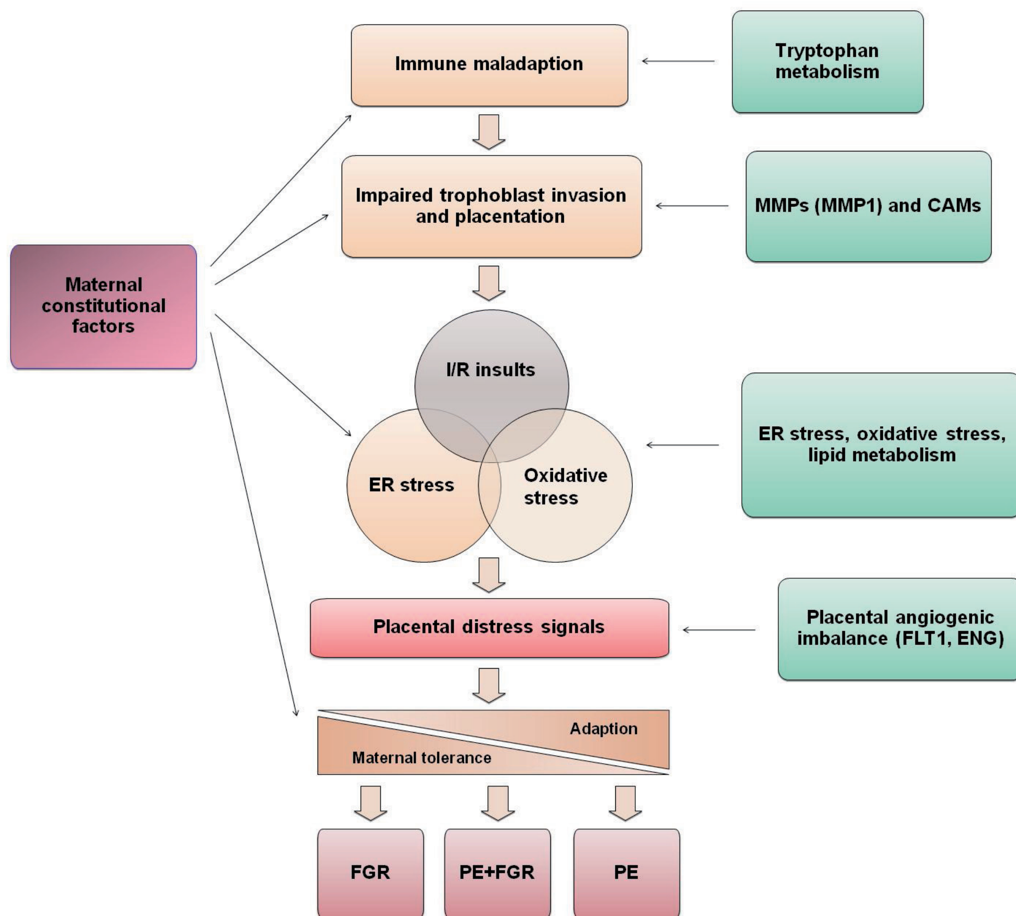

Figure 6. Summary of main findings of this thesis in relation to the proposed model for the pathogenesis of PE and FGR.

7. CONCLUSIONS AND FUTURE PERSPECTIVES

PE and FGR are among the most important causes of maternal and perinatal morbidity and mortality worldwide. Advances in the understanding of pathophysiological mechanisms in PE and FGR can guide preventive and therapeutic strategies to improve maternal and fetal health. The work included in this thesis has shown that reduced trophoblast invasion in PE and/or FGR may be associated with decreased trophoblast expression of MMPs, and that immunological perturbations in PE and/or FGR can be linked to disturbed metabolism of tryptophan. We have also confirmed that utero-placental tissue from pregnancies complicated by PE and/or FGR is characterised by increased levels of oxidative stress, ER stress, and an anti-angiogenic state. One interesting finding in our studies, the decidual downregulation of ZEB2, located at a previously identified PE susceptibility locus, warrants further investigation to determine whether polymorphisms or other variations within this gene is associated with a genetic susceptibility to impaired trophoblast inavasion. In addition, it would be interesting to determine whether high levels of XBP1(U) can protect cells against ER stress.

Some have proposed that PE and FGR may result from different pathogenic mechanisms. However, the work presented in this thesis (and by others) indicates that it does not seem to be a fundamental difference in *which* mechanisms that are involved in PE or FGR, but rather to what *degree* they are altered. Emerging data also indicate that many of the major pathophysiological components of PE and FGR, such as impaired trophoblast invasion, oxidative stress, ER stress, and an angiogenic imbalance are features of several, if not most other obstetrical syndromes. This may imply that the body has a limited reaction pattern to damage or stress, and that future therapeutic strategies aimed at disturbances in these processes could be applied to a broad range of pregnancy complications.

Over the last decades, it has become apparent that PE and FGR are not homogenous conditions with single causes. Rather, they are very heterogeneous syndromes of multifactorial origin, where different contributions of maternal, fetal and placental factors can lead to clinical disease in different women, demonstrating that if not all, then at least many roads lead to Rome. To elucidate mechanisms involved in development of PE and FGR, dissecting these phenotypes into specific subgroups which may have more homogenous aetiologies (be it according to onset or severity of disease etc.) is necessary, and in doing so, large study groups are needed to overcome problems in statistical analyses pertaining to large phenotypic variations. With regards to future prediction and therapeutic strategies, it is unlikely to find a single prognostic or therapeutic measure [228], and prophylactic strategies will need to target to the needs of different women.

8. REFERENCES

1. Chesley LC. History and epidemiology of preeclampsia-eclampsia. Clin Obstet Gynecol 1984;27:801-20.
2. Altman D, Carroli G, Duley L, Farrell B, Moodley J, Neilson J, et al. Do women with pre-eclampsia, and their babies, benefit from magnesium sulphate? The Magpie Trial: a randomised placebo-controlled trial. Lancet 2002;359:1877-90.
3. Basso O, Rasmussen S, Weinberg CR, Wilcox AJ, Irgens LM, Skjaerven R. Trends in fetal and infant survival following preeclampsia. JAMA 2006;296:1357-62.
4. Khan KS, Wojdyla D, Say L, Gulmezoglu AM, Van Look PF. WHO analysis of causes of maternal death: a systematic review. Lancet 2006;367:1066-74.
5. Geographic variation in the incidence of hypertension in pregnancy. World Health Organization International Collaborative Study of Hypertensive Disorders of Pregnancy. Am J Obstet Gynecol 1988;158:80-3.
6. Saftlas AF, Olson DR, Franks AL, Atrash HK, Pokras R. Epidemiology of preeclampsia and eclampsia in the United States, 1979-1986. Am J Obstet Gynecol 1990;163:460-5.
7. Sibai BM, Caritis SN, Thom E, Klebanoff M, McNellis D, Rocco L, et al. Prevention of preeclampsia with low-dose aspirin in healthy, nulliparous pregnant women. The National Institute of Child Health and Human Development Network of Maternal-Fetal Medicine Units. N Engl J Med 1993;329:1213-8.
8. Wallis AB, Saftlas AF, Hsia J, Atrash HK. Secular trends in the rates of preeclampsia, eclampsia, and gestational hypertension, United States, 1987-2004. Am J Hypertens 2008;21:521-6.
9. Dahlstrøm BL, Engh ME, Bukholm G, Øian P. Changes in the prevalence of pre-eclampsia in Akershus County and the rest of Norway during the past 35 years. Acta Obstet Gynecol Scand 2006;85:916-21.
10. Bodnar LM, Ness RB, Markovic N, Roberts JM. The risk of preeclampsia rises with increasing prepregnancy body mass index. Ann Epidemiol 2005;15:475-82.
11. Berg CJ, Mackay AP, Qin C, Callaghan WM. Overview of maternal morbidity during hospitalization for labor and delivery in the United States: 1993-1997 and 2001-2005. Obstet Gynecol 2009;113:1075-81.
12. Young BC, Levine RJ, Karumanchi SA. Pathogenesis of preeclampsia. Annu Rev Pathol 2010;5:173-92.
13. Vatten LJ, Skjærven R. Is pre-eclampsia more than one disease? BJOG 2004;111:298-302.
14. Huppertz B. Placental origins of preeclampsia: challenging the current hypothesis. Hypertension 2008;51:970-5.
15. Walker JJ. Pre-eclampsia. Lancet 2000;356:1260-5.
16. Ødegård RA, Vatten LJ, Nilsen ST, Salvesen KA, Austgulen R. Preeclampsia and fetal growth. Obstet Gynecol 2000;96:950-5.
17. Cnattingius S, Mills JL, Yuen J, Eriksson O, Salonen H. The paradoxical effect of smoking in preeclamptic pregnancies: smoking reduces the incidence but increases the rates of perinatal mortality, abruptio placentae, and intrauterine growth restriction. Am J Obstet Gynecol 1997;177:156-61.

18. Lin CC, Santolaya-Forgas J. Current concepts of fetal growth restriction: part II. Diagnosis and management. Obstet Gynecol 1999;93:140-6.
19. Kramer MS, Olivier M, McLean FH, Willis DM, Usher RH. Impact of intrauterine growth retardation and body proportionality on fetal and neonatal outcome. Pediatrics 1990;86:707-13.
20. Cnattingius S, Haglund B, Kramer MS. Differences in late fetal death rates in association with determinants of small for gestational age fetuses: population based cohort study. BMJ 1998;316:1483-7.
21. Brodsky D, Christou H. Current concepts in intrauterine growth restriction. J Intensive Care Med 2004;19:307-19.
22. Cox P, Marton T. Pathological assessment of intrauterine growth restriction. Best Pract Res Clin Obstet Gynaecol 2009;23:751-64.
23. Lee PA, Chernausek SD, Hokken-Koelega AC, Czernichow P. International Small for Gestational Age Advisory Board consensus development conference statement: management of short children born small for gestational age, April 24-October 1, 2001. Pediatrics 2003;111:1253-61.
24. ACOG practice bulletin 'Intrauterine growth restriction'. No. 12. Clinical management guidelines for obstetrician-gynecologists. Int J Gynecol Obstet 2001;72: 85-96.
25. Gardosi J, Francis A. Controlled trial of fundal height measurement plotted on customised antenatal growth charts. Br J Obstet Gynaecol 1999;106:309-17.
26. Platz E, Newman R. Diagnosis of IUGR: traditional biometry. Semin Perinatol 2008;32:140-7.
27. Karsdorp VH, van Vugt JM, van Geijn HP, Kostense PJ, Arduini D, Montenegro N, et al. Clinical significance of absent or reversed end diastolic velocity waveforms in umbilical artery. Lancet 1994;344:1664-8.
28. Maršál K. Obstetric management of intrauterine growth restriction. Best Pract Res Clin Obstet Gynaecol 2009;23:857-70.
29. Lie RT, Rasmussen S, Brunborg H, Gjessing HK, Lie-Nielsen E, Irgens LM. Fetal and maternal contributions to risk of pre-eclampsia: population based study. BMJ 1998;316:1343-7.
30. Esplin MS, Fausett MB, Fraser A, Kerber R, Mineau G, Carrillo J, et al. Paternal and maternal components of the predisposition to preeclampsia. N Engl J Med 2001;344:867-72.
31. Skjærven R, Vatten LJ, Wilcox AJ, Rønning T, Irgens LM, Lie RT. Recurrence of pre-eclampsia across generations: exploring fetal and maternal genetic components in a population based cohort. BMJ 2005;331:877.
32. Ødegård RA, Vatten LJ, Nilsen ST, Salvesen KA, Austgulen R. Risk factors and clinical manifestations of pre-eclampsia. BJOG 2000;107:1410-6.
33. Cnattingius S, Reilly M, Pawitan Y, Lichtenstein P. Maternal and fetal genetic factors account for most of familial aggregation of preeclampsia: a population-based Swedish cohort study. Am J Med Genet A 2004;130A:365-71.
34. Villar J, Carroli G, Wojdyla D, Abalos E, Giordano D, Ba'aqeel H, et al. Preeclampsia, gestational hypertension and intrauterine growth restriction, related or independent conditions? Am J Obstet Gynecol 2006;194:921-31.

35. Magnussen EB, Vatten LJ, Lund-Nilsen TI, Salvesen KA, Davey Smith G, Romundstad PR. Prepregnancy cardiovascular risk factors as predictors of pre-eclampsia: population based cohort study. BMJ 2007;335:978.
36. Yogev Y, Melamed N, Bardin R, Tenenbaum-Gavish K, Ben-Shitrit G, Ben-Haroush A. Pregnancy outcome at extremely advanced maternal age. Am J Obstet Gynecol 2010;203:558 e1-7.
37. Svensson AC, Pawitan Y, Cnattingius S, Reilly M, Lichtenstein P. Familial aggregation of small-for-gestational-age births: the importance of fetal genetic effects. Am J Obstet Gynecol 2006;194:475-9.
38. Yunis KA, Beydoun H, Tamim H, Nassif Y, Khogali M. Risk factors for term or near-term fetal growth restriction in the absence of maternal complications. Am J Perinatol 2004;21:227-34.
39. Tveit JV, Saastad E, Stray-Pedersen B, Børdahl PE, Frøen JF. Concerns for decreased foetal movements in uncomplicated pregnancies - increased risk of foetal growth restriction and stillbirth among women being overweight, advanced age or smoking. J Matern Fetal Neonatal Med 2010;23:1129-35.
40. Vedmedovska N, Rezeberga D, Teibe U, Zodzika J, Donders GG. Preventable maternal risk factors and association of genital infection with fetal growth restriction. Gynecol Obstet Invest 2010;70:291-8.
41. Odibo AO, Nelson D, Stamilio DM, Sehdev HM, Macones GA. Advanced maternal age is an independent risk factor for intrauterine growth restriction. Am J Perinatol 2006;23:325-8.
42. Wolf M, Sandler L, Muñoz K, Hsu K, Ecker JL, Thadhani R. First trimester insulin resistance and subsequent preeclampsia: a prospective study. J Clin Endocrinol Metab 2002;87:1563-8.
43. Czeizel AE, Bánhidy F. Chronic hypertension in pregnancy. Curr Opin Obstet Gynecol 2011;23:76-81.
44. Manten GT, Sikkema MJ, Voorbij HA, Visser GH, Bruinse HW, Franx A. Risk factors for cardiovascular disease in women with a history of pregnancy complicated by preeclampsia or intrauterine growth restriction. Hypertens Pregnancy 2007;26:39-50.
45. Berends AL, de Groot CJ, Sijbrands EJ, Sie MP, Benneheij SH, Pal R, et al. Shared constitutional risks for maternal vascular-related pregnancy complications and future cardiovascular disease. Hypertension 2008;51:1034-41.
46. Magnussen EB, Vatten LJ, Smith GD, Romundstad PR. Hypertensive disorders in pregnancy and subsequently measured cardiovascular risk factors. Obstet Gynecol 2009;114:961-70.
47. Romundstad PR, Magnussen EB, Smith GD, Vatten LJ. Hypertension in pregnancy and later cardiovascular risk: common antecedents? Circulation 2010;122:579-84.
48. Smith GC, Pell JP, Walsh D. Pregnancy complications and maternal risk of ischaemic heart disease: a retrospective cohort study of 129,290 births. Lancet 2001;357:2002-6.
49. Irgens HU, Reisæter L, Irgens LM, Lie RT. Long term mortality of mothers and fathers after pre-eclampsia: population based cohort study. BMJ 2001;323:1213-7.

50. McDonald SD, Malinowski A, Zhou Q, Yusuf S, Devereaux PJ. Cardiovascular sequelae of preeclampsia/eclampsia: a systematic review and meta-analyses. Am Heart J 2008;156:918-30.
51. Sattar N, Greer IA. Pregnancy complications and maternal cardiovascular risk: opportunities for intervention and screening? BMJ 2002;325:157-60.
52. Williams D. Pregnancy: a stress test for life. Curr Opin Obstet Gynecol 2003;15:465-71.
53. Craici I, Wagner S, Garovic VD. Preeclampsia and future cardiovascular risk: formal risk factor or failed stress test? Ther Adv Cardiovasc Dis 2008;2:249-59.
54. Johansson Å, Curran JE, Johnson MP, Freed KA, Fenstad MH, Bjørge L, et al. Identification of ACOX2 as a shared genetic risk factor for preeclampsia and cardiovascular disease. Eur J Hum Genet 2011;19:796-800.
55. Rasmussen S, Irgens LM. History of fetal growth restriction is more strongly associated with severe rather than milder pregnancy-induced hypertension. Hypertension 2008;51:1231-8.
56. Lykke JA, Paidas MJ, Langhoff-Roos J. Recurring complications in second pregnancy. Obstet Gynecol 2009;113:1217-24.
57. Wikström AK, Stephansson O, Cnattingius S. Previous preeclampsia and risks of adverse outcomes in subsequent nonpreeclamptic pregnancies. Am J Obstet Gynecol 2011;204:148 e1-6.
58. Redman CW. Current topic: pre-eclampsia and the placenta. Placenta 1991;12:301-8.
59. Redman CW, Sargent IL. Latest advances in understanding preeclampsia. Science 2005;308:1592-4.
60. Cindrova-Davies T. Gabor Than Award Lecture 2008: pre-eclampsia - from placental oxidative stress to maternal endothelial dysfunction. Placenta 2009;30 Suppl A:S55-65.
61. Brosens I, Robertson WB, Dixon HG. The physiological response of the vessels of the placental bed to normal pregnancy. J Pathol Bacteriol 1967;93:569-79.
62. Brosens IA, Robertson WB, Dixon HG. The role of the spiral arteries in the pathogenesis of preeclampsia. Obstet Gynecol Annu 1972;1:177-91.
63. Khong TY, De Wolf F, Robertson WB, Brosens I. Inadequate maternal vascular response to placentation in pregnancies complicated by pre-eclampsia and by small-for-gestational age infants. Br J Obstet Gynaecol 1986;93:1049-59.
64. Brosens I, Dixon HG, Robertson WB. Fetal growth retardation and the arteries of the placental bed. Br J Obstet Gynaecol 1977;84:656-63.
65. Brosens IA. Morphological changes in the utero-placental bed in pregnancy hypertension. Clin Obstet Gynaecol 1977;4:573-93.
66. Kim YM, Bujold E, Chaiworapongsa T, Gomez R, Yoon BH, Thaler HT, et al. Failure of physiologic transformation of the spiral arteries in patients with preterm labor and intact membranes. Am J Obstet Gynecol 2003;189:1063-9.
67. Madazli R, Somunkiran A, Calay Z, Ilvan S, Aksu MF. Histomorphology of the placenta and the placental bed of growth restricted foetuses and correlation with the Doppler velocimetries of the uterine and umbilical arteries. Placenta 2003;24:510-6.

68. Lyall F. Mechanisms regulating cytotrophoblast invasion in normal pregnancy and pre-eclampsia. Aust N Z J Obstet Gynaecol 2006;46:266-73.
69. Pijnenborg R, Vercruysse L, Hanssens M. The uterine spiral arteries in human pregnancy: facts and controversies. Placenta 2006;27:939-58.
70. Harris LK, Jones CJ, Aplin JD. Adhesion molecules in human trophoblast - a review. II. extravillous trophoblast. Placenta 2009;30:299-304.
71. Kokkinos MI, Murthi P, Wafai R, Thompson EW, Newgreen DF. Cadherins in the human placenta - epithelial-mesenchymal transition (EMT) and placental development. Placenta 2010;31:747-55.
72. Zhou Y, Damsky CH, Chiu K, Roberts JM, Fisher SJ. Preeclampsia is associated with abnormal expression of adhesion molecules by invasive cytotrophoblasts. J Clin Invest 1993;91:950-60.
73. Zhou Y, Damsky CH, Fisher SJ. Preeclampsia is associated with failure of human cytotrophoblasts to mimic a vascular adhesion phenotype. One cause of defective endovascular invasion in this syndrome? J Clin Invest 1997;99:2152-64.
74. Zygmunt M, Boving B, Wienhard J, Münstedt K, Braems G, Bohle RM, et al. Expression of cell adhesion molecules in the extravillous trophoblast is altered in IUGR. Am J Reprod Immunol 1997;38:295-301.
75. Fisher SJ, Cui TY, Zhang L, Hartman L, Grahl K, Zhang GY, et al. Adhesive and degradative properties of human placental cytotrophoblast cells in vitro. J Cell Biol 1989;109:891-902.
76. Librach CL, Werb Z, Fitzgerald ML, Chiu K, Corwin NM, Esteves RA, et al. 92-kD type IV collagenase mediates invasion of human cytotrophoblasts. J Cell Biol 1991;113:437-49.
77. Lim KH, Zhou Y, Janatpour M, McMaster M, Bass K, Chun SH, et al. Human cytotrophoblast differentiation/invasion is abnormal in pre-eclampsia. Am J Pathol 1997;151:1809-18.
78. Campbell S, Rowe J, Jackson CJ, Gallery ED. Interaction of cocultured decidual endothelial cells and cytotrophoblasts in preeclampsia. Biol Reprod 2004;71:244-52.
79. Reister F, Kingdom JC, Ruck P, Marzusch K, Heyl W, Pauer U, et al. Altered protease expression by periarterial trophoblast cells in severe early-onset preeclampsia with IUGR. J Perinat Med 2006;34:272-9.
80. Bulmer JN. Immune aspects of pathology of the placental bed contributing to pregnancy pathology. Baillière's Clin Obstet Gynaecol 1992;6:461-88.
81. Dekker GA, Sibai BM. The immunology of preeclampsia. Semin Perinatol 1999;23:24-33.
82. Nishizawa H, Hasegawa K, Suzuki M, Kamoshida S, Kato T, Saito K, et al. The etiological role of allogeneic fetal rejection in pre-eclampsia. Am J Reprod Immunol 2007;58:11-20.
83. Lee GK, Park HJ, Macleod M, Chandler P, Munn DH, Mellor AL. Tryptophan deprivation sensitizes activated T cells to apoptosis prior to cell division. Immunology 2002;107:452-60.
84. Mellor AL, Keskin DB, Johnson T, Chandler P, Munn DH. Cells expressing indoleamine 2,3-dioxygenase inhibit T cell responses. J Immunol 2002;168:3771-6.

85. Mellor AL, Munn DH. Tryptophan catabolism prevents maternal T cells from activating lethal anti-fetal immune responses. J Reprod Immunol 2001;52:5-13.
86. Santoso DI, Rogers P, Wallace EM, Manuelpillai U, Walker D, Subakir SB. Localization of indoleamine 2,3-dioxygenase and 4-hydroxynonenal in normal and pre-eclamptic placentae. Placenta 2002;23:373-9.
87. Kudo Y, Boyd CA, Sargent IL, Redman CW. Decreased tryptophan catabolism by placental indoleamine 2,3-dioxygenase in preeclampsia. Am J Obstet Gynecol 2003;188:719-26.
88. Meekins JW, Pijnenborg R, Hanssens M, McFadyen IR, van Asshe A. A study of placental bed spiral arteries and trophoblast invasion in normal and severe pre-eclamptic pregnancies. Br J Obstet Gynaecol 1994;101:669-74.
89. Brosens I, Pijnenborg R, Vercruysse L, Romero R. The "Great Obstetrical Syndromes" are associated with disorders of deep placentation. Am J Obstet Gynecol 2011;204:193-201.
90. Khong Y, Brosens I. Defective deep placentation. Best Pract Res Clin Obstet Gynaecol 2011;25:301-11.
91. Hung TH, Skepper JN, Burton GJ. In vitro ischemia-reperfusion injury in term human placenta as a model for oxidative stress in pathological pregnancies. Am J Pathol 2001;159:1031-43.
92. Hung TH, Burton GJ. Hypoxia and reoxygenation: a possible mechanism for placental oxidative stress in preeclampsia. Taiwan J Obstet Gynecol 2006;45:189-200.
93. Olofsson P, Laurini RN, Marsál K. A high uterine artery pulsatility index reflects a defective development of placental bed spiral arteries in pregnancies complicated by hypertension and fetal growth retardation. Eur J Obstet Gynecol Reprod Biol 1993;49:161-8.
94. Hubel CA. Oxidative stress in the pathogenesis of preeclampsia. Proc Soc Exp Biol Med 1999;222:222-35.
95. Takagi Y, Nikaido T, Toki T, Kita N, Kanai M, Ashida T, et al. Levels of oxidative stress and redox-related molecules in the placenta in preeclampsia and fetal growth restriction. Virchows Arch 2004;444:49-55.
96. Fujimaki A, Watanabe K, Mori T, Kimura C, Shinohara K, Wakatsuki A. Placental oxidative DNA damage and its repair in preeclamptic women with fetal growth restriction. Placenta 2011;32:367-72.
97. Staff AC, Halvorsen B, Ranheim T, Henriksen T. Elevated level of free 8-iso-prostaglandin F2α in the decidua basalis of women with preeclampsia. Am J Obstet Gynecol 1999;181:1211-5.
98. Staff AC, Ranheim T, Khoury J, Henriksen T. Increased contents of phospholipids, cholesterol, and lipid peroxides in decidua basalis in women with preeclampsia. Am J Obstet Gynecol 1999;180:587-92.
99. Zusterzeel PL, Rütten H, Roelofs HM, Peters WH, Steegers EA. Protein carbonyls in decidua and placenta of pre-eclamptic women as markers for oxidative stress. Placenta 2001;22:213-9.

100. Eide IP, Isaksen CV, Salvesen KA, Langaas M, Schønberg SA, Austgulen R. Decidual expression and maternal serum levels of heme oxygenase 1 are increased in pre-eclampsia. Acta Obstet Gynecol Scand 2008;87:272-9.
101. Khong TY. Acute atherosis in pregnancies complicated by hypertension, small-for-gestational-age infants, and diabetes mellitus. Arch Pathol Lab Med 1991;115:722-5.
102. DeGracia DJ, Montie HL. Cerebral ischemia and the unfolded protein response. J Neurochem 2004;91:1-8.
103. Yung HW, Korolchuk S, Tolkovsky AM, Charnock-Jones DS, Burton GJ. Endoplasmic reticulum stress exacerbates ischemia-reperfusion-induced apoptosis through attenuation of Akt protein synthesis in human choriocarcinoma cells. FASEB J 2007;21:872-84.
104. Kaufman RJ, Scheuner D, Schröder M, Shen X, Lee K, Liu CY, et al. The unfolded protein response in nutrient sensing and differentiation. Nat Rev Mol Cell Biol 2002;3:411-21.
105. Burton GJ, Yung HW, Cindrova-Davies T, Charnock-Jones DS. Placental endoplasmic reticulum stress and oxidative stress in the pathophysiology of unexplained intrauterine growth restriction and early onset preeclampsia. Placenta 2009;30 Suppl A:S43-8.
106. Yung HW, Calabrese S, Hynx D, Hemmings BA, Cetin I, Charnock-Jones DS, et al. Evidence of placental translation inhibition and endoplasmic reticulum stress in the etiology of human intrauterine growth restriction. Am J Pathol 2008;173:451-62.
107. Austin RC. The unfolded protein response in health and disease. Antioxid Redox Signal 2009;11:2279-87.
108. Zhang K, Kaufman RJ. From endoplasmic-reticulum stress to the inflammatory response. Nature 2008;454:455-62.
109. Yoshida H. ER stress and diseases. FEBS J 2007;274:630-58.
110. Tabas I. The role of endoplasmic reticulum stress in the progression of atherosclerosis. Circ Res 2010;107:839-50.
111. Charnock-Jones DS, Kaufmann P, Mayhew TM. Aspects of human fetoplacental vasculogenesis and angiogenesis. I. Molecular regulation. Placenta 2004;25:103-13.
112. Torry DS, Mukherjea D, Arroyo J, Torry RJ. Expression and function of placenta growth factor: implications for abnormal placentation. J Soc Gynecol Investig 2003;10:178-88.
113. Zhu X, Wu S, Dahut WL, Parikh CR. Risks of proteinuria and hypertension with bevacizumab, an antibody against vascular endothelial growth factor: systematic review and meta-analysis. Am J Kidney Dis 2007;49:186-93.
114. Clark DE, Smith SK, He Y, Day KA, Licence DR, Corps AN, et al. A vascular endothelial growth factor antagonist is produced by the human placenta and released into the maternal circulation. Biol Reprod 1998;59:1540-8.
115. Kendall RL, Thomas KA. Inhibition of vascular endothelial cell growth factor activity by an endogenously encoded soluble receptor. Proc Natl Acad Sci U S A 1993;90:10705-9.
116. Maynard SE, Min JY, Merchan J, Lim KH, Li J, Mondal S, et al. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. J Clin Invest 2003;111:649-58.

117. Venkatesha S, Toporsian M, Lam C, Hanai J, Mammoto T, Kim YM, et al. Soluble endoglin contributes to the pathogenesis of preeclampsia. Nat Med 2006;12:642-9.
118. Maynard S, Epstein FH, Karumanchi SA. Preeclampsia and angiogenic imbalance. Annu Rev Med 2008;59:61-78.
119. Shibata E, Rajakumar A, Powers RW, Larkin RW, Gilmour C, Bodnar LM, et al. Soluble fms-like tyrosine kinase 1 is increased in preeclampsia but not in normotensive pregnancies with small-for-gestational-age neonates: relationship to circulating placental growth factor. J Clin Endocrinol Metab 2005;90:4895-903.
120. Crispi F, Domínguez C, Llurba E, Martín-Gallán P, Cabero L, Gratacós E. Placental angiogenic growth factors and uterine artery Doppler findings for characterization of different subsets in preeclampsia and in isolated intrauterine growth restriction. Am J Obstet Gynecol 2006;195:201-7.
121. Wikström AK, Larsson A, Eriksson UJ, Nash P, Nordén-Lindeberg S, Olovsson M. Placental growth factor and soluble FMS-like tyrosine kinase-1 in early-onset and late-onset preeclampsia. Obstet Gynecol 2007;109:1368-74.
122. Romero R, Nien JK, Espinoza J, Todem D, Fu W, Chung H, et al. A longitudinal study of angiogenic (placental growth factor) and anti-angiogenic (soluble endoglin and soluble vascular endothelial growth factor receptor-1) factors in normal pregnancy and patients destined to develop preeclampsia and deliver a small for gestational age neonate. J Matern Fetal Neonatal Med 2008;21:9-23.
123. Staff AC, Harsem NK, Braekke K, Hyer M, Hoover RN, Troisi R. Maternal, gestational and neonatal characteristics and maternal angiogenic factors in normotensive pregnancies. Eur J Obstet Gynecol Reprod Biol 2009;143:29-33.
124. Levine RJ, Lam C, Qian C, Yu KF, Maynard SE, Sachs BP, et al. Soluble endoglin and other circulating antiangiogenic factors in preeclampsia. N Engl J Med 2006;355:992-1005.
125. Stepan H, Krämer T, Faber R. Maternal plasma concentrations of soluble endoglin in pregnancies with intrauterine growth restriction. J Clin Endocrinol Metab 2007;92:2831-4.
126. Chaiworapongsa T, Romero R, Espinoza J, Bujold E, Mee Kim Y, Gonçalves LF, et al. Evidence supporting a role for blockade of the vascular endothelial growth factor system in the pathophysiology of preeclampsia. Young Investigator Award. Am J Obstet Gynecol 2004;190:1541-7; discussion 7-50.
127. Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, Yu KF, et al. Circulating angiogenic factors and the risk of preeclampsia. N Engl J Med 2004;350:672-83.
128. Tsatsaris V, Goffin F, Munaut C, Brichant JF, Pignon MR, Noel A, et al. Overexpression of the soluble vascular endothelial growth factor receptor in preeclamptic patients: pathophysiological consequences. J Clin Endocrinol Metab 2003;88:5555-63.
129. Jeyabalan A, McGonigal S, Gilmour C, Hubel CA, Rajakumar A. Circulating and placental endoglin concentrations in pregnancies complicated by intrauterine growth restriction and preeclampsia. Placenta 2008;29:555-63.
130. Yinon Y, Nevo O, Xu J, Many A, Rolfo A, Todros T, et al. Severe intrauterine growth restriction pregnancies have increased placental endoglin levels: hypoxic regulation via transforming growth factor-β3. Am J Pathol 2008;172:77-85.

131. Vatten LJ, Eskild A, Nilsen TI, Jeansson S, Jenum PA, Staff AC. Changes in circulating level of angiogenic factors from the first to second trimester as predictors of preeclampsia. Am J Obstet Gynecol 2007;196:239 e1-6.
132. Åsvold BO, Vatten LJ, Romundstad PR, Jenum PA, Karumanchi SA, Eskild A. Angiogenic factors in maternal circulation and the risk of severe fetal growth restriction. Am J Epidemiol 2011;173:630-9.
133. Teasdale F. Histomorphometry of the human placenta in maternal preeclampsia. Am J Obstet Gynecol 1985;152:25-31.
134. Egbor M, Ansari T, Morris N, Green CJ, Sibbons PD. Pre-eclampsia and fetal growth restriction: how morphometrically different is the placenta? Placenta 2006;27:727-34.
135. Mayhew TM, Manwani R, Ohadike C, Wijesekara J, Baker PN. The placenta in pre-eclampsia and intrauterine growth restriction: studies on exchange surface areas, diffusion distances and villous membrane diffusive conductances. Placenta 2007;28:233-8.
136. Mayhew TM, Charnock-Jones DS, Kaufmann P. Aspects of human fetoplacental vasculogenesis and angiogenesis. III. Changes in complicated pregnancies. Placenta 2004;25:127-39.
137. Roberts JM, Hubel CA. The two stage model of preeclampsia: variations on the theme. Placenta 2009;30 Suppl A:S32-7.
138. Burton GJ, Jauniaux E. Oxidative stress. Best Pract Res Clin Obstet Gynaecol 2011;25:287-99.
139. Sacks GP, Studena K, Sargent K, Redman CW. Normal pregnancy and preeclampsia both produce inflammatory changes in peripheral blood leukocytes akin to those of sepsis. Am J Obstet Gynecol 1998;179:80-6.
140. Ness RB, Sibai BM. Shared and disparate components of the pathophysiologies of fetal growth restriction and preeclampsia. Am J Obstet Gynecol 2006;195:40-9.
141. Wiktor H, Kankofer M, Schmerold I, Dadak A, Lopucki M, Niedermüller H. Oxidative DNA damage in placentas from normal and pre-eclamptic pregnancies. Virchows Arch 2004;445:74-8.
142. Laskowska M, Laskowska K, Leszczyńska-Gorzelak B, Oleszczuk J. Comparative analysis of the maternal and umbilical interleukin-8 levels in normal pregnancies and in pregnancies complicated by preeclampsia with intrauterine normal growth and intrauterine growth retardation. J Matern Fetal Neonatal Med 2007;20:527-32.
143. Haig D. Genetic conflicts in human pregnancy. Q Rev Biol 1993;68:495-532.
144. Göring HH, Curran JE, Johnson MP, Dyer TD, Charlesworth J, Cole SA, et al. Discovery of expression QTLs using large-scale transcriptional profiling in human lymphocytes. Nat Genet 2007;39:1208-16.
145. Moses EK, Fitzpatrick E, Freed KA, Dyer TD, Forrest S, Elliott K, et al. Objective prioritization of positional candidate genes at a quantitative trait locus for pre-eclampsia on 2q22. Mol Hum Reprod 2006;12:505-12.
146. Founds SA, Dorman JS, Conley YP. Microarray technology applied to the complex disorder of preeclampsia. J Obstet Gynecol Neonatal Nurs 2008;37:146-57.

147. McMinn J, Wei M, Schupf N, Cusmai J, Johnson EB, Smith AC, et al. Unbalanced placental expression of imprinted genes in human intrauterine growth restriction. Placenta 2006;27:540-9.
148. Sitras V, Paulssen R, Leirvik J, Vårtun Å, Acharya G. Placental gene expression profile in intrauterine growth restriction due to placental insufficiency. Reprod Sci 2009;16:701-11.
149. Nishizawa H, Ota S, Suzuki M, Kato T, Sekiya T, Kurahashi H, et al. Comparative gene expression profiling of placentas from patients with severe pre-eclampsia and unexplained fetal growth restriction. Reprod Biol Endocrinol 2011;9:107.
150. Enquobahrie DA, Meller M, Rice K, Psaty BM, Siscovick DS, Williams MA. Differential placental gene expression in preeclampsia. Am J Obstet Gynecol 2008;199:566 e1-11.
151. Founds SA, Conley YP, Lyons-Weiler JF, Jeyabalan A, Hogge WA, Conrad KP. Altered global gene expression in first trimester placentas of women destined to develop preeclampsia. Placenta 2009;30:15-24.
152. Tsai S, Hardison NE, James AH, Motsinger-Reif AA, Bischoff SR, Thames BH, et al. Transcriptional profiling of human placentas from pregnancies complicated by preeclampsia reveals disregulation of sialic acid acetylesterase and immune signalling pathways. Placenta 2011;32:175-82.
153. Soleymanlou N, Jurisica I, Nevo O, Ietta F, Zhang X, Zamudio S, et al. Molecular evidence of placental hypoxia in preeclampsia. J Clin Endocrinol Metab 2005;90:4299-308.
154. Sitras V, Paulssen RH, Grønaas H, Leirvik J, Hanssen TA, Vårtun Å, et al. Differential placental gene expression in severe preeclampsia. Placenta 2009;30:424-33.
155. Freed KA, Brennecke SP, Moses EK. Gene expression of the constant region of the heavy chain of immunoglobulin G (IgG CRHC) is down-regulated in human decidua in association with preeclampsia. J Reprod Immunol 2005;68:105-20.
156. Herse F, Dechend R, Harsem NK, Wallukat G, Janke J, Qadri F, et al. Dysregulation of the circulating and tissue-based renin-angiotensin system in preeclampsia. Hypertension 2007;49:604-11.
157. Winn VD, Gormley M, Paquet AC, Kjaer-Sorensen K, Kramer A, Rumer KK, et al. Severe preeclampsia-related changes in gene expression at the maternal-fetal interface include sialic acid-binding immunoglobulin-like lectin-6 and pappalysin-2. Endocrinology 2009;150:452-62.
158. Nishizawa H, Pryor-Koishi K, Kato T, Kowa H, Kurahashi H, Udagawa Y. Microarray analysis of differentially expressed fetal genes in placental tissue derived from early and late onset severe pre-eclampsia. Placenta 2007;28:487-97.
159. Centlow M, Wingren C, Borrebaeck C, Brownstein MJ, Hansson SR. Differential gene expression analysis of placentas with increased vascular resistance and pre-eclampsia using whole-genome microarrays. J Pregnancy 2011;2011:472354.
160. Srinivas SK, Morrison AC, Andrela CM, Elovitz MA. Allelic variations in angiogenic pathway genes are associated with preeclampsia. Am J Obstet Gynecol 2010;202:445 e1-11.

161. Rigó J, Szendei G, Rosta K, Fekete A, Bögi K, Molvarec A, et al. Leptin receptor gene polymorphisms in severely pre-eclamptic women. Gynecol Endocrinol 2006;22:521-5.
162. Sugathadasa BH, Tennekoon KH, Karunanayake EH, Kumarasiri JM, Wijesundere AP. Association of -2548 G/A polymorphism in the leptin gene with preeclampsia/pregnancy-induced hypertension. Hypertens Pregnancy 2010;29:366-74.
163. Report of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy. Am J Obstet Gynecol 2000;183:S1-S22.
164. Maršál K, Persson PH, Larsen T, Lilja H, Selbing A, Sultan B. Intrauterine growth curves based on ultrasonically estimated foetal weights. Acta Paediatr 1996;85:843-8.
165. Sibai B, Dekker G, Kupferminc M. Pre-eclampsia. Lancet 2005;365:785-99.
166. Viero S, Chaddha V, Alkazaleh F, Simchen MJ, Malik A, Kelly E, et al. Prognostic value of placental ultrasound in pregnancies complicated by absent end-diastolic flow velocity in the umbilical arteries. Placenta 2004;25:735-41.
167. Harsem NK, Staff AC, He L, Roald B. The decidual suction method: a new way of collecting decidual tissue for functional and morphological studies. Acta Obstet Gynecol Scand 2004;83:724-30.
168. Butte A. The use and analysis of microarray data. Nat Rev Drug Discov 2002;1:951-60.
169. Lockhart DJ, Dong H, Byrne MC, Follettie MT, Gallo MV, Chee MS, et al. Expression monitoring by hybridization to high-density oligonucleotide arrays. Nat Biotechnol 1996;14:1675-80.
170. Gunderson KL, Kruglyak S, Graige MS, Garcia F, Kermani BG, Zhao C, et al. Decoding randomly ordered DNA arrays. Genome Res 2004;14:870-7.
171. Shi L, Reid LH, Jones WD, Shippy R, Warrington JA, Baker SC, et al. The MicroArray Quality Control (MAQC) project shows inter- and intraplatform reproducibility of gene expression measurements. Nat Biotechnol 2006;24:1151-61.
172. Maouche S, Poirier O, Godefroy T, Olaso R, Gut I, Collet JP, et al. Performance comparison of two microarray platforms to assess differential gene expression in human monocyte and macrophage cells. BMC Genomics 2008;9:302.
173. Chen JJ, Hsueh HM, Delongchamp RR, Lin CJ, Tsai CA. Reproducibility of microarray data: a further analysis of microarray quality control (MAQC) data. BMC Bioinformatics 2007;8:412.
174. Bustin SA, Mueller R. Real-time reverse transcription PCR (qRT-PCR) and its potential use in clinical diagnosis. Clin Sci (Lond) 2005;109:365-79.
175. Nakane PK, Pierce GB, Jr. Enzyme-labeled antibodies: preparation and application for the localization of antigens. J Histochem Cytochem 1966;14:929-31.
176. Carlton SJ. Immunofluorescent techniques. In: Theory and Practice of Histological Techniques. Bancroft JD (ed). London: Churchill Livingstone 2002;579-91.
177. Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci U S A 1979;76:4350-4.

178. Renart J, Reiser J, Stark GR. Transfer of proteins from gels to diazobenzyloxymethyl-paper and detection with antisera: a method for studying antibody specificity and antigen structure. Proc Natl Acad Sci U S A 1979;76:3116-20.
179. Irizarry RA, Hobbs B, Collin F, Beazer-Barclay YD, Antonellis KJ, Scherf U, et al. Exploration, normalization, and summaries of high density oligonucleotide array probe level data. Biostatistics 2003;4:249-64.
180. Smyth GK. Linear models and empirical bayes methods for assessing differential expression in microarray experiments. Stat Appl Genet Mol Biol 2004;3:Article3.
181. Benjamini Y, Hochberg Y. Controlling the false discovery rate; a practical and powerful approach to multiple testing. J Royal Stat Soc Ser B 1995;289-300.
182. Almasy L, Blangero J. Multipoint quantitative-trait linkage analysis in general pedigrees. Am J Hum Genet 1998;62:1198-211.
183. Wu D, Lim E, Vaillant F, Asselin-Labat ML, Visvader JE, Smyth GK. ROAST: rotation gene set tests for complex microarray experiments. Bioinformatics 2010;26:2176-82.
184. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A 2005;102:15545-50.
185. Langsrud Ø. Rotation tests. Statistics and Computing 2005;15:53-60.
186. Fu WJ, Hu J, Spencer T, Carroll R, Wu G. Statistical models in assessing fold change of gene expression in real-time RT-PCR experiments. Comput Biol Chem 2006;30:21-6.
187. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta Ct}$ method. Methods 2001;25:402-8.
188. Abebe J, Eigbefoh J, Isabu P, Okogbenin S, Eifediyi R, Okusanya B. Accuracy of urine dipsticks, 2-h and 12-h urine collections for protein measurement as compared with the 24-h collection. J Obstet Gynaecol 2008;28:496-500.
189. Terwilliger JD, Göring HH. Gene mapping in the 20th and 21st centuries: statistical methods, data analysis, and experimental design. Hum Biol 2000;72:63-132.
190. Sood R, Zehnder JL, Druzin ML, Brown PO. Gene expression patterns in human placenta. Proc Natl Acad Sci U S A 2006;103:5478-83.
191. Wyatt SM, Kraus FT, Roh CR, Elchalal U, Nelson DM, Sadovsky Y. The correlation between sampling site and gene expression in the term human placenta. Placenta 2005;26:372-9.
192. Winn VD, Haimov-Kochman R, Paquet AC, Yang YJ, Madhusudhan MS, Gormley M, et al. Gene expression profiling of the human maternal-fetal interface reveals dramatic changes between midgestation and term. Endocrinology 2007;148:1059-79.
193. Mikheev AM, Nabekura T, Kaddoumi A, Bammler TK, Govindarajan R, Hebert MF, et al. Profiling gene expression in human placentae of different gestational ages: an OPRU Network and UW SCOR Study. Reprod Sci 2008;15:866-77.
194. Biological Pathwyas Leading to Preterm Birth. In: Preterm Birth: Causes, Consequences, and Prevention. Behrman RE, Butler AS (eds). Washington; National Academies Press 2007;169-206.

195. Cindrova-Davies T, Yung HW, Johns J, Spasic-Boskovic O, Korolchuk S, Jauniaux E, et al. Oxidative stress, gene expression, and protein changes induced in the human placenta during labor. Am J Pathol 2007;171:1168-79.
196. Sitras V, Paulssen RH, Grønaas H, Vårtun Å, Acharya G. Gene expression profile in labouring and non-labouring human placenta near term. Mol Hum Reprod 2008;14:61-5.
197. Millenaar FF, Okyere J, May ST, van Zanten M, Voesenek LA, Peeters AJ. How to decide? Different methods of calculating gene expression from short oligonucleotide array data will give different results. BMC Bioinformatics 2006;7:137.
198. Tarca AL, Romero R, Draghici S. Analysis of microarray experiments of gene expression profiling. Am J Obstet Gynecol 2006;195:373-88.
199. Allison DB, Cui X, Page GP, Sabripour M. Microarray data analysis: from disarray to consolidation and consensus. Nat Rev Genet 2006;7:55-65.
200. Ackermann M, Strimmer K. A general modular framework for gene set enrichment analysis. BMC Bioinformatics 2009;10:47.
201. Czechowski T, Bari RP, Stitt M, Scheible WR, Udvardi MK. Real-time RT-PCR profiling of over 1400 Arabidopsis transcription factors: unprecedented sensitivity reveals novel root- and shoot-specific genes. Plant J 2004;38:366-79.
202. Wang Y, Barbacioru C, Hyland F, Xiao W, Hunkapiller KL, Blake J, et al. Large scale real-time PCR validation on gene expression measurements from two commercial long-oligonucleotide microarrays. BMC Genomics 2006;7:59.
203. Liu Q, Yan X, Li Y, Zhang Y, Zhao X, Shen Y. Pre-eclampsia is associated with the failure of melanoma cell adhesion molecule (MCAM/CD146) expression by intermediate trophoblast. Lab Invest 2004;84:221-8.
204. Imamichi Y, König A, Gress T, Menke A. Collagen type I-induced Smad-interacting protein 1 expression downregulates E-cadherin in pancreatic cancer. Oncogene 2007;26:2381-5.
205. Katoh M, Katoh M. Integrative genomic analyses of ZEB2: Transcriptional regulation of ZEB2 based on SMADs, ETS1, HIF1α, POU/OCT, and NF-κB. Int J Oncol 2009;34:1737-42.
206. Gallery ED, Campbell S, Arkell J, Nguyen M, Jackson CJ. Preeclamptic decidual microvascular endothelial cells express lower levels of matrix metalloproteinase-1 than normals. Microvasc Res 1999;57:340-6.
207. Galewska Z, Bańkowski E, Romanowicz L, Jaworski S. Pre-eclampsia (EPH-gestosis)-induced decrease of MMP-s content in the umbilical cord artery. Clin Chim Acta 2003;335:109-15.
208. Huppertz B, Kertschanska S, Demir AY, Frank HG, Kaufmann P. Immunohistochemistry of matrix metalloproteinases (MMP), their substrates, and their inhibitors (TIMP) during trophoblast invasion in the human placenta. Cell Tissue Res 1998;291:133-48.
209. Goyal R, Yellon SM, Longo LD, Mata-Greenwood E. Placental gene expression in a rat 'model' of placental insufficiency. Placenta 2010;31:568-75.
210. Ho IA, Chan KY, Ng WH, Guo CM, Hui KM, Cheang P, et al. Matrix metalloproteinase 1 is necessary for the migration of human bone marrow-derived mesenchymal stem cells toward human glioma. Stem Cells 2009;27:1366-75.

211. Harris LK, Smith SD, Keogh RJ, Jones RL, Baker PN, Knöfler M, et al. Trophoblast- and vascular smooth muscle cell-derived MMP-12 mediates elastolysis during uterine spiral artery remodeling. Am J Pathol 2010;177:2103-15.
212. Munn DH, Zhou M, Attwood JT, Bondarev I, Conway SJ, Marshall B, et al. Prevention of allogeneic fetal rejection by tryptophan catabolism. Science 1998;281:1191-3.
213. Opitz CA, Wick W, Steinman L, Platten M. Tryptophan degradation in autoimmune diseases. Cell Mol Life Sci 2007;64:2542-63.
214. Moses EK, Johnson MP, Tømmerdal L, Forsmo S, Curran JE, Abraham LJ, et al. Genetic association of preeclampsia to the inflammatory response gene SEPS1. Am J Obstet Gynecol 2008;198:336.e1-5.
215. Johnson MP, Roten LT, Dyer TD, East CE, Forsmo S, Blangero J, et al. The ERAP2 gene is associated with preeclampsia in Australian and Norwegian populations. Hum Genet 2009;126:655-66.
216. Cullinan SB, Zhang D, Hannink M, Arvisais E, Kaufman RJ, Diehl JA. Nrf2 is a direct PERK substrate and effector of PERK-dependent cell survival. Mol Cell Biol 2003;23:7198-209.
217. Ishii T, Itoh K, Takahashi S, Sato H, Yanagawa T, Katoh Y, et al. Transcription factor Nrf2 coordinately regulates a group of oxidative stress-inducible genes in macrophages. J Biol Chem 2000;275:16023-9.
218. Meekins JW, Pijnenborg R, Hanssens M, van Assche A, McFadyen IR. Immunohistochemical detection of lipoprotein(a) in the wall of placental bed spiral arteries in normal and severe preeclamptic pregnancies. Placenta 1994;15:511-24.
219. Feng B, Yao PM, Li Y, Devlin CM, Zhang D, Harding HP, et al. The endoplasmic reticulum is the site of cholesterol-induced cytotoxicity in macrophages. Nat Cell Biol 2003;5:781-92.
220. Colgan SM, Tang D, Werstuck GH, Austin RC. Endoplasmic reticulum stress causes the activation of sterol regulatory element binding protein-2. Int J Biochem Cell Biol 2007;39:1843-51.
221. Hotamisligil GS. Endoplasmic reticulum stress and the inflammatory basis of metabolic disease. Cell 2010;140:900-17.
222. Hotamisligil GS. Inflammation and metabolic disorders. Nature 2006;444:860-7.
223. Yoshida H, Oku M, Suzuki M, Mori K. pXBP1(U) encoded in XBP1 pre-mRNA negatively regulates unfolded protein response activator pXBP1(S) in mammalian ER stress response. J Cell Biol 2006;172:565-75.
224. Yoshida H, Uemura A, Mori K. pXBP1(U), a negative regulator of the unfolded protein response activator pXBP1(S), targets ATF6 but not ATF4 in proteasome-mediated degradation. Cell Struct Funct 2009;34:1-10.
225. Guo F, Lin EA, Liu P, Lin J, Liu C. XBP1U inhibits the XBP1S-mediated upregulation of the iNOS gene expression in mammalian ER stress response. Cell Signal 2010;22:1818-28.
226. Lin JH, Li H, Zhang Y, Ron D, Walter P. Divergent effects of PERK and IRE1 signaling on cell viability. PLoS One 2009;4:e4170.
227. Borell U, Fernstroem I, Ohlson L, Wiqvist N. Influence of uterine contractions on the uteroplacental blood flow at term. Am J Obstet Gynecol 1965;93:44-57.

228. Cetin I, Huppertz B, Burton G, Cuckle H, Gonen R, Lapaire O, et al. Pregenesys pre-eclampsia markers consensus meeting: What do we require from markers, risk assessment and model systems to tailor preventive strategies? Placenta 2011;32 Suppl:S4-16.

Paper I

Is not included due to copyright

Paper II

Placenta 31 (2010) 615–620

Contents lists available at ScienceDirect

Placenta

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Matrix Metalloproteinase 1 in Pre-eclampsia and Fetal Growth Restriction: Reduced Gene Expression in Decidual Tissue and Protein Expression in Extravillous Trophoblasts

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ARTICLE INFO

Article history:
Accepted 6 April 2010

Keywords:
Trophoblast invasion
MMP1
Gene expression
Decidua
Pre-eclampsia
Fetal growth restriction

ABSTRACT

Superficial invasion of extravillous trophoblasts (EVTs) and impaired spiral artery remodelling are characterizing phenomena in pregnancies complicated by pre-eclampsia (PE) and fetal growth restriction (FGR). However, the underlying causes remain unclear. In this study, gene expression in decidua basalis tissue from pregnancies complicated with PE and/or FGR ($n = 18$) and normal pregnancies ($n = 17$) was assessed by Affymetrix HG Focus microarray to obtain hints of mechanisms involved in the pathogenesis. A total of 200 differentially expressed transcripts were detected at a false discovery rate (FDR) ≤ 0.1. Several genes involved in trophoblast differentiation and invasion were downregulated, including the matrix metalloproteinases (MMPs) MMP1, -7 and -12. MMPs are a family of enzymes involved in degradation of extracellular matrix and have been ascribed a permissive role in trophoblast invasion. MMP1 had the highest fold change among the differentially expressed genes (four-fold downregulated) and was chosen for further investigation. Reduced MMP1 mRNA in decidual tissue was confirmed by RT-qPCR. MMP1 protein expression in EVTs was assessed by double immunofluorescence analysis, using antibodies against pro-MMP1 and cytokeratin 7. The proportion of MMP1 positive EVTs was reduced in all subgroups of cases (PE: $n = 18$, FGR: $n = 11$ and PE + FGR: $n = 30$) compared to controls ($n = 23$) (all p's < 0.05). Based on these findings, we hypothesize that reduced levels of MMP1 protein in EVTs could be linked to the impaired trophoblast invasion in PE and/or FGR.
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1. Introduction

Superficial trophoblast invasion and impaired spiral artery remodelling characterize the placental disease in both pre-eclampsia (PE) [1] and fetal growth restriction (FGR) [2], where the depth of trophoblast invasion as well as the number of vessels invaded by trophoblasts is reduced [2]. A complex molecular dialogue between extravillous trophoblasts (EVTs), maternal immune cells, decidual cells and extracellular matrix (ECM) components is important for successful trophoblast invasion and implantation. Proper expression of cell adhesion molecules (CAMs) is necessary for trophoblast migration, and abnormal CAM expression has been detected in EVTs from pregnancies complicated by PE and FGR [reviewed by Lyall et al.] [3]. Throughout the process of invading and remodelling spiral arteries, extracellular matrix must be broken down and matrix metalloproteinases (MMPs) are major participants in this disintegration [4,5]. Based on substrate specificities, MMPs are divided in four subgroups; collagenases (MMP1, -8, -13), gelatinases (MMP2, -9), stromelysins (MMP3, -7, -10, −11, −12) and membrane-type MMPs (MT-MMPs) [5]. A number of studies have demonstrated that the invasive capacity of trophoblasts is strongly associated with their ability to produce and secrete MMPs [4,6]. Reduced trophoblasts expression of MMP2, -3, -7 and -9 protein has been reported in PE and FGR [7,8]. MMP1 mainly degrades fibrillar collagen [5] and is expressed in a variety of cells at the maternal–fetal interface; decidual cells, cytotrophoblasts, syncytiotrophoblast [9], decidual endothelial cells [8,10] and EVTs [11], and thus, MMP1 has been ascribed a central role in the process of trophoblast invasion. In accordance with such an assumption, reduced levels of MMP1 protein in umbilical cord blood [12] as well as decreased levels of MMP1 mRNA and protein in cultured decidual

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doi:10.1016/j.placenta.2010.04.003

endothelial cells [8,10] from pre-eclamptic pregnancies has been reported. However, the specific expression of MMP1 in EVTs from PE and FGR pregnancies has not been evaluated so far.

Despite extensive research on trophoblast invasion in PE and FGR, the mechanisms underlying reduced invasion and subsequent placental disease remain unclear [13]. To study possible mechanisms involved at the maternal–fetal interface, a global microarray analysis on decidual tissue from PE and/or FGR pregnancies (cases) and normal pregnancies (controls) was performed. Altered expression of several genes involved in trophoblast invasion and differentiation was identified, including a four-fold downregulation of MMP1 in cases. To explore whether reduced MMP1 expression could be related to shallow trophoblast invasion, MMP1 protein expression in EVTs from cases and controls was compared by immunohistochemical analysis.

2. Materials and methods

2.1. Study groups

Women with singleton pregnancies complicated by PE and/or FGR were consecutively recruited at St. Olavs University Hospital and Haukeland University Hospital, Norway, from 2002 to 2006. PE was defined as persistent hypertension (blood pressure of ≥140 mmHg systolic or 90 mmHg diastolic), plus proteinuria (≥0.3 g in a 24 h urine collection or ≥2 + according to a dipstick test), developing after 20 weeks of gestation [14]. Birthweight ≤2 standard deviations (2SD), corresponding to the 2.5 percentile for gestational age at delivery according to a Scandinavian normogram, was used as an FGR criterion [15]. In addition, *restricted* fetal growth was documented by requiring that at least one of the following additional criteria was fulfilled; 1) reduced fundal height in serial measurements throughout pregnancy, 2) serial ultrasound biometry identifying failure to grow along a consistent centile or 3) abnormal umbilical artery wave form. Determination of gestational age of both cases and controls was based on ultrasound measurements.

Due to tissue sampling procedures, only women delivering by caesarean section (CS) were included. Women with diabetes, cardiovascular, renal or rheumatologic diseases, or pregnancies with fetal chromosomal or congenital abnormalities were excluded. Healthy women with normal singleton pregnancies undergoing CS for various reasons considered irrelevant to the aim of this study (i.e. breech presentation, previous CS and fear of labour) were recruited as controls. Women with previous pregnancy complications (such as PE or FGR) were not enrolled as controls.

The study was approved by the Regional Committee for Medical Research Ethics (REK no.054-02), and informed consent was obtained from all participants.

2.2. Decidual tissue

Decidual tissue was collected by vacuum suction of the placental bed during CS after the placenta was delivered [16,17], and the procedure was performed by a trained surgeon. Collected tissue was immediately flushed with room-tempered saline solution to remove excess blood. Tissue for microarray and RT-qPCR analyses was immediately placed in RNAlater solution (Ambion, Huntington, UK) to prevent RNA degradation and stored at −80 °C until used. Tissue used for immunofluorescence analyses was fixed in 10% neutral-buffered formalin, paraffin embedded and cut in sections of 4 μm on a motorized rotation microtome. To verify that the collected samples in fact represented decidua basalis, the presence of EVTs was evaluated by an experienced pathologist. Monoclonal antibody against cytokeratin 7 was used to detect EVTs, and only specimens with confirmed presence of EVT were included for further studies, as previously described in Ref. [18].

2.3. RNA preparation

Frozen decidual tissue in RNAlater solution was homogenized using a Rotor-Stator homogenizator (Janke & Kunkel, IKA Labortechnik, Staufen, Germany). Total RNA isolation and purification was performed using RNeasy Midi kit (Qiagen, Hilden, Germany), according to manufacturers' protocol. RNA quality was checked by standard denaturating agarosis gel electrophoresis, and RNA concentration and purity were assessed by NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Rocland, USA). Only RNA of high quality was used in subsequent analyses.

2.4. Affymetrix microarray analysis of decidual tissue

The microarray experiments were conducted as described in the Affymetrix GeneChip® Expression Analysis Technical Manual (*2004 revision*). Total RNA extracted from decidual tissue was hybridized to the HG Focus Arrays (Affymetrix, Santa Clara, USA), containing 8,793 probesets, and scanned on the Agilent GeneArray Scanner (Agilent Technologies, Santa Clara, USA). All experiments were performed at the Norwegian Microarray Consortium, NTNU, in accordance with the Minimum Information About a Microarray Experiment (MIAME) guidelines [19]. Experimental data have been submitted to ArrayExpress (www.ebi.ac.uk/arrayexpress), accession number E-MEXP-1050.

2.5. Pathway analysis of microarray data

To identify potential pathways involved in the pathogenesis of PE and/or FGR, differentially expressed transcripts ($p \leq 0.1$, false discovery rate (FDR) corrected) were imported to the bioinformatics tool Ingenuity Pathways Analysis (IPA) version 7.5 (Ingenuity® Systems, www.ingenuity.com). Using Fisher's exact test, IPAs Functional Analysis was used to identify the most significant biological functions among the top differentially expressed genes. The functions that were identified using IPA were further reanalyzed using ROAST (Rotation Gene Set Tests) and ROMER (Rotation Gene Set Enrichment Analysis) pathway analyses, using the limma package available via the Bioconductor Project (www.r-project.org) [20,21]. ROAST tests whether any of the genes in the gene set are differentially expressed, and ROMER tests whether the set of *n* genes in the biological function of interest is more significantly differentially expressed than other set of *n* genes in total dataset of 8793 transcripts. ROMER is an implementation of gene set enrichment analysis (GSEA) [22]. For both methods, genes are ranked by moderated *t*-statistics, and rotation [22], which is a smoothed version of permutation suitable for linear models, is used to assess significance.

2.6. Reverse transcription quantitative PCR analysis of MMP1

Total RNA extracted from decidual tissue was reverse transcribed using a cDNA synthesis kit (Bio-Rad Laboratories, Hercules, USA) and subjected to (real time) qPCR analysis on a Chromo4 detector (Bio-Rad Laboratories), using the iQ SYBR Green supermix (Bio-Rad Laboratories). The specificity of amplification was assayed by melting curve analysis. The following primer-pairs were used; MMP1: 5′-tggacctg-gaggaaatcttg-3′ (forward), 5′-agaatggccgagttcatgag-3′ (reverse) and TATA-box binding protein (TBP): 5′-ttgctgcggtaatcatgagg-3′ (forward), 5′-gccagtctggactgttcttc-3′ (reverse) (Sigma–Aldrich, St. Louis, USA).

2.7. Immunohistochemical analysis of MMP1

The MMP1 protein is produced as an inactive pro-form (pro-MMP1) which is cleaved and activated after secretion [5]. A monoclonal (mice-anti-human) antibody (mAb) against pro-MMP1 (clone 36660.11, diluted 1:5; R&D Systems, Minneapolis, USA) was used to assess cellular expression of the protein. Since we aimed to focus on MMP1 expression in EVTs, mAb pro-MMP1 was used in combination with a polyclonal (rabbit–anti-human) antibody (Ab) against cytokeratin 7 (anti-cytokeratin 7 (CK7), ab31014, diluted 1:20; Abcam, Cambridge, UK). Double immunostaining was performed in an automated slide stainer (Autostainer Plus, Dako, Glostrup, Denmark) after deparaffination in xylen, rehydration and heat-induced antigen retrieval in tris-buffered saline (TRS), pH 6 (Dako). Bovine serum albumin (BSA) (2%) was added for 10 min to inhibit non-specific binding. A primary antibody mixture was added and the slides were incubated overnight at 4 °C. The slides were incubated with secondary antibodies (tetramethyl rhodamine iso-thiocyanate (TRITC)-conjugated (swine-anti-rabbit) Ab (catalog no. R0156, diluted 1:10; Dako) and fluorescein iso-thiocyanate (FITC)-conjugated (goat-anti-mice) Ab (catalog no. F0479, diluted 1:10; Dako) for 30 min in a dark chamber. All sections were counterstained with 4′-6-Diamidino-2-phenylindole (DAPI) (Abbott Molecular Inc, Illinois, USA) and examined using a fluorescent microscope (Nixon Eclipse E600 with CytoVision 3.7 software from Applied Imaging, New Milton Hampshire, UK) at magnification × 600. Decidual glands were used as a positive control for Ab against CK7. Negative controls were performed by omission of primary antibodies as well as their substitution by isotype-matched rabbit- (catalog no. 000-000-120, Jackson ImmunoResearch, Pennsylvania, USA) and mouse serum (catalog no. X0931, Dako).

2.8. Assessment of MMP1 protein expression in EVTs

The proportion of EVTs expressing MMP1 protein was independently assessed by two of the authors *(IAL and JHT*), blinded for group status, after reaching agreement on inclusion criteria for positively stained cells. Cells were regarded as positive if the staining of CK7 or MMP1 covered >20% of the areal/membrane of the cell (less positive signal than this was considered unspecific binding). Cells were scored as either positive or negative. Slides were evaluated by counting systematically in 15 adjacent fields, starting in the upper left corner of each slide (600 × magnification). Only slides with >250 CK7 positive cells were evaluated. The number of trophoblasts in each slide demonstrated a great variation, i.e. the total number of CK7 positive cells counted varied from 323 to 2084 (mean 1027.2 ± 259.8). The variation in cell numbers did not differ between cases and controls. The proportion of MMP1 positive EVTs was calculated as the number of MMP1 positive cells among the total number of CK7 positive cells. Slides were reevaluated if the percentage scores (% MMP1 positive EVTs) diverged (> 15%) between the two observers. The proportion

of MMP1 positive EVTs on each slide was calculated as the average of the scores given by the two observers, and this value was used for statistical analyses.

2.9. Statistical analysis

Statistical analyses of clinical data were performed using the unpaired two-sample student's *t*-test (two-sided) for continuous variables after testing for Gaussian distribution, while Fisher's exact test was used for categorical variables (SPSS 15, SPSS Inc., Illinois, USA).

The pre-processing of microarray data was performed using the Robust Multi-chip Average (RMA) method [23]. The method uses a global background correction method, a quantile normalization method, and the expression values (one for each probeset) are produced with the aid of a linear model. Differential mRNA expression between cases and controls was tested using a moderated *t*-statistic [20]. The original *p*-values were corrected for multiple testing using the FDR method [24], as implemented using the Benjamini–Hochberg step-up procedure. The proportion of non-differentially expressed genes was estimated using the convex decreasing estimator of Langaas et al. [25]. The RMA method, the moderated *t*-test and the FDR procedure are available via the Bioconductor Project (www.r-project.org) and described in BOOK [20].

Relative quantification of (real time) qPCR data was performed using a variant of the $2^{-\Delta\Delta C_t}$ method [26]. Each sample was put on the plate in triplicates for both the target gene MMP1 and the reference gene, TBP. The average Ct values for the triplicates were constructed separately for the target gene and the reference gene. Further statistical analyses were based on the difference in average C_t values, (ΔC_t) between the target gene and the reference gene for each sample. Differences in ΔC_t values between the total case group vs. controls, as well as the three subgroups of cases vs. controls, were tested using the Mann–Whitney *U* test.

Analysis of immunohistochemical data was performed using the unpaired two-sample student's *t*-test (two-sided) after testing for Gaussian distribution (SPSS 15, SPSS Inc.).

3. Results

3.1. Study groups

Decidual tissue was collected between 2002 and 2006, and the microarray, RT-qPCR and immunofluorescence analysis were performed at different time points during this period. At the time when the microarray experiments were performed, the total number of cases and controls was 35. The number of cases was estimated to be too small to divide individuals into subgroups. Thus, in the microarray analysis, a pooled group of cases (PE, FGR and PE+FGR) ($n = 18$) were compared to controls ($n = 17$).

At the time when the RT-qPCR analysis was performed, more women had been enrolled in the study (PE ($n = 8$), FGR ($n = 7$), PE + FGR ($n = 16$) and controls ($n = 19$)), and finally, when the immunofluorescence analysis was done, samples from 82 women were available. Of these, four slides were excluded from analyses due to blood contamination. In addition, two slides were not included due to disagreement between the two observers regarding the evaluation of the staining results (see Section 3.5). The 76 women finally included in the immunofluorescence study subgrouped into PE ($n = 18$), FGR ($n = 11$), PE + FGR ($n = 27$) and controls ($n = 20$). The clinical data on patients included in the immunofluorescence analysis are presented in Table 1. The clinical parameters on cases and controls included in the microarray and RT-qPCR analysis did not differ statistically from those presented in Table 1.

3.2. Affymetrix gene expression

Microarray analysis revealed five differentially expressed transcripts between the total case group (PE, FGR and PE + FGR) and controls (FDR adjusted, $p < 0.05$). Among these, MMP1 was four-fold downregulated in cases. Using a less stringent FDR cutoff ($p \leq 0.1$), 200 differentially expressed transcripts were identified (Table 1S). A total of 88 transcripts were upregulated in cases compared to controls, among these transcripts were laminin γ3 and integrin β8 (fold changes 1.66 and 1.31, respectively). Downregulated transcripts in cases compared to controls included MMP7, MMP12 and serpine peptidase inhibitor, clade E, member 2 (SERPINE2) (fold changes −1.72, −3.29 and −3.52, respectively).

3.3. Pathway analysis of microarray data

The functions "cell movement" and "cell-to-cell signalling and interaction" were among the top biological functions identified by IPA pathway analysis. These two functions were also found significant in the dataset using the tests ROAST and ROMER. The function "cell movement" contained 44 transcripts, where 33 were downregulated in cases (Table 2S). The function "cell-to-cell signalling and interaction" contained 39 transcripts, of which 27 were downregulated in cases (Table 3S). A total of 23 genes were involved in both functions. The genes with highest relevance to trophoblast invasion, migration and ECM breakdown are presented in Table 2, and includes transcripts such as integrin α5, melanoma cell adhesion molecule (MCAM), leptin receptor (LEPR), insulin-like growth factor 1 (IGF1), as well as MMP1, -7 and -12.

3.4. Real-time quantitative PCR

To validate the decreased MMP1 mRNA expression identified by microarray analysis, a RT-qPCR analysis was performed. The MMP1 mRNA levels in decidual tissue were downregulated in PE, FGR and PE + FGR cases (all *p's* ≤ 0.01) (fold change as compared to controls were −13.2, −7.1 and −14.2, respectively). No significant differences in MMP1 mRNA were observed between the case subgroups (*data not shown*).

3.5. MMP1 protein expression in EVTs

The proportion of MMP1 positive EVTs in the control group was 33.1±12.9%. A lower proportion was observed in all case groups (all *p*'s < 0.05). The lowest proportion was found in the FGR group (15.5 ± 10.1%), whereas the PE and PE + FGR group showed 19.7 ± 10.7% and 21.4 ± 12.5% MMP1 positive EVTs, respectively. The proportion of MMP1 positive EVTs did not differ statistically between these three case groups.

Table 1
Clinical characteristics of the study groups included for immunofluorescence analysis.

	PE ($n = 18$)	FGR ($n = 11$)	PE + FGR ($n = 27$)	Controls ($n = 20$)
GA (weeks)	33.4 ± 3.2	32.6 ± 5.0	30.0 ± 3.1	38.6 ± 0.5
BW (g)	2136.7 ± 677.4	1379.2 ± 636.4	1097.2 ± 409.5	3503.5 ± 311.5
Systolic BP (mmHg)	156.3 ± 19.8	127.7 ± 13.4	157.7 ± 15.4	110.5 ± 8.3
Diastolic BP (mmHg)	96.9 ± 10.7	74.6 ± 10.0*	98.1 ± 11.0	68.0 ± 6.1
Primipara	11/18	4/11	15/27	3/20

PE, pre-eclampsia; FGR, fetal growth restriction; GA, gestational age; BW, birthweight; BP, blood pressure. All but one* characteristic were significant in comparison between cases and controls ($p < 0.05$). Data are presented as mean ± standard deviation. The characteristics presented in Table 1 are also representative for the study groups included in the microarray- and RT-qPCR analyses.

Table 2
Genes involved in the IPA annotated functions “cell migration” and “cell-to-cell signaling and interaction”.

Fold Change	*p*-value	Probeset ID	Symbol	Entrez Gene Name	Chromosome
Cell adhesion molecules					
−1.44	0.082	211340_s_at	MCAM	Melanoma cell adhesion molecule	11q23.3
−1.43	0.109	204726_at	CDH13	Cadherin 13, H-cadherin	16q24.2–24.3
−1.34	0.083	216331_at	ITGA7	Integrin α7	12q13
−1.22	0.096	202351_at	ITGAV	Integrin α5	2q31–32
−1.39	0.029	203780_at	EVA1	Epithelial V-like antigen	11q24
−1.31	0.082	201952_at	ALCAM	Activated leukocyte cell adhesion molecule	3q13.1
−1.26	0.076	200602_at	APP	Amyloid beta (A4) precursor protein	21q21.2–21.3
1.13	0.104	211867_s_at	PCDHA10	Protocadherin α10	5q31
1.11	0.089	221410_x_at	PCDHB3	Protocadherin β3	5q31
1.07	0.109	211877_s_at	PCDHGA11	Protocadherin γ subfamily A, 11	5q31
1.11	0.076	211807_x_at	PCDHGB5	Protocadherin γ subfamily B, 5	5q31
1.31	0.109	211488_s_at	ITGB8	Integrin β8	7p21
1.13	0.101	201511_at	AAMP	Angio-associated, migratory cell protein	2q35
1.23	0.095	200924_s_at	SLC3A2	Solute carrier family 3, member 2	11q13
Extracellular matrix components					
−4.07	0.029	204475_at	MMP1	Matrix metallopeptidase 1	11q22.3
−3.29	0.098	204580_at	MMP12	Matrix metallopeptidase 12	11q22.3
−1.72	0.076	204259_at	MMP7	Matrix metallopeptidase 7	11q21-22
−3.52	0.104	212190_at	SERPINE2	Serpin peptidase inhibitor, clade E, member 2	2q33-35
−2.10	0.109	201645_at	TNC	Tenascin C	9q33
−1.55	0.104	201110_s_at	THBS1	Thrombospondin 1	15q15
−1.22	0.109	209561_at	THBS3	Thrombospondin 3	1q21
−1.45	0.087	206101_at	ECM2	Extracellular matrix protein 2	9q22.3
−1.19	0.099	200665_s_at	SPARC	Secreted protein, acidic, cysteine-rich	5q31.3–32
1.09	0.099	210731_s_at	LGALS8	Lectin, galactoside-binding, soluble,- 8	1q42–q43
1.66	0.076	219407_s_at	LAMC3	Laminin γ3	9q31–q34
Genes associated with cell movement					
−1.11	0.109	212586_at	CAST	Calpastatin	5q15
−1.64	0.109	209541_at	IGF1	Insulin-like growth factor 1	12q22–23
−1.46	0.076	209894_at	LEPR	Leptin receptor	1p31
−1.45	0.095	205392_s_at	CCL14	Chemokine (C–C motif) ligand 14	17q11.2
−1.19	0.109	203218_at	MAPK9	Mitogen-activated protein kinase 9	5q35
−1.15	0.100	207738_s_at	NCKAP1	NCK-associated protein 1	2q32
−1.09	0.096	210355_at	PTHLH	Parathyroid hormone-like hormone	12p11.2–12.1
−1.89	0.105	206023_at	NMU	Neuromedin U	4q12
−1.18	0.094	200958_s_at	SDCBP	Syndecan binding protein	8q12
−1.25	0.094	204042_at	WASF3	WAS protein family, member 3	13q12
−1.38	0.076	202133_at	WWTR1	WW domain containing transcription regulator 1	3q23-24
−1.43	0.076	203603_s_at	ZEB2	Zinc finger E-box binding homeobox 2	2q22.3

The *p*-values are false discovery rate (FDR)-corrected. Fold change values show the magnitude of change in gene expression in cases vs. controls.

The scores given by the two observers did not differ significantly; the cell numbers obtained were in agreement in 63 of 78 slides (80.8%) after the primary evaluation. Fifteen slides (19.2%) were revaluated, and consensus was obtained in assessment of all but two (97%). These two slides were discarded from further analysis.

The intracellular localization of MMP1 staining in EVTs varied. Most EVTs demonstrated a scattered cytoplasmatic pattern (Fig. 1B and C), whereas in some EVTs (<5%), a distinct polarized cytoplasmatic MMP1 staining pattern was observed (Fig. 1D and E). In areas where EVTs displayed polarized staining, adjacent EVTs were often found to be polarized in the same direction (Fig. 1D and E). No qualitative difference in staining pattern was observed between subgroups of cases or controls. The polarized staining phenomenon was observed in both cases and controls, but was too rare for quantification and comparison between groups.

4. Discussion

Gene expression profiling of the maternal–fetal interface revealed 200 differentially expressed transcripts between cases with PE and/or FGR and controls, including decreased expression of MMP1, -7 and -12 in cases. Among the differentially expressed transcripts, MMP1 had the highest fold change (four-fold down-regulated in cases). The reduced MMP1 gene expression was confirmed in all case groups (PE, FGR and PE+FGR) by RT-qPCR analysis.

MMPs are proteolytic enzymes and the main mediators of ECM degradation. MMP1 especially degrades interstitial collagen [5], which is abundant in decidual tissue [11,27]. The invasive capacity of trophoblasts has been related to their secretion of MMPs [4,6]. In accordance with this, invasive, but not proliferative trophoblasts express MMP1 protein, and the strongest MMP1 staining in EVTs is observed where the interstitial collagen concentration is highest, i.e. in the deeper layers of decidua [11]. MMP1 expression has also been shown to be crucial for the migratory capacity of mesenchymal stem cells [28]. Previous studies have provided evidence suggesting that impaired trophoblast invasion in PE and FGR is linked to reduced production of MMPs in EVTs [7,8,29]. Reduced MMP1 levels in decidual endothelial cells from pre-eclamptic pregnancies have been reported [8,10], however, to our knowledge, the present study is the first to investigate whether MMP1 levels in EVTs are decreased in pregnancies complicated with PE and/or FGR.

We observed 33.1% MMP1 immunopositive EVTs in the control group. Decreased expression of MMP1 in EVTs was seen in all case groups compared to controls (15.5%, 19.7% and 21.4% in FGR, PE and PE + FGR, respectively). Our results are in contrast to the findings obtained by Huppertz et al. [11], who reported that all EVTs in third trimester placental biopsies were MMP1 immunopositive [11]. However, since they examined placenta, and not decidua, and used

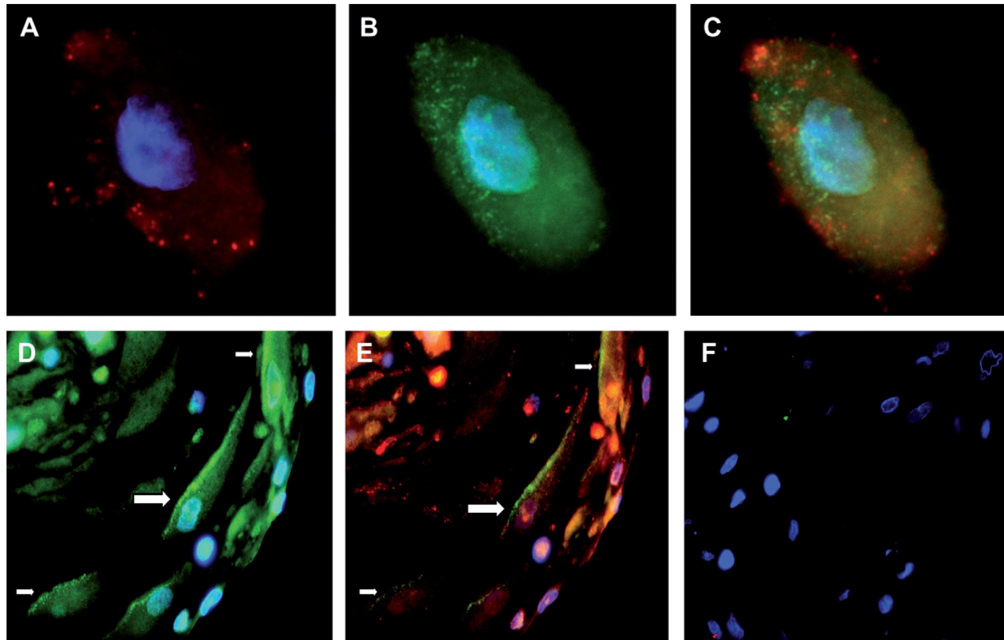

Fig. 1. Expression of matrix metalloproteinase 1 (MMP1) in extravillous trophoblasts (EVTs), stained with mAbs against cytokeratin 7 (CK7) (red signal) and against pro-MMP1 (green signal). Antibodies were added alone (A, B and D) and in combination (C and E) on formalin-fixed decidua basalis tissue. The nuclei were counterstained with 4′-6-Diamidino-2-phenylindole (DAPI) (blue signal). (A–C) A typical pro-MMP1 staining pattern in a single EVT is shown, magnification × 1000. (D–E) Polarized staining of MMP1 was observed in a few EVTs (large arrows), with similar polarized staining of adjacent EVTs (small arrows), magnification × 600. The EVTs in D and E are localized in the edge of a decidual tissue fragment. (F) A representative image of the staining obtained using isotype-matched irrelevant mouse- and rabbit IgG as negative controls, magnification × 600.

different antibodies (detecting both intra- and extracellular MMP1) and used different immunohistochemical techniques, direct comparisons are difficult. The collected decidual tissue was not suited for assessment of extracellular (active) MMP1 levels since sampling procedures included rinsing of the decidual tissue fragments in saline solution, thus the present study does not allow any conclusions as to whether or not the levels of active MMP1 in decidual tissue are altered in cases.

Most MMP1 positive EVTs demonstrated a scattered cytoplasmatic staining (Fig. 1B and C), but polarized MMP1 staining was observed in some (Fig. 1D and E). Polarized MMP1 secretion in EVTs has not previously been reported, but it is tempting to hypothesize that MMP1 secretion and subsequent ECM degradation occurs in the direction of invasion. However, this assumption could not be evaluated since the histological orientation and potential landmarks of the tissue were lost due to the tissue sampling method.

In this study, we were not able to match cases and controls for gestational age as the deliveries of the cases occurred at a lower gestational age (average 31.4 weeks, range 27–38) than that of controls (average 38.8 weeks, range 38–40). Previous studies have demonstrated that the expression of a number of genes change during gestation [30,31], which implies that some of the differences in gene expression between cases and controls may be ascribed to differences in gestational age. Winn et al. compared gene expression in decidual biopsies from normal pregnancies at different gestational ages and found that 418 of 39,000 studied transcripts were differently expressed between midgestation (weeks 14–24) and term (weeks 37–40) [30]. Seven (CMAH, MAP4, NBN, PLOD2, TNFRSF21, TNMD and vWF) of the 200 differentially expressed transcripts reported in our work were among these gestational age affected genes, and thus, interpretation of these findings should be done with care. On the other hand, Winn et al. reported that 189 of the 200 genes we found to be differently expressed did not change between midgestation and term [30]. Based on this, it is tempting to ascribe the differential expression of these 189 genes to disease related mechanisms. It should be noted that the datasets are not completely comparable, as the cases in our study delivered after midgestation, and no comparison was made in Winn's study between weeks 27–38 vs. 38–40 [30]. Regarding MMP1, the expression in EVTs has been reported stable throughout normal gestation in several publications [6,9,11].

IPA pathway analysis revealed that several genes involved in “cell movement” and “cell-to-cell signalling and interaction” were overrepresented in the dataset, supporting the notion that trophoblast invasion and migration have central roles in the pathogenesis underlying the pregnancy complications studied. It has previously been reported that trophoblasts from pre-eclamptic pregnancies fail to undergo the normal change in cell adhesion repertoire that is linked to the acquisition of an invasive trophoblast phenotype; they retain their expression of E-cadherin and integrin $\alpha6/\beta4$, and fail to upregulate MCAM and integrin $\alpha5/\beta1$ [reviewed by Harris et al.] [32]. In accordance with this, we found decreased expression of MCAM, integrin $\alpha5$ and zinc finger E-box binding homeobox 2 (ZEB2) (a transcription factor that is shown to decrease E-cadherin expression) [33] in cases (Table 2).

Gene expression analysis may provide both new hypotheses about disease mechanisms and suggest candidate genes for further single nucleotide polymorphisms (SNP) analysis. The latter is exemplified by the fact that several of the differential expressed genes found here are located at previously identified PE susceptibility loci (ZEB2 on chromosome 2q22.3 [34], calpastatin (CAST) on chromosome 5q15 [35] and MMP1, -7, -12 and MCAM on

chromosome 11q21–24 [36]. Thus, the observations obtained in the present study may prove helpful in prioritizing among genes located in the quantitative trait loci in future candidate gene studies of PE.

In conclusion, pregnancies complicated by PE and/or FGR demonstrate altered decidual expression of several genes involved in trophoblast invasion, including MMP1. MMP1 protein expression in EVTs is reduced in cases compared to controls, and we suggest that this may be a contributing factor to impaired trophoblast invasion.

Acknowledgements

This study was supported by the Norwegian University of Science and Technology (NTNU), Trondheim and Haukeland University Hospital, Bergen, Norway. Affymetrix GeneChip Analysis was supported by the Norwegian Microarray Consortium (NTNU). We thank the Department of Pathology, Haukeland University Hospital for help in optimizing the immunostaining protocol. Finally, we would like to thank Borgny Ytterhus and Unn Granli at Department of Laboratory Medicine, Children's and Women's Health, NTNU, for performing the immunofluorescence staining.

Appendix. Supplementary data

The supplementary data associated with this article can be found in the online version at doi:10.1016/j.placenta.2010.04.003

References

[1] Brosens IA, Robertson WB, Dixon HG. The role of the spiral arteries in the pathogenesis of preeclampsia. Obstet Gynecol Annu 1972;1:177–91.
[2] Khong TY, De Wolf F, Robertson WB, Brosens I. Inadequate maternal vascular response to placentation in pregnancies complicated by pre-eclampsia and by small-for-gestational age infants. Br J Obstet Gynaecol 1986;93:1049–59.
[3] Lyall F. Mechanisms regulating cytotrophoblast invasion in normal pregnancy and pre-eclampsia. Aust N Z J Obstet Gynaecol 2006;46:266–73.
[4] Fisher SJ, Cui TY, Zhang L, Hartman L, Grahl K, Zhang GY, et al. Adhesive and degradative properties of human placental cytotrophoblast cells in vitro. J Cell Biol 1989;109:891–902.
[5] Hulboy DL, Rudolph LA, Matrisian LM. Matrix metalloproteinases as mediators of reproductive function. Mol Hum Reprod 1997;3:27–45.
[6] Librach CL, Werb Z, Fitzgerald ML, Chiu K, Corwin NM, Esteves RA, et al. 92-kD type IV collagenase mediates invasion of human cytotrophoblasts. J Cell Biol 1991;113:437–49.
[7] Reister F, Kingdom JC, Ruck P, Marzusch K, Heyl W, Pauer U, et al. Altered protease expression by periarterial trophoblast cells in severe early-onset preeclampsia with IUGR. J Perinat Med 2006;34:272–9.
[8] Campbell S, Rowe J, Jackson CJ, Gallery ED. Interaction of cocultured decidual endothelial cells and cytotrophoblasts in preeclampsia. Biol Reprod 2004;71:244–52.
[9] Vettraino IM, Roby J, Tolley T, Parks WC. Collagenase-I, stromelysin-I, and matrilysin are expressed within the placenta during multiple stages of human pregnancy. Placenta 1996;17:557–63.
[10] Gallery ED, Campbell S, Arkell J, Nguyen M, Jackson CJ. Preeclamptic decidual microvascular endothelial cells express lower levels of matrix metalloproteinase-1 than normals. Microvasc Res 1999;57:340–6.
[11] Huppertz B, Kertschanska S, Demir AY, Frank HG, Kaufmann P. Immunohistochemistry of matrix metalloproteinases (MMP), their substrates, and their inhibitors (TIMP) during trophoblast invasion in the human placenta. Cell Tissue Res 1998;291:133–48.
[12] Galewska Z, Bankowski E, Romanowicz L, Jaworski S. Pre-eclampsia (EPH-gestosis)-induced decrease of MMP-s content in the umbilical cord artery. Clin Chim Acta 2003;335:109–15.
[13] Red-Horse K, Zhou Y, Genbacev O, Prakobphol A, Foulk R, McMaster M, et al. Trophoblast differentiation during embryo implantation and formation of the maternal-fetal interface. J Clin Invest 2004;114:744–54.
[14] Report of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy. Am J Obstet Gynecol 2000;183: S1–22.
[15] Marsal K, Persson PH, Larsen T, Lilja H, Selbing A, Sultan B. Intrauterine growth curves based on ultrasonically estimated foetal weights. Acta Paediatr 1996;85:843–8.
[16] Staff AC, Ranheim T, Khoury J, Henriksen T. Increased contents of phospholipids, cholesterol, and lipid peroxides in decidua basalis in women with preeclampsia. Am J Obstet Gynecol 1999;180:587–92.
[17] Harsem NK, Staff AC, He L, Roald B. The decidual suction method: a new way of collecting decidual tissue for functional and morphological studies. Acta Obstet Gynecol Scand 2004;83:724–30.
[18] Eide IP, Isaksen CV, Salvesen KA, Langaas M, Gunther CC, Iversen AC, et al. Fetal growth restriction is associated with reduced FasL expression by decidual cells. J Reprod Immunol 2007;74:7–14.
[19] Brazma A, Hingamp P, Quackenbush J, Sherlock G, Spellman P, Stoeckert C, et al. Minimum information about a microarray experiment (MIAME)-toward standards for microarray data. Nat Genet 2001;29:365–71.
[20] Smyth GK. Limma: linear models for microarray data. In: Bioinformatics and computional biology solutions using R and bioconductor. Gentleman R, Carey S, Dudoit R, Irizarry W, Huber W (Editors). 2005, Springer: New York p. 397–420.
[21] Goeman JJ, Buhlmann P. Analyzing gene expression data in terms of gene sets: methodological issues. Bioinformatics 2007;23:980–7.
[22] Langsrud Ø. Rotation tests. Statistics and Computing 2005;15:53–60.
[23] Irizarry RA, Hobbs B, Collin F, Beazer-Barclay YD, Antonellis KJ, Scherf U, et al. Exploration, normalization, and summaries of high density oligonucleotide array probe level data. Biostatistics 2003;4:249–64.
[24] Benjamini Y. Controlling the false discovery rate; a practical and powerful approach to multiple testing. J Royal Stat Soc 1995;57:289–300.
[25] Langaas F, Lindqvist. Estimating the proportion of true null hypotheses, with application to DNA microarray data. J Royal Stat Soc 2005;67:555–72.
[26] Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 2001;25:402–8.
[27] Amenta PS, Gay S, Vaheri A, Martinez-Hernandez A. The extracellular matrix is an integrated unit: ultrastructural localization of collagen types I, III, IV, V, VI, fibronectin, and laminin in human term placenta. Coll Relat Res 1986;6:125–52.
[28] Ho IA, Chan KY, Ng WH, Guo CM, Hui KM, Cheang P, et al. Matrix metalloproteinase 1 is necessary for the migration of human bone marrow-derived mesenchymal stem cells toward human glioma. Stem Cells 2009;27:1366–75.
[29] Lim KH, Zhou Y, Janatpour M, McMaster M, Bass K, Chun SH, et al. Human cytotrophoblast differentiation/invasion is abnormal in pre-eclampsia. Am J Pathol 1997;151:1809–18.
[30] Winn VD, Haimov-Kochman R, Paquet AC, Yang YJ, Madhusudhan MS, Gormley M, et al. Gene expression profiling of the human maternal–fetal interface reveals dramatic changes between midgestation and term. Endocrinology 2007;148:1059–79.
[31] Mikheev AM, Nabekura T, Kaddoumi A, Bammler TK, Govindarajan R, Hebert MF, et al. Profiling gene expression in human placentae of different gestational ages: an OPRU Network and UW SCOR. Study. Reprod Sci 2008;15:866–77.
[32] Harris LK, Jones CJ, Aplin JD. Adhesion molecules in human trophoblast – a review. II. Extravillous trophoblast. Placenta 2009;30:299–304.
[33] Imamichi Y, Konig A, Gress T, Menke A. Collagen type I-induced Smad-interacting protein 1 expression downregulates E-cadherin in pancreatic cancer. Oncogene 2007;26:2381–5.
[34] Moses EK, Fitzpatrick E, Freed KA, Dyer TD, Forrest S, Elliott K, et al. Objective prioritization of positional candidate genes at a quantitative trait locus for pre-eclampsia on 2q22. Mol Hum Reprod 2006;12:505–12.
[35] Johnson MP, Fitzpatrick E, Dyer TD, Jowett JB, Brennecke SP, Blangero J, et al. Identification of two novel quantitative trait loci for pre-eclampsia susceptibility on chromosomes 5q and 13q using a variance components-based linkage approach. Mol Hum Reprod 2007;13:61–7.
[36] Moses EK, Lade JA, Guo G, Wilton AN, Grehan M, Freed K, et al. A genome scan in families from Australia and New Zealand confirms the presence of a maternal susceptibility locus for pre-eclampsia, on chromosome 2. Am J Hum Genet 2000;67:1581–5.

Paper III

GENETICS

A transcriptional profile of the decidua in preeclampsia

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OBJECTIVE: We sought to obtain insight into possible mechanisms underlying preeclampsia using genomewide transcriptional profiling in decidua basalis.

STUDY DESIGN: Genomewide transcriptional profiling was performed on decidua basalis tissue from preeclamptic (n = 37) and normal (n = 58) pregnancies. Differentially expressed genes were identified and merged into canonical pathways and networks.

RESULTS: Of the 26,504 expressed transcripts detected, 455 were differentially expressed ($P < .05$; false discovery rate, $P < .1$). Both novel (ARL5B, SLITRK4) and previously reported preeclampsia-associated (PLA2G7, HMOX1) genes were identified. Pathway analysis revealed that tryptophan metabolism, endoplasmic reticulum stress, linoleic acid metabolism, notch signaling, fatty acid metabolism, arachidonic acid metabolism, and NRF2-mediated oxidative stress response were overrepresented canonical pathways.

CONCLUSION: In the present study single genes, canonical pathways, and gene-gene networks that are likely to play an important role in the pathogenesis of preeclampsia have been identified. Future functional studies are needed to accomplish a greater understanding of the mechanisms involved.

Key words: decidua, genomewide gene expression, microarray, preeclampsia

Cite this article as: Løset M, Mundal SB, Johnson MP, et al. A transcriptional profile of the decidua in preeclampsia. Am J Obstet Gynecol 2011;204:84.e1-27.

The etiology of preeclampsia is not fully understood, but a number of observations suggest that divergent abnormalities may be involved (immunological, inflammatory, vascular/ischemic).[1] In a normal pregnancy extravillous trophoblasts (of fetal origin) invade decidua basalis and modify the spiral arteries. In preeclampsia, this pregnancy-associated adaption of spiral arteries may fail, with a hypoperfused placenta as a result. Oxidative stress is suggested to play a central role in the pathogenesis of preeclampsia,[2] and may be generated in the decidua basalis.[3,4] Heritability of the disease has been estimated to be >50%,[5,6] with both maternal and fetal (paternal) contributions.[7]

Microarray-based transcriptional profiling can be a powerful strategy for identification of disease-related genes and pathways,[8] and this approach has been used for analysis of placental[9] as well as decidual[6,10,11] tissues from preeclamptic pregnancies. However, the data obtained have been inconsistent. In the case of the 3 decidual studies reported, the diverging results may be due to the relatively small number of samples analyzed (≤12 preeclamptic samples included).[6,10,11] In the current study, we have applied genomewide transcriptional profiling (measuring ≥48,000 transcripts from all known genes) on a large collection of decidual samples (from 37 preeclamptic and 58 normal pregnancies) to comprehensively investigate how gene expression at the maternal-fetal interface may be contributing to the pathogenesis of preeclampsia. We further aimed to identify the genetic canonical pathways and gene-gene interaction networks represented by the differently expressed genes using contemporary bioinformatic approaches.

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Received April 14, 2010; revised July 9, 2010; accepted Aug. 24, 2010.

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This work was supported by grants from the Norwegian University of Science and Technology (Drs Løset, Mundal, and Austgulen); the Central Norway Regional Health Authority, the Research Council of Norway, and the Fulbright Foundation for Educational Exchange (Dr Fenstad); National Institutes of Health Grants R01 HD049847 (Drs Blangero and Moses) and R01 MH059490 (Dr Blangero); and a grant from the Southwest Foundation Forum (Dr Johnson). This investigation was conducted, in part, in facilities constructed with support for the Research Facilities Improvement Program Grant C06 RR017515 from the National Center for Research Resources, National Institutes of Health.

The first 2 authors contributed equally to this work.

0002-9378/$36.00 • © 2011 Mosby, Inc. All rights reserved. • doi: 10.1016/j.ajog.2010.08.043

Materials and Methods

Human subjects

Women with pregnancies complicated by preeclampsia (n = 43) and women with normal pregnancies (n = 59) were recruited at St. Olav's University Hospital (Trondheim, Norway) and Haukeland University Hospital (Bergen, Norway) from 2002 through 2006. Preeclampsia was defined as persistent hypertension (blood pressure of ≥140/90 mm Hg) plus

proteinuria (≥0.3 g/L or ≥1+ by dipstick) developing >20 weeks of pregnancy.[12] Due to tissue sampling procedures, only pregnancies delivered by cesarean section were included. Women with preeclamptic pregnancies had cesarean section performed for medical indications, whereas women with normal pregnancies underwent cesarean section for reasons considered irrelevant to the aim of the study (eg, breech presentation, cephalopelvic disproportion in previous delivery, and fear of vaginal delivery). None of the included mothers were in labor prior to cesarean section. Exclusively healthy women with no history of preeclampsia were accepted in the normal pregnancy group. Multiple pregnancies, pregnancies with chromosomal aberrations, fetal and placental structural abnormalities, or suspected perinatal infections were excluded from both study groups. The study was approved by the Norwegian Regional Committee for Medical Research Ethics. Informed consent was obtained from all participants prior to collection of decidual samples.

Decidual tissue collection

Samples of decidua basalis tissue were obtained by vacuum suction of the placental bed, a procedure that allows the collection of tissue from the whole placental bed.[13] Collected samples were flushed with saline solution to remove excessive blood. The decidual tissue was immediately submerged in RNA-later (Ambion, Austin, TX).

Total RNA isolation

Total RNA was isolated using a TRIzol (Invitrogen, Carlsbad, CA) extraction protocol with chloroform interphase separation, isopropanol precipitation, and ethanol wash steps. Precipitated total RNA was resuspended in Rnase-free water and purified with an RNeasy Mini Kit using spin technology (Qiagen, Valencia, CA). Spectrophotometric determination of purified total RNA yield (μg) was performed using the NanoDrop ND-1000 (Thermo Scientific, Wilmington, DE). Total RNA quality was measured using RNA 6000 Nano Series II Kit on a BioAnalyzer 2100 (Agilent Technologies, Santa Clara, CA). Ethical approval for total RNA processing and decidua expression analysis was obtained from the institutional review board at the University of Texas Health Science Center in San Antonio.

Synthesis, amplification, and purification of antisense RNA

Antisense RNA (aRNA) was synthesized, amplified, and purified using the Illumina TotalPrep RNA Amplification Kit according to manufacturer's instructions (Ambion, Austin, TX). Synthesis of aRNA was performed using a T7 Oligo(dT) primer, and the amplification underwent in vitro transcription with a T7 RNA polymerase to generate multiple copies of biotinylated aRNA from a double-stranded complementary DNA (cDNA) template. Purified aRNA yield was determined spectrophotometrically using the NanoDrop ND-1000.

Microarray data

Purified aRNA was hybridized to Illumina's HumanWG-6 v2 Expression BeadChip (Illumina Inc, San Diego, CA). Washing, blocking, and transcript signal detection (streptavidin-Cy3) was performed using Illumina's 6 × 2 BeadChip protocol. Samples were scanned on the Illumina BeadArray 500GX Reader using Illumina BeadScan image data acquisition software (version 2.3.0.13). Illumina's BeadStudio Gene Expression software module (version 3.2.7) was used to subtract background noise signals and generate an output file for statistical analysis.

Real-time quantitative polymerase chain reaction

We performed a verification of the microarray experiment with quantitative real-time (RT)-polymerase chain reaction (PCR) on 6 of the most differentially expressed transcripts using a 7900HT Fast RT-PCR instrument (Applied Biosystems, Foster City, CA). The 6 genes were prioritized for RT-PCR based on beta values, false discovery rate (FDR) *P* values, and manual literature searches. RT quantitative PCR was run with 93 samples. Two of the total collection of 95 samples were excluded due to shortage of biological material. Preoptimized TaqMan Gene Expression Assays (Applied Biosystems) were run, in triplicate, to measure messenger RNA expression levels relative to the reference genes, TATA box binding protein and glyceraldehyde-3-phosphate dehydrogenase. Reverse transcription and PCR amplification was performed in a 2-step procedure, following Applied Biosystems High-Capacity cDNA ReverseTranscription Kit Protocol and TaqMan Gene Expression Master Mix Protocol. Negative controls were run, in triplicate, without RT enzyme or no cDNA template.

Statistical analysis

Transcript data for each sample were preprocessed and analyzed using our Sequential Oligogenic Linkage Analysis Routines (SOLAR) statistical analysis software program,[14] as previously described.[15] To evaluate the magnitude of differential gene expression the displacement of each detected transcript's mean expression value was measured between the 2 groups. A standard regression analysis was performed on the preeclamptic group to test whether the mean transcription level differed from that of the normal pregnancy group.

The messenger RNA expression levels were calculated by the Comparative threshold cycle (CT) method, as described elsewhere.[16] For each target gene, the mean CT value for each sample was used for analysis, after exclusion of outliers. Outliers were determined as values >2SD from the mean. Delta CT (ΔCT) values were computed as the difference between the given mean value for a target gene and the mean of the CT values for the 2 reference genes.[17] Fold change values were calculated, based on the differences in ΔCT values between tissue from preeclamptic women and women with normal pregnancy ($2^{-\Delta\Delta CT}$).[16] A *t* test statistic (SPSS, version 16; SPSS, Inc, Chicago, IL) evaluated the difference between the ΔCT values of the preeclamptic pregnancies, compared with the normal pregnancy group. Analyzing for the 2 reference genes separately did not change the results.

Canonical pathway and network identification

Differentially expressed transcripts in the preeclamptic group ($P < .05$; FDR,[18]

$P < .1$) were imported into Ingenuity Pathways Analysis (IPA, v7.5; Ingenuity Systems, Redwood City, CA). Transcripts' gene identifiers were mapped to their corresponding gene object in the Ingenuity Pathways Knowledge Base. IPA was used to bioinformatically identify canonical (ie, cell signaling and metabolic) pathways and gene-gene interaction networks potentially involved in preeclampsia within our dataset. IPA gene-gene networks were constructed from the published literature, and they diagrammatically represent molecular relationships between gene-gene products.

Significant IPA pathways were further analyzed with Rotation Gene Set Enrichment Analysis (ROMER; Fred Hutchinson Cancer Research Center, Seattle, WA) pathway analysis, using the *limma* package, available via the Bioconductor Project (Fred Hutchinson Cancer Research Center).[19]

TABLE 1
Clinical characteristics of study groups

Variable	Preeclamptic pregnancies[a] (n = 37)	Normal pregnancies[a] (n = 58)
Gestational age, wk	32 ± 4[b]	39 ± 1
Systolic blood pressure, mm Hg	152 ± 16[b]	116 ± 10
Diastolic blood pressure, mm Hg	96 ± 10[b]	70 ± 9.0
Birthweight, g	1555 ± 769[b]	3619 ± 469
Body mass index, kg/m^{2}[c]	27.7 ± 6.2	25.3 ± 5.7

Values are means ± SD.

[a] In all, 43 women with pregnancies complicated by preeclampsia and 59 women with normal pregnancies were included in the study. Six samples from preeclamptic pregnancies and 1 sample from normal pregnancy were excluded from gene expression analysis due to low RNA quality; [b] $P < .001$ obtained with *t* test statistics with software (SPSS, version 16; SPSS, Inc, Chicago, IL); [c] Body mass index was measured at first antenatal care visit.

Løset. A transcriptional profile of the decidua in preeclampsia. Am J Obstet Gynecol 2011.

RESULTS

Human subjects

The clinical information of women/pregnancies enrolled is presented in Table 1. Only those samples of sufficient RNA quality for gene expression analysis have been included. In the preeclamptic pregnancies, both mean gestational age and birthweight were lower than in the normal pregnancies (Table 1). As expected, the mean blood pressure was higher among preeclamptic than normal pregnancies (Table 1).

Decidual genomewide transcriptional profiling

In total, 43 women with pregnancies complicated by preeclampsia and 59 women with normal pregnancies were included in the study. Six samples from preeclamptic pregnancies and 1 sample from a normal pregnancy were excluded from gene expression analyses due to low RNA quality. The 95 samples with good RNA quality were hybridized onto Illumina's HumanWG-6 v2 genomewide Expression BeadChip.

The nonnormalized decidua basalis transcriptional profile data (n = 48,095) may be found at ArrayExpress (European Molecular Biology Laboratory-European Bioinformatics Institute, Hinxton, UK) (accession code E-TABM-682). We detected 26,504 significantly expressed transcripts (55.1%), of which 455 were differentially expressed after FDR correction ($P < .05$; FDR, $P < .1$); 285 were down-regulated and 170 were up-regulated. The significant differentially expressed transcripts are presented in Table 2, together with the corresponding *P* values (raw and FDR adjusted) and preeclampsia-correlated expression. The RT quantitative PCR for the 6 genes (PLA2G7, ANGPTL2, MAN1A2, SLITRK4, FZD4, and ARL5B) tested showed a high grade of correlation with the microarray data (Table 3).

Canonical pathways and network

The 455 differentially expressed transcripts were analyzed using IPA. The significant canonical pathways ($P < .01$) are shown in Table 4, along with the included genes and *P* values. They included tryptophan metabolism, endoplasmic reticulum (ER) stress, linoleic acid metabolism, notch signaling, fatty acid metabolism, arachidonic acid metabolism, and NRF2-mediated oxidative stress response. All the canonical pathways identified in IPA were also found to be significant ($P < .01$) using ROMER (Table 4), with the exception of the NRF2-mediated oxidative stress response canonical pathway (IPA, $P = .009$; ROMER, $P = .067$).

Using network analysis in IPA, 59 of the preeclampsia-associated genes could be connected into a single network of gene-gene product interactions (Figure). The genes in this network were among others involved in the function of ER, oxidative stress, notch signaling, and cell migration. The network included a cluster of 15 up-regulated genes (ATP2A2, TRAM1, FKBP2, HMOX1, SPCS2, ATF6, DNAJC3, EIF2AK3, PIGA, SEC23B, SEC24D, DNAJB9, SRPRB, DNAJB11, and X-box binding protein 1 [XBP1]) associated with ER stress and oxidative stress (Figure). All these genes were in a direct relationship to XBP1. Epidermal growth factor receptor (EGFR) was another focus molecule with a direct relationship to 7 other genes (PLCG1, NGF, MET, LRIG1, SLN, ATP2A2, and SHC2) in the network.

COMMENT

In this study, 455 differentially expressed transcripts were found when decidua basalis tissue from preeclamptic and normal pregnancies was compared. Some transcripts were novel findings (ie, ARL5B and SLITRK4), whereas others, such as PLA2G7[20] and HMOX1,[21,22] have been reported to be associated with preeclampsia previously. Pathway analysis identified 7 significant canonical pathways.

In our patient cohort, a lower gestational age was found in the preeclamptic group (average, 32 weeks; range, 28–36) compared with the normal pregnancy group (39 weeks; range, 38–40). This is not unexpected due to the need for early delivery in patients with severe preeclampsia. Since gene expression in uteroplacental tissues

TABLE 2
Differentially expressed transcripts[a]

Illumina ID	GenBank ID	Symbol	Definition	Ch	Beta value[b]	*P* value[c]	FDR *P* value[d]
ILMN_1782259	NM_173078.2	SLITRK4	SLIT and NTRK-like family, member 4	X	−1.0363	4.6×10^{-8}	.0012
ILMN_1680465	NM_178815.3	ARL5B	ADP-ribosylation factor-like 5B	10	0.9122	4.5×10^{-7}	.0039
ILMN_1743367	NM_012193.2	FZD4	Frizzled homolog 4 (Drosophila)	11	−0.9122	4.1×10^{-7}	.0054
ILMN_1726210	NM_178172.2	LOC338328	High density lipoprotein–binding protein	8	−0.8672	3.7×10^{-6}	.0088
ILMN_1709222	NM_005692.3	ABCF2	ATP-binding cassette, subfamily F (GCN20), member 2, nuclear gene encoding mitochondrial protein, transcript variant 2	7	−0.8634	3.5×10^{-6}	.0093
ILMN_1772612	NM_012098.2	ANGPTL2	Angiopoietin-like 2	9	−0.8884	4.4×10^{-6}	.0097
ILMN_1659792	NM_014213.2	HOXD9	Homeobox D9	2	−0.8541	3.5×10^{-6}	.0102
ILMN_1813295	NM_018640.3	LMO3	LIM domain only 3 (rhombotin-like 2), transcript variant 1	12	−0.8992	3.3×10^{-6}	.0110
ILMN_1669023	NM_020482.3	FHL5	Four and a half LIM domains 5	6	−0.8489	3.2×10^{-6}	.0123
ILMN_1658677	NM_178502.2	DTX3	Deltex 3 homolog (Drosophila)	12	−0.9038	2.9×10^{-6}	.0130
ILMN_1812461	NM_003881.2	WISP2	WNT1 inducible signaling pathway protein 2	20	−0.8717	6.6×10^{-6}	.0134
ILMN_1776157	NM_080415.1	SEPT4	Septin 4, transcript variant 2	17	−0.8882	2.6×10^{-6}	.0140
ILMN_1794370	NM_001031702.2	SEMA5B	Sema domain, 7 thrombospondin repeats (type 1 and type 1-like), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 5B, transcript variant 1	3	−0.8695	8.0×10^{-6}	.0141
ILMN_1719069	NM_213596.1	FOXN4	Forkhead box N4	12	−0.8803	7.8×10^{-6}	.0147
ILMN_1733667	NM_021931.2	DHX35	DEAH (Asp-Glu-Ala-His) box polypeptide 35	20	−0.8537	9.0×10^{-6}	.0149
ILMN_1734276	NM_199169.1	TMEPAI	Transmembrane, prostate androgen-induced RNA, transcript variant 2	20	−0.8360	1.6×10^{-5}	.0153
ILMN_1701195	NM_005084.2	PLA2G7	Phospholipase A2, group VII (platelet-activating factor acetylhydrolase, plasma)	6	0.8305	1.6×10^{-5}	.0155
ILMN_1687821	NM_033201.1	C16orf45	Chromosome 16 open reading frame 45	16	−0.8218	1.4×10^{-5}	.0156
ILMN_1736911	NM_003275.2	TMOD1	Tropomodulin 1	9	−0.8178	1.5×10^{-5}	.0157
ILMN_1744487	NM_015645.2	C1QTNF5	C1q and tumor necrosis factor-related protein 5	11	−0.8113	1.7×10^{-5}	.0157
ILMN_1767556	NM_007021.2	C10orf10	Chromosome 10 open reading frame 10	10	−0.7966	1.3×10^{-5}	.0158
ILMN_1668249	NM_022773.2	TMEM112	Transmembrane protein 112	16	−0.8079	1.6×10^{-5}	.0158
ILMN_1788462	NM_001033059.1	AMD1	Adenosylmethionine decarboxylase 1, transcript variant 2	6	0.8027	1.4×10^{-5}	.0160
ILMN_1665945	NM_022735.3	ACBD3	acyl-Coenzyme A binding domain containing 3	1	0.8297	1.3×10^{-5}	.0164

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(continued)

TABLE 2
Differentially expressed transcripts[a] *(continued)*

Illumina ID	GenBank ID	Symbol	Definition	Ch	Beta value[b]	*P* value[c]	FDR *P* value[d]
ILMN_1657803	NM_001014975.1	CFH	Complement factor H, transcript variant 2	1	−0.8780	2.5×10^{-6}	.0164
ILMN_1880012	NM_003966.2	SEMA5A	Sema domain, 7 thrombospondin repeats (type 1 and type 1-like), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 5A	5	0.8208	2.7×10^{-5}	.0168
ILMN_1763036	NM_001286.2	CLCN6	Chloride channel 6, transcript variant ClC-6a	1	−0.8027	2.4×10^{-5}	.0170
ILMN_1710962	NM_014573.2	TMEM97	Transmembrane protein 97	17	0.8236	2.6×10^{-5}	.0171
ILMN_1801927	NM_001004311.2	FIGLA	Folliculogenesis-specific basic helix-loop-helix	2	−0.8616	1.1×10^{-5}	.0171
ILMN_1673773	NM_198516.1	GALNTL4	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetyl-galactosaminyltransferase-like 4	11	−0.7998	2.3×10^{-5}	.0172
ILMN_1711516	NM_001690.2	ATP6V1A	ATPase, H+ transporting, lysosomal 70 kDa, V1 subunit A	3	0.8101	1.2×10^{-5}	.0172
ILMN_1715555	NM_001352.2	DBP	D site of albumin promoter (albumin D-box) binding protein	19	−0.7916	1.3×10^{-5}	.0172
ILMN_1779632	NM_001001723.1	TMEM1	Transmembrane protein 1, transcript variant 2	21	0.8054	2.6×10^{-5}	.0172
ILMN_1685703	NM_003500.2	ACOX2	acyl-Coenzyme A oxidase 2, branched chain	3	−0.8253	2.2×10^{-5}	.0173
ILMN_1711157	NM_004557.3	NOTCH4	Notch homolog 4 (Drosophila)	6	−0.7709	2.5×10^{-5}	.0174
ILMN_1740160	NM_182811.1	PLCG1	Phospholipase C, gamma 1, transcript variant 2	20	−0.8077	2.1×10^{-5}	.0176
ILMN_1834017	N25708	Hs.573236	yx79f04s1 Soares melanocyte 2NbHM cDNA clone IMAGE: 267967 3 sequence		0.8058	2.3×10^{-5}	.0176
ILMN_1798076	NM_006898.4	HOXD3	Homeobox D3	2	−0.8238	2.3×10^{-5}	.0176
ILMN_1705985	NM_020473.2	PIGA	Phosphatidylinositol glycan anchor biosynthesis, class A (paroxysmal nocturnal hemoglobinuria), transcript variant 3	X	0.7983	2.6×10^{-5}	.0177
ILMN_1772302	NM_006441.1	MTHFS	5,10-Methenyltetrahydrofolate synthetase (5-formyltetrahydrofolate cyclo-ligase)	15	0.7802	2.9×10^{-5}	.0178
ILMN_1781791	NM_000950.1	PRRG1	Proline-rich Gla (G-carboxyglutamic acid) 1	X	0.7681	3.2×10^{-5}	.0179
ILMN_1748812	NM_152913.1	TMEM130	Transmembrane protein 130	7	−0.7814	3.0×10^{-5}	.0179
ILMN_1680774	XM_001132373.1	LOC730994	Similar to NACHT, leucine-rich repeat and PYD (pyrin domain) containing 1, transcript variant 1	17	−0.8034	2.0×10^{-5}	.0179
ILMN_1755120	NM_006699.3	MAN1A2	Mannosidase, alpha, class 1A, member 2	1	0.8519	1.3×10^{-5}	.0180

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(continued)

TABLE 2
Differentially expressed transcripts[a] *(continued)*

Illumina ID	GenBank ID	Symbol	Definition	Ch	Beta value[b]	*P* value[c]	FDR *P* value[d]
ILMN_1788166	NM_003318.3	TTK	TTK protein kinase	6	0.8169	2.1×10^{-5}	.0181
ILMN_1685608	NM_002523.1	NPTX2	Neuronal pentraxin II	7	−0.7865	3.1×10^{-5}	.0181
ILMN_1678842	NM_003247.2	THBS2	Thrombospondin 2	6	−0.8054	3.2×10^{-5}	.0182
ILMN_1813430	NM_182985.3	TRIM69	Tripartite motif-containing 69, transcript variant a	15	−0.8120	3.5×10^{-5}	.0192
ILMN_1675936	NM_016438.2	HIGD1B	HIG1 domain family, member 1B	17	−0.8060	3.9×10^{-5}	.0202
ILMN_1877909	BX105647	Hs.125533	BX105647 Soares_NFL_T_GBC_S1 cDNA clone IMAGp998F143713 sequence		−0.7992	3.7×10^{-5}	.0202
ILMN_1803279	NM_016040.3	TMED5	Transmembrane emp24 protein transport domain containing 5	1	0.7904	3.8×10^{-5}	.0202
ILMN_1700202	NM_022918.2	TMEM135	Transmembrane protein 135	11	0.7615	4.1×10^{-5}	.0206
ILMN_1727589	NM_004605.2	SULT2B1	Sulfotransferase family, cytosolic, 2B, member 1, transcript variant 1	19	0.7826	4.2×10^{-5}	.0209
ILMN_1811873	NM_002889.2	RARRES2	Retinoic acid receptor responder (tazarotene induced) 2	7	−0.7690	4.4×10^{-5}	.0214
ILMN_1703955	NM_148177.1	FBXO32	F-box protein 32, transcript variant 2	8	−0.8049	4.8×10^{-5}	.0225
ILMN_1731358	NM_018181.4	ZNF532	Zinc finger protein 532	18	−0.7974	4.7×10^{-5}	.0226
ILMN_1682937	NM_001038633.2	RSPO1	R-spondin homolog (Xenopus laevis)	1	−0.7973	5.0×10^{-5}	.0230
ILMN_1695947	NM_174934.2	SCN4B	Sodium channel, voltage-gated, type IV, beta	11	−0.7948	5.8×10^{-5}	.0234
ILMN_1707342	NM_015541.2	LRIG1	Leucine-rich repeats and immunoglobulin-like domains 1	3	−0.7679	5.8×10^{-5}	.0235
ILMN_1781626	NM_001734.2	C1S	Complement component 1, s subcomponent, transcript variant 1	12	−0.7833	5.7×10^{-5}	.0236
ILMN_1676215	NM_001364.2	DLG2	Discs, large homolog 2, chapsyn-110 (Drosophila)	11	−0.7928	5.6×10^{-5}	.0238
ILMN_1880210	BC038188	Hs.179213	Homo sapiens, clone IMAGE: 3451765		0.7666	5.7×10^{-5}	.0239
ILMN_1767225	NM_006092.1	NOD1	Nucleotide-binding oligomerization domain containing 1	7	−0.7808	5.3×10^{-5}	.0239
ILMN_1793410	NM_021021.2	SNTB1	Syntrophin, beta 1 (dystrophin-associated protein A1, 59 kDa, basic component 1)	8	0.7636	5.5×10^{-5}	.0239
ILMN_1752837	NM_018184.2	ARL8B	ADP-ribosylation factor-like 8B	3	0.7644	5.3×10^{-5}	.0241
ILMN_1791949	NM_032507.2	PGBD1	PiggyBac transposable element-derived 1	6	−0.7478	5.5×10^{-5}	.0243
ILMN_1859863	BM458075	Hs.555181	AGENCOURT_6411402 NIH_MGC_71 cDNA clone IMAGE:5530423 5 sequence		0.7667	6.4×10^{-5}	.0248

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(continued)

TABLE 2
Differentially expressed transcripts[a] *(continued)*

Illumina ID	GenBank ID	Symbol	Definition	Ch	Beta value[b]	*P* value[c]	FDR *P* value[d]
ILMN_1782788	NM_003651.3	CSDA	Cold shock domain protein A	12	−0.7756	6.3×10^{-5}	.0251
ILMN_1727740	NM_006372.3	SYNCRIP	Synaptotagmin binding, cytoplasmic RNA interacting protein	6	0.6949	6.7×10^{-5}	.0253
ILMN_1677396	NM_019080.1	NDFIP2	Nedd4 family interacting protein 2	13	0.7591	6.6×10^{-5}	.0253
ILMN_1744191	NM_003042.2	SLC6A1	Solute carrier family 6 (neurotransmitter transporter, GABA), member 1	3	−0.7914	6.9×10^{-5}	.0253
ILMN_1656129	NM_020342.1	SLC39A10	Solute carrier family 39 (zinc transporter), member 10	2	0.7306	6.8×10^{-5}	.0253
ILMN_1809639	NM_178505.5	TMEM26	Transmembrane protein 26	10	0.7732	7.9×10^{-5}	.0287
ILMN_1786326	NM_024076.1	KCTD15	Potassium channel tetramerization domain containing 15	19	−0.7853	8.2×10^{-5}	.0291
ILMN_1651343	NM_001004439.1	ITGA11	Integrin, alpha 11	15	−0.7812	8.2×10^{-5}	.0292
ILMN_1739887	NM_031491.2	RBP5	Retinol-binding protein 5, cellular	12	−0.7607	8.7×10^{-5}	.0304
ILMN_1716247	NM_203371.1	FIBIN	Fin bud initiation factor	11	−0.7760	8.9×10^{-5}	.0307
ILMN_1752668	NM_015345.2	DAAM2	Disheveled-associated activator of morphogenesis 2	6	−0.7617	1.0×10^{-4}	.0309
ILMN_1789243	NM_018668.3	VPS33B	Vacuolar protein sorting 33 homolog B (yeast)	15	−0.7368	1.0×10^{-4}	.0312
ILMN_1763852	NM_001093.3	ACACB	acetyl-Coenzyme A carboxylase beta	12	−0.7651	9.6×10^{-5}	.0314
ILMN_1731561	NM_022370.2	ROBO3	Roundabout, axon guidance receptor, homolog 3 (Drosophila)	11	−0.7335	1.0×10^{-4}	.0314
ILMN_1672635	NM_182947.2	GEFT	RhoA/RAC/CDC42 exchange factor, transcript variant 1	12	−0.7711	9.3×10^{-5}	.0315
ILMN_1691181	NM_030755.4	TXNDC1	Thioredoxin domain containing 1	14	0.7498	1.1×10^{-4}	.0315
ILMN_1742034	NM_000261.1	MYOC	Myocilin, trabecular meshwork-inducible glucocorticoid response	1	−0.7416	1.0×10^{-4}	.0315
ILMN_1761968	NM_033256.1	PPP1R14A	Protein phosphatase 1, regulatory (inhibitor) subunit 14A	19	−0.7785	9.5×10^{-5}	.0315
ILMN_1703142	NM_001005416.1	MARCH2	Membrane-associated ring finger (C3HC4) 2, transcript variant 3	19	−0.7337	1.0×10^{-4}	.0316
ILMN_1752225	NR_002330.1	ST7OT1	ST7 overlapping transcript 1 (antisense noncoding RNA)	7	−0.7606	9.8×10^{-5}	.0318
ILMN_1667692	NM_000961.3	PTGIS	Prostaglandin I2 (prostacyclin) synthase	20	−0.7787	9.5×10^{-5}	.0318
ILMN_1691457	NM_004900.3	APOBEC3B	Apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3B	22	0.7343	1.0×10^{-4}	.0319

Løset. A transcriptional profile of the decidua in preeclampsia. Am J Obstet Gynecol 2011. *(continued)*

TABLE 2
Differentially expressed transcripts[a] *(continued)*

Illumina ID	GenBank ID	Symbol	Definition	Ch	Beta value[b]	*P* value[c]	FDR *P* value[d]
ILMN_1728979	NM_207310.1	CCDC74B	Coiled-coil domain containing 74B	2	−0.7428	1.2×10^{-4}	.0320
ILMN_1688346	NM_176814.3	ZNF800	Zinc finger protein 800	7	0.7259	1.2×10^{-4}	.0323
ILMN_1682428	NM_144584.1	C1orf59	Chromosome 1 open reading frame 59	1	0.7635	1.2×10^{-4}	.0323
ILMN_1755173	NM_020904.1	PLEKHA4	Pleckstrin homology domain containing, family A (phosphoinositide binding specific) member 4	19	−0.7470	1.1×10^{-4}	.0324
ILMN_1782954	NM_005339.3	UBE2K	Ubiquitin-conjugating enzyme E2-25K	4	0.7190	1.2×10^{-4}	.0324
ILMN_1735996	NM_016931.2	NOX4	NADPH oxidase 4	11	−0.7504	1.3×10^{-4}	.0325
ILMN_1680110	NM_006829.2	C10orf116	Chromosome 10 open reading frame 116	10	−0.7497	1.2×10^{-4}	.0325
ILMN_1755832	NM_000435.2	NOTCH3	Notch homolog 3 (Drosophila)	19	−0.7589	1.1×10^{-4}	.0325
ILMN_1800463	NM_017859.2	UCKL1	Uridine-cytidine kinase 1-like 1	20	−0.7338	1.2×10^{-4}	.0326
ILMN_1674337	NM_004470.2	FKBP2	FK506 binding protein 2, 13 kDa, transcript variant 1	11	0.7401	1.2×10^{-4}	.0327
ILMN_1807171	NM_000929.2	PLA2G5	Phospholipase A2, group V	1	−0.7349	1.3×10^{-4}	.0327
ILMN_1724671	NM_207577.1	MAP6	Microtubule-associated protein 6, transcript variant 2	11	−0.7623	1.2×10^{-4}	.0328
ILMN_1655117	NM_025132.3	WDR19	WD repeat domain 19	4	−0.7425	1.3×10^{-4}	.0328
ILMN_1706511	NM_003216.2	TEF	Thyrotrophic embryonic factor	22	−0.7288	1.1×10^{-4}	.0328
ILMN_1677018	NM_002141.4	HOXA4	Homeobox A4	7	−0.7424	1.3×10^{-4}	.0333
ILMN_1785646	NM_153321.1	PMP22	Peripheral myelin protein 22, transcript variant 2	17	−0.7487	1.3×10^{-4}	.0334
ILMN_1709661	NM_145276.1	ZNF563	Zinc finger protein 563	19	−0.7481	1.4×10^{-4}	.0334
ILMN_1736863	NM_018295.2	TMEM140	Transmembrane protein 140	7	−0.7336	1.3×10^{-4}	.0337
ILMN_1807379	NM_023034.1	WHSC1L1	Wolf-Hirschhorn syndrome candidate 1-like 1, transcript variant long	8	0.7237	1.4×10^{-4}	.0338
ILMN_1740842	NM_005407.1	SALL2	Sal-like 2 (Drosophila)	14	−0.7458	1.4×10^{-4}	.0340
ILMN_1734229	NM_032802.3	SPPL2A	Signal peptide peptidase-like 2A	15	0.7168	1.4×10^{-4}	.0343
ILMN_1696003	NM_006496.1	GNAI3	Guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 3	1	0.7101	1.5×10^{-4}	.0343
ILMN_1793770	NM_058246.3	DNAJB6	DnaJ (Hsp40) homolog, subfamily B, member 6, transcript variant 1	7	−0.7448	1.4×10^{-4}	.0343
ILMN_1797861	NM_002184.2	IL6ST	Interleukin 6 signal transducer (gp130, oncostatin M receptor), transcript variant 1	5	0.7406	1.6×10^{-4}	.0353
ILMN_1720865	NM_145798.2	OSBPL7	Oxysterol binding protein-like 7, transcript variant 1	17	−0.7298	1.6×10^{-4}	.0355
ILMN_1713978	NM_006923.2	SDF2	Stromal cell-derived factor 2	17	0.7243	1.6×10^{-4}	.0356

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(continued)

TABLE 2
Differentially expressed transcripts[a] *(continued)*

Illumina ID	GenBank ID	Symbol	Definition	Ch	Beta value[b]	*P* value[c]	FDR *P* value[d]
ILMN_1682231	NM_001003682.2	TTMB	TTMB protein	1	−0.7581	1.6×10^{-4}	.0356
ILMN_1684554	NM_001856.3	COL16A1	Collagen, type XVI, alpha 1	1	−0.7316	1.5×10^{-4}	.0356
ILMN_1778595	NM_003063.2	SLN	Sarcolipin	11	−0.7375	1.6×10^{-4}	.0356
ILMN_1811790	NM_004118.3	FKHL18	Forkhead-like 18 (Drosophila)	20	−0.7197	1.6×10^{-4}	.0357
ILMN_1712461	NM_004352.1	CBLN1	Cerebellin 1 precursor	16	−0.7413	1.5×10^{-4}	.0358
ILMN_1815874	NM_018946.2	NANS	N-acetylneuraminic acid synthase (sialic acid synthase)	9	0.7205	1.7×10^{-4}	.0359
ILMN_1720819	XM_934796.2	LOC653566	Similar to signal peptidase complex subunit 2 (microsomal signal peptidase 25-kDa subunit) (SPase 25-kDa subunit), transcript variant 3	1	0.6515	1.7×10^{-4}	.0359
ILMN_1669898	NM_201446.1	EGFL7	EGF-like-domain, multiple 7, transcript variant 2	9	−0.6935	1.5×10^{-4}	.0359
ILMN_1740441	NM_000398.4	CYB5R3	Cytochrome b5 reductase 3, transcript variant M	22	−0.7263	1.7×10^{-4}	.0360
ILMN_1700274	NM_031442.2	TMEM47	Transmembrane protein 47	X	−0.7303	1.6×10^{-4}	.0360
ILMN_1720889	NM_001017369.1	SC4MOL	Sterol-C4-methyl oxidase-like, transcript variant 2	4	0.6822	1.7×10^{-4}	.0367
ILMN_1793543	NM_144697.2	C1orf51	Chromosome 1 open reading frame 51	1	−0.7115	1.8×10^{-4}	.0376
ILMN_1734288	NM_152511.3	DUSP18	Dual specificity phosphatase 18	22	−0.7243	1.9×10^{-4}	.0383
ILMN_1678998	NM_014665.1	LRRC14	Leucine-rich repeat containing 14	8	−0.7119	1.9×10^{-4}	.0383
ILMN_1791508	NM_024302.3	MMP28	Matrix metallopeptidase 28, transcript variant 1	17	−0.7246	1.9×10^{-4}	.0385
ILMN_1688295	NM_016423.1	ZNF219	Zinc finger protein 219	14	−0.7437	1.9×10^{-4}	.0388
ILMN_1770293	NM_001730.3	KLF5	Kruppel-like factor 5 (intestinal)	13	0.7122	1.9×10^{-4}	.0388
ILMN_1886424	BG621061	Hs.559870	602616941F1 NIH_MGC_79 cDNA clone IMAGE:4730410 5 sequence		−0.7236	1.9×10^{-4}	.0388
ILMN_1697006	XM_930748.2	LOC642361	Hypothetical protein LOC642361	10	−0.6926	2.2×10^{-4}	.0400
ILMN_1673543	NM_018290.2	PGM2	Phosphoglucomutase 2	4	0.6845	2.0×10^{-4}	.0401
ILMN_1742230	NM_182648.1	BAZ1A	Bromodomain adjacent to zinc finger domain, 1A, transcript variant 2	14	0.7376	2.1×10^{-4}	.0401
ILMN_1659843	NM_006260.2	DNAJC3	DnaJ (Hsp40) homolog, subfamily C, member 3	13	0.7094	2.1×10^{-4}	.0401
ILMN_1696585	NM_017671.4	C20orf42	Chromosome 20 open reading frame 42	20	0.7335	2.1×10^{-4}	.0402
ILMN_1763641	NM_025040.2	ZNF614	Zinc finger protein 614	19	0.7013	2.1×10^{-4}	.0402
ILMN_1726678	NM_014147.1	HSPC047	HSPC047 protein	7	−0.7153	2.0×10^{-4}	.0402
ILMN_1779034	NM_018161.4	NADSYN1	NAD synthetase 1	11	−0.6854	2.1×10^{-4}	.0402
ILMN_1705253	NM_130393.2	PTPRD	Protein tyrosine phosphatase, receptor type, D, transcript variant 4	9	0.7342	2.1×10^{-4}	.0403

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(continued)

TABLE 2
Differentially expressed transcripts[a] *(continued)*

Illumina ID	GenBank ID	Symbol	Definition	Ch	Beta value[b]	*P* value[c]	FDR *P* value[d]
ILMN_1837017	CB269825	Hs.543359	1008732 Human Fat Cell 5-Stretch Plus cDNA Library cDNA 5' sequence		−0.7281	2.1×10^{-4}	.0405
ILMN_1829490	BX106357	Hs.445732	BX106357 Soares_NFL_T_GBC_S1 cDNA clone IMAGp998B055155 sequence		0.6957	2.2×10^{-4}	.0409
ILMN_1714691	NM_002148.3	HOXD10	Homeobox D10	2	−0.7265	2.3×10^{-4}	.0413
ILMN_1803213	NM_015419.2	MXRA5	Matrix-remodeling-associated 5	X	−0.7061	2.3×10^{-4}	.0416
ILMN_1732158	NM_001460.2	FMO2	Flavin containing monooxygenase 2 (nonfunctional)	1	−0.6950	2.4×10^{-4}	.0424
ILMN_1681938	NM_022568.2	ALDH8A1	Sldehyde dehydrogenase 8 family, member A1, transcript variant 1	6	0.6875	2.4×10^{-4}	.0424
ILMN_1753243	NM_016306.4	DNAJB11	DnaJ (Hsp40) homolog, subfamily B, member 11	3	0.7183	2.5×10^{-4}	.0431
ILMN_1793846	NM_014670.2	BZW1	Basic leucine zipper and W2 domains 1	2	0.7033	2.7×10^{-4}	.0431
ILMN_1852159	BF753039	Hs.557431	RC3-BN0425-011200-022-c08 BN0425 cDNA sequence		−0.7234	2.4×10^{-4}	.0432
ILMN_1805992	NM_018330.4	KIAA1598	KIAA1598	10	0.7077	2.4×10^{-4}	.0433
ILMN_1740512	XM_936687.1	MGC39900	Hypothetical protein MGC39900	X	−0.7227	2.6×10^{-4}	.0433
ILMN_1708916	NM_032512.2	PDZD4	PDZ domain containing 4	X	−0.7075	2.7×10^{-4}	.0434
ILMN_1773563	NM_015927.3	TGFB1I1	Transforming growth factor beta 1-induced transcript 1, transcript variant 2	16	−0.7335	2.6×10^{-4}	.0435
ILMN_1674184	NM_153022.2	C12orf59	Chromosome 12 open reading frame 59	12	0.7122	2.6×10^{-4}	.0436
ILMN_1657483	NM_032985.4	SEC23B	Sec23 homolog B (S cerevisiae), transcript variant 2	20	0.6717	2.7×10^{-4}	.0436
ILMN_1772540	NM_015251.2	ASCIZ	ATM/ATR-Substrate Chk2-Interacting Zn2+-finger protein	16	0.6872	2.6×10^{-4}	.0438
ILMN_1756862	NM_145641.1	APOL3	Apolipoprotein L, 3, transcript variant beta/a	22	−0.7021	2.8×10^{-4}	.0438
ILMN_1685413	NM_024079.4	ALG8	Asparagine-linked glycosylation 8 homolog (S cerevisiae, alpha-1,3-glucosyltransferase), transcript variant 1	11	0.6986	2.8×10^{-4}	.0439
ILMN_1686645	NM_021645.4	UTP14C	UTP14, U3 small nucleolar ribonucleoprotein, homolog C (yeast)	13	0.6746	2.8×10^{-4}	.0440
ILMN_1813746	NM_003389.2	CORO2A	Coronin, actin-binding protein, 2A, transcript variant 1	9	0.7135	2.6×10^{-4}	.0440
ILMN_1765557	NM_015441.1	OLFML2B	Olfactomedin-like 2B	1	−0.6714	2.7×10^{-4}	.0441
ILMN_1740586	NM_000300.2	PLA2G2A	Phospholipase A2, group IIA (platelets, synovial fluid)	1	−0.7049	2.6×10^{-4}	.0443

Løset. A transcriptional profile of the decidua in preeclampsia. Am J Obstet Gynecol 2011. *(continued)*

TABLE 2
Differentially expressed transcripts[a] *(continued)*

Illumina ID	GenBank ID	Symbol	Definition	Ch	Beta value[b]	*P* value[c]	FDR *P* value[d]
ILMN_1758750	NR_003501.1	EARS2	Glutamyl-tRNA synthetase 2, mitochondrial (putative), transcript variant 2, transcribed RNA	16	0.7199	2.6×10^{-4}	.0444
ILMN_1703178	NM_003469.3	SCG2	Secretogranin II (chromogranin C)	2	−0.7239	2.6×10^{-4}	.0444
ILMN_1710522	NM_175635.1	RUNX1T1	Runt-related transcription factor 1; translocated to, 1 (cyclin D-related), transcript variant 3	8	−0.6886	3.1×10^{-4}	.0444
ILMN_1730048	NM_024067.2	C7orf26	Chromosome 7 open reading frame 26	7	−0.6943	2.9×10^{-4}	.0444
ILMN_1722855	NM_003377.3	VEGFB	Vascular endothelial growth factor B	11	−0.7039	3.0×10^{-4}	.0445
ILMN_1752915	NM_004124.2	GMFB	Glia maturation factor, beta	14	0.6872	2.6×10^{-4}	.0445
ILMN_1702124	NM_153371.3	LNX2	Ligand of numb-protein X 2	13	0.7044	3.0×10^{-4}	.0445
ILMN_1695299	NM_014476.1	PDLIM3	PDZ and LIM domain 3	4	−0.7140	3.1×10^{-4}	.0445
ILMN_1666364	NM_144576.3	COQ10A	Coenzyme Q10 homolog A (S cerevisiae), transcript variant 1	12	−0.6949	2.6×10^{-4}	.0445
ILMN_1756942	NM_001017371.3	SP3	Sp3 transcription factor, transcript variant 2	2	0.6849	3.1×10^{-4}	.0445
ILMN_1750386	NM_006172.2	NPPA	Natriuretic peptide precursor A	1	−0.6947	3.1×10^{-4}	.0445
ILMN_1685433	NM_020351.2	COL8A1	Collagen, type VIII, alpha 1, transcript variant 2	3	−0.6900	2.9×10^{-4}	.0445
ILMN_1665095	NM_015537.3	NELF	Nasal embryonic LHRH factor	9	−0.7203	2.9×10^{-4}	.0445
ILMN_1695316	NM_022154.5	SLC39A8	Solute carrier family 39 (zinc transporter), member 8	4	0.6843	2.9×10^{-4}	.0446
ILMN_1749338	NM_173505.2	ANKRD29	Ankyrin repeat domain 29	18	−0.6916	3.0×10^{-4}	.0446
ILMN_1692340	NM_207404.2	ZNF662	Zinc finger protein 662	3	−0.7117	2.9×10^{-4}	.0447
ILMN_1730612	NM_001048223.1	DBNDD2	Dysbindin (dystrobrevin binding protein 1) domain containing 2, transcript variant 3	20	−0.7208	3.1×10^{-4}	.0447
ILMN_1778523	NM_001206.2	KLF9	Kruppel-like factor 9	9	−0.6988	3.0×10^{-4}	.0447
ILMN_1813175	NM_014921.3	LPHN1	Latrophilin 1, transcript variant 2	19	−0.6905	3.0×10^{-4}	.0447
ILMN_1800103	XM_001128785.1	LOC731196	Similar to proprotein convertase subtilisin/kexin type 7 precursor (proprotein convertase PC7) (subtilisin/kexin-like protease PC7) (prohormone convertase PC7) (PC8) (hPC8) (lymphoma proprotein convertase)	11	0.6977	2.9×10^{-4}	.0447
ILMN_1801583	NM_017680.3	ASPN	Asporin	9	−0.7233	2.9×10^{-4}	.0447
ILMN_1740024	NM_005467.2	NAALAD2	N-acetylated alpha-linked acidic dipeptidase 2	11	−0.6949	3.0×10^{-4}	.0449
ILMN_1683133	NM_014079.2	KLF15	Kruppel-like factor 15	3	−0.6842	3.2×10^{-4}	.0451
ILMN_1801441	NM_144629.1	RFTN2	Raftlin family member 2	2	−0.6948	3.2×10^{-4}	.0452

Løset. A transcriptional profile of the decidua in preeclampsia. Am J Obstet Gynecol 2011. *(continued)*

TABLE 2
Differentially expressed transcripts[a] *(continued)*

Illumina ID	GenBank ID	Symbol	Definition	Ch	Beta value[b]	*P* value[c]	FDR *P* value[d]
ILMN_1719097	NM_013326.3	C18orf8	Chromosome 18 open reading frame 8	18	−0.7098	3.2×10^{-4}	.0452
ILMN_1723689	NM_003624.1	RANBP3	RAN binding protein 3, transcript variant RANBP3-a	19	−0.6899	3.2×10^{-4}	.0452
ILMN_1790052	NM_004659.1	MMP23A	Matrix metallopeptidase 23A	1	−0.7011	3.3×10^{-4}	.0456
ILMN_1679262	NM_001387.2	DPYSL3	Dihydropyrimidinase-like 3	5	−0.7208	3.3×10^{-4}	.0458
ILMN_1683487	NM_003444.1	ZNF154	Zinc finger protein 154 (pHZ-92)	19	−0.6905	3.3×10^{-4}	.0460
ILMN_1710284	NM_005524.2	HES1	Hairy and enhancer of split 1, (Drosophila)	3	−0.7019	3.4×10^{-4}	.0462
ILMN_1728710	NM_001031665.1	ZNF816A	Zinc finger protein 816A	19	0.6975	3.5×10^{-4}	.0462
ILMN_1685156	NM_020983.2	ADCY6	Adenylate cyclase 6, transcript variant 2	12	−0.6890	3.5×10^{-4}	.0464
ILMN_1721087	NM_012435.1	SHC2	SHC (Src homology 2 domain containing) transforming protein 2	19	−0.6788	3.5×10^{-4}	.0465
ILMN_1700811	NM_019116.2	UBFD1	Ubiquitin family domain containing 1	16	0.6888	3.5×10^{-4}	.0466
ILMN_1661066	XM_927710.1	LOC644596	Hypothetical protein LOC644596	X	−0.6663	3.5×10^{-4}	.0466
ILMN_1733769	NM_001033047.1	NPNT	Nephronectin	4	−0.7029	3.5×10^{-4}	.0466
ILMN_1784948	NM_144569.4	SPOCD1	SPOC domain containing 1	1	−0.7223	3.6×10^{-4}	.0467
ILMN_1727574	NM_178835.3	LOC152485	Hypothetical protein LOC152485		−0.6904	3.5×10^{-4}	.0467
ILMN_1724984	NM_004836.4	EIF2AK3	Eukaryotic translation initiation factor 2-alpha kinase 3	2	0.6981	3.7×10^{-4}	.0467
ILMN_1660305	NM_177966.4	2'-PDE	2'-Phosphodiesterase	3	0.7031	3.5×10^{-4}	.0468
ILMN_1782057	NM_020452.2	ATP8B2	ATPase, class I, type 8B, member 2, transcript variant 1	1	−0.7041	3.6×10^{-4}	.0468
ILMN_1751072	NM_021203.2	SRPRB	Signal recognition particle receptor, B subunit	3	0.6672	3.7×10^{-4}	.0468
ILMN_1740609	NM_032964.2	CCL15	Chemokine (C-C motif) ligand 15, transcript variant 1	17	−0.6697	3.7×10^{-4}	.0468
ILMN_1669982	NM_001080433.1	CCDC85A	Coiled-coil domain containing 85A	2	−0.6858	3.6×10^{-4}	.0468
ILMN_1807515	NM_015235.2	CSTF2T	Cleavage stimulation factor, 3' pre-RNA, subunit 2, 64 kDa, tau variant	10	0.6892	3.5×10^{-4}	.0469
ILMN_1657361	NM_175709.2	CBX7	Chromobox homolog 7	22	−0.6904	3.8×10^{-4}	.0469
ILMN_1801043	NM_198252.2	GSN	Gelsolin (amyloidosis, Finnish type), transcript variant 2	9	−0.7028	3.7×10^{-4}	.0469
ILMN_1738116	NM_181724.1	TMEM119	Transmembrane protein 119	12	−0.6425	3.7×10^{-4}	.0470
ILMN_1760890	NM_206926.1	SEPN1	Selenoprotein N, 1, transcript variant 2	1	−0.6762	3.8×10^{-4}	.0473
ILMN_1728785	NM_015234.4	GPR116	G protein-coupled receptor 116, transcript variant 1	6	−0.6903	3.8×10^{-4}	.0475

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(continued)

TABLE 2
Differentially expressed transcripts[a] *(continued)*

Illumina ID	GenBank ID	Symbol	Definition	Ch	Beta value[b]	*P* value[c]	FDR *P* value[d]
ILMN_1744647	NM_018448.2	CAND1	Cullin-associated and neddylation-dissociated 1	12	0.6883	3.9×10^{-4}	.0479
ILMN_1757440	XM_001130258.1	FAM69B	Family with sequence similarity 69, member B	9	−0.6706	3.9×10^{-4}	.0480
ILMN_1783805	NM_013364.4	PNMA3	Paraneoplastic antigen MA3	X	−0.7005	3.9×10^{-4}	.0482
ILMN_1809098	NM_019599.2	TAS2R1	Taste receptor, type 2, member 1	5	0.7013	4.1×10^{-4}	.0490
ILMN_1719759	NM_002160.2	TNC	Tenascin C (hexabrachion)	9	−0.7107	4.1×10^{-4}	.0491
ILMN_1811313	NM_003062.1	SLIT3	Slit homolog 3 (Drosophila)	5	−0.6810	4.0×10^{-4}	.0491
ILMN_1700432	NM_002221.2	ITPKB	Inositol 1,4,5-trisphosphate 3-kinase B	1	−0.6983	4.1×10^{-4}	.0495
ILMN_1809488	NM_014752.1	SPCS2	Signal peptidase complex subunit 2 homolog (S cerevisiae)	11	0.6204	4.2×10^{-4}	.0498
ILMN_1795338	NM_013313.3	YPEL1	Yippee-like 1 (Drosophila)	22	−0.6528	4.3×10^{-4}	.0505
ILMN_1736242	NM_015432.2	PLEKHG4	Pleckstrin homology domain containing, family G (with RhoGef domain) member 4	16	−0.6879	4.3×10^{-4}	.0506
ILMN_1696568	NM_014382.2	ATP2C1	ATPase, Ca++ transporting, type 2C, member 1, transcript variant 1	3	0.6572	4.3×10^{-4}	.0507
ILMN_1766925	NM_001257.3	CDH13	Cadherin 13, H-cadherin (heart)	16	−0.7020	4.3×10^{-4}	.0509
ILMN_1698252	NM_152633.2	FANCB	Fanconi anemia, complementation group B, transcript variant 2	X	0.6928	4.5×10^{-4}	.0526
ILMN_1781149	NM_006774.4	INMT	Indolethylamine N-methyltransferase	7	−0.6688	4.6×10^{-4}	.0530
ILMN_1665437	NM_000773.3	CYP2E1	Cytochrome P450, family 2, subfamily E, polypeptide 1	10	−0.6839	4.6×10^{-4}	.0531
ILMN_1773395	NM_002905.2	RDH5	Retinol dehydrogenase 5 (11-cis/9-cis)	12	−0.6860	4.6×10^{-4}	.0533
ILMN_1665483	NM_014878.4	KIAA0020	KIAA0020	9	0.6892	4.6×10^{-4}	.0534
ILMN_1666545	NM_001097635.1	GCNT1	Glucosaminyl (N-acetyl) transferase 1, core 2 (beta-1,6-N-acetylglucosaminyltransferase), transcript variant 4	9	0.6953	4.6×10^{-4}	.0535
ILMN_1743864	NM_001453.2	FOXC1	Forkhead box C1	6	−0.6694	4.8×10^{-4}	.0542
ILMN_1709486	NM_006307.3	SRPX	Sushi-repeat-containing protein, X-linked	X	−0.6834	4.8×10^{-4}	.0543
ILMN_1676088	NM_198080.2	MSRB3	Methionine sulfoxide reductase B3, transcript variant 1	12	−0.6889	4.8×10^{-4}	.0543
ILMN_1771238	NM_000390.2	CHM	Choroideremia (Rab escort protein 1), transcript variant 1	X	0.6744	4.9×10^{-4}	.0544
ILMN_1656807	NM_000988.3	RPL27	Ribosomal protein L27	17	−0.6987	4.9×10^{-4}	.0545
ILMN_1711826	NM_020344.1	SLC24A2	Solute carrier family 24 (sodium/potassium/calcium exchanger), member 2	9	−0.6804	5.0×10^{-4}	.0545

Løset. A transcriptional profile of the decidua in preeclampsia. Am J Obstet Gynecol 2011.

(continued)

TABLE 2
Differentially expressed transcripts[a] *(continued)*

Illumina ID	GenBank ID	Symbol	Definition	Ch	Beta value[b]	*P* value[c]	FDR *P* value[d]
ILMN_1660730	NM_032803.4	SLC7A3	Solute carrier family 7 (cationic amino acid transporter, y+ system), member 3, transcript variant 1	X	−0.6816	4.9×10^{-4}	.0546
ILMN_1849218	BX451947	Hs.559564	BX451947 FETAL BRAIN cDNA clone CS0DF008YL16 5-PRIME sequence		−0.6910	4.9×10^{-4}	.0547
ILMN_1726752	NM_175071.1	APTX	Aprataxin, transcript variant 5	9	0.6732	4.9×10^{-4}	.0548
ILMN_1739640	NM_003737.2	DCHS1	Dachsous 1 (Drosophila)	11	−0.6808	5.0×10^{-4}	.0550
ILMN_1686968	NM_152493.2	FLJ25476	FLJ25476 protein	1	−0.6856	5.1×10^{-4}	.0557
ILMN_1718044	NM_018127.5	ELAC2	elaC Homolog 2 (E coli)	17	−0.6695	5.3×10^{-4}	.0562
ILMN_1799836	NM_006735.3	HOXA2	Homeobox A2	7	−0.7036	5.3×10^{-4}	.0563
ILMN_1658847	XM_939432.1	MGC61598	Similar to ankyrin-repeat protein Nrarp	9	−0.6363	5.3×10^{-4}	.0565
ILMN_1764619	NM_207443.1	FLJ45244	FLJ45244 protein	14	−0.6691	5.3×10^{-4}	.0567
ILMN_1739521	NM_014932.2	NLGN1	Neuroligin 1	3	0.6893	5.4×10^{-4}	.0568
ILMN_1710675	NM_005080.2	XBP1	X-box binding protein 1, transcript variant 1	22	0.6814	5.3×10^{-4}	.0568
ILMN_1772810	XM_946142.2	SHANK3	SH3 and multiple ankyrin repeat domains 3, transcript variant 4	22	−0.6733	5.4×10^{-4}	.0570
ILMN_1693481	NM_021949.2	ATP2B3	ATPase, Ca++ transporting, plasma membrane 3, transcript variant 1	X	0.6669	5.3×10^{-4}	.0570
ILMN_1671106	NM_002060.2	GJA4	Gap junction protein, alpha 4, 37 kDa	1	−0.6706	5.3×10^{-4}	.0572
ILMN_1773757	NM_138718.1	SLC26A8	Solute carrier family 26, member 8, transcript variant 2	6	0.6936	5.5×10^{-4}	.0573
ILMN_1680652	NM_003944.2	SELENBP1	Selenium binding protein 1	1	−0.6566	5.6×10^{-4}	.0585
ILMN_1813528	NM_133459.1	CCBE1	Collagen and calcium binding EGF domains 1	18	−0.6806	5.7×10^{-4}	.0587
ILMN_1715175	NM_000245.2	MET	Met protooncogene (hepatocyte growth factor receptor)	7	0.6834	5.7×10^{-4}	.0587
ILMN_1688160	NM_182552.3	WDR27	WD repeat domain 27	6	−0.6906	5.7×10^{-4}	.0587
ILMN_1805842	NM_001449.3	FHL1	Four and a half LIM domains 1	X	−0.6833	5.6×10^{-4}	.0587
ILMN_1806301	NM_002077.2	GOLGA1	Golgi autoantigen, golgin subfamily a, 1	9	−0.6603	5.8×10^{-4}	.0595
ILMN_1734653	NM_032532.2	FNDC1	Fibronectin type III domain containing 1	6	−0.6810	5.9×10^{-4}	.0596
ILMN_1706935	NM_022742.3	CCDC136	Coiled-coil domain containing 136	7	−0.6766	5.9×10^{-4}	.0597
ILMN_1727091	NM_138326.2	ACMSD	Aminocarboxymuconate semialdehyde decarboxylase	2	0.6688	5.9×10^{-4}	.0597
ILMN_1740385	NM_014956.4	CEP164	Centrosomal protein 164 kDa	11	−0.6244	5.9×10^{-4}	.0598
ILMN_1746517	NM_003937.2	KYNU	Kynureninase (L-kynurenine hydrolase), transcript variant 1	2	0.6445	6.0×10^{-4}	.0598

Løset. A transcriptional profile of the decidua in preeclampsia. Am J Obstet Gynecol 2011.

(continued)

TABLE 2
Differentially expressed transcripts[a] *(continued)*

Illumina ID	GenBank ID	Symbol	Definition	Ch	Beta value[b]	*P* value[c]	FDR *P* value[d]
ILMN_1801246	NM_003641.3	IFITM1	Interferon-induced transmembrane protein 1 (9-27)	11	−0.6716	6.0×10^{-4}	.0599
ILMN_1756784	NM_014286.2	FREQ	Frequenin homolog (Drosophila)	9	−0.6824	6.1×10^{-4}	.0599
ILMN_1652389	NM_001031733.2	CALML4	Calmodulin-like 4, transcript variant 2	15	−0.6783	6.1×10^{-4}	.0600
ILMN_1794038	NM_030797.2	FAM49A	Family with sequence similarity 49, member A	2	0.6333	6.1×10^{-4}	.0601
ILMN_1758731	NM_000775.2	CYP2J2	Cytochrome P450, family 2, subfamily J, polypeptide 2	1	0.6767	6.1×10^{-4}	.0602
ILMN_1707380	NM_002725.3	PRELP	Proline/arginine-rich end leucine-rich repeat protein, transcript variant 1	1	−0.6844	6.1×10^{-4}	.0603
ILMN_1801226	NM_020812.1	DOCK6	Dedicator of cytokinesis 6	19	−0.6576	6.1×10^{-4}	.0605
ILMN_1766386	XR_017805.1	LOC401433	Hypothetical gene supported by AK127717, misc RNA	7	−0.6178	6.2×10^{-4}	.0607
ILMN_1763657	NM_025212.1	CXXC4	CXXC finger 4	4	−0.6560	6.3×10^{-4}	.0608
ILMN_1777221	NM_058182.2	C21orf51	Chromosome 21 open reading frame 51	21	−0.6266	6.3×10^{-4}	.0612
ILMN_1712199	NM_024738.1	C12orf49	Chromosome 12 open reading frame 49	12	0.6531	6.4×10^{-4}	.0619
ILMN_1741801	NM_003503.2	CDC7	Cell division cycle 7 homolog (S cerevisiae)	1	0.6725	6.6×10^{-4}	.0631
ILMN_1891067	AK127526	Hs.553187	cDNA FLJ45619 fis, clone BRTHA3027318		0.6413	6.6×10^{-4}	.0632
ILMN_1663843	NM_004161.3	RAB1A	RAB1A, member RAS oncogene family	2	0.6529	6.7×10^{-4}	.0632
ILMN_1792571	NM_173728.2	ARHGEF15	Rho guanine nucleotide exchange factor (GEF) 15	17	−0.6508	6.7×10^{-4}	.0632
ILMN_1790315	NM_001039706.1	FLJ21062	Hypothetical protein FLJ21062	7	−0.6657	6.6×10^{-4}	.0634
ILMN_1733756	NM_080645.2	COL12A1	Collagen, type XII, alpha 1, transcript variant short	6	−0.6799	6.8×10^{-4}	.0638
ILMN_1812701	NM_001099783.1	C4orf33	Chromosome 4 open reading frame 33, transcript variant 2	4	0.6666	6.8×10^{-4}	.0640
ILMN_1782257	NM_022734.2	METT11D1	Methyltransferase 11 domain containing 1, transcript variant 2	14	−0.6653	6.9×10^{-4}	.0643
ILMN_1691112	NM_176787.4	PIGN	Phosphatidylinositol glycan anchor biosynthesis, class N, transcript variant 1	18	0.6741	6.9×10^{-4}	.0646
ILMN_1756086	NM_023015.3	INTS3	Integrator complex subunit 3	1	−0.6306	6.9×10^{-4}	.0648
ILMN_1710303	NM_031421.2	TTC25	Tetratricopeptide repeat domain 25	17	−0.6482	7.0×10^{-4}	.0651
ILMN_1785765	NM_004800.1	TM9SF2	Transmembrane 9 superfamily member 2	13	0.6617	7.1×10^{-4}	.0656
ILMN_1684321	NM_030579.2	CYB5B	Cytochrome b5 type B (outer mitochondrial membrane)	16	0.6858	7.1×10^{-4}	.0658

Løset. A transcriptional profile of the decidua in preeclampsia. Am J Obstet Gynecol 2011.

(continued)

TABLE 2
Differentially expressed transcripts[a] *(continued)*

Illumina ID	GenBank ID	Symbol	Definition	Ch	Beta value[b]	*P* value[c]	FDR *P* value[d]
ILMN_1722244	NM_001018011.1	ZBTB16	Zinc finger and BTB domain containing 16, transcript variant 2	11	−0.6734	7.2×10^{-4}	.0660
ILMN_1787906	NM_014629.2	ARHGEF10	Rho guanine nucleotide exchange factor (GEF) 10	8	−0.6634	7.3×10^{-4}	.0668
ILMN_1736974	NM_006943.2	SOX12	SRY (sex determining region Y)-box 12	20	−0.6435	7.4×10^{-4}	.0668
ILMN_1808590	NM_000856.3	GUCY1A3	Guanylate cyclase 1, soluble, alpha 3	4	−0.6252	7.3×10^{-4}	.0669
ILMN_1751559	NM_024600.2	C16orf30	Chromosome 16 open reading frame 30	16	−0.6442	7.4×10^{-4}	.0671
ILMN_1774427	NM_020898.1	CALCOCO1	Calcium binding and coiled-coil domain 1	12	−0.6804	7.7×10^{-4}	.0682
ILMN_1657502	NM_001098515.1	MRGPRF	MAS-related GPR, member F, transcript variant 1	11	−0.6781	7.6×10^{-4}	.0682
ILMN_1652128	NM_018368.2	LMBRD1	LMBR1 domain containing 1	6	0.6306	7.8×10^{-4}	.0683
ILMN_1808417	NM_015102.2	NPHP4	Nephronophthisis 4	1	−0.6615	7.7×10^{-4}	.0684
ILMN_1657194	NM_018430.2	TSNAXIP1	Translin-associated factor X interacting protein 1	16	−0.6513	7.7×10^{-4}	.0684
ILMN_1680948	NM_012134.2	LMOD1	Leiomodin 1 (smooth muscle)	1	−0.6757	7.7×10^{-4}	.0684
ILMN_1703471	NM_007348.2	ATF6	Activating transcription factor 6	1	0.6569	7.6×10^{-4}	.0684
ILMN_1728742	NM_032385.3	C5orf4	Chromosome 5 open reading frame 4, transcript variant 2	5	−0.6564	7.8×10^{-4}	.0686
ILMN_1702861	NM_172244.2	SGCD	Sarcoglycan, delta (35 kDa dystrophin-associated glycoprotein), transcript variant 2	5	−0.6460	7.6×10^{-4}	.0686
ILMN_1868150	BX537697	Hs.98581	mRNA; cDNA DKFZp686D0853 (from clone DKFZp686D0853)		−0.6645	7.9×10^{-4}	.0686
ILMN_1694325	NM_002501.2	NFIX	Nuclear factor I/X (CCAAT-binding transcription factor)	19	−0.6525	7.8×10^{-4}	.0687
ILMN_1748432	XM_375646.3	ZNF525	Zinc finger protein 525	19	0.6710	8.0×10^{-4}	.0691
ILMN_1743357	NM_003399.5	XPNPEP2	X-prolyl aminopeptidase (aminopeptidase P) 2, membrane-bound	X	−0.6388	8.0×10^{-4}	.0694
ILMN_1782125	NM_024422.2	DSC2	Desmocollin 2, transcript variant Dsc2a	18	0.6142	8.0×10^{-4}	.0695
ILMN_1687967	NM_001007156.1	NTRK3	Neurotrophic tyrosine kinase, receptor, type 3, transcript variant 3	15	−0.6630	8.1×10^{-4}	.0699
ILMN_1685286	NM_017607.2	PPP1R12C	Protein phosphatase 1, regulatory (inhibitor) subunit 12C	19	−0.6754	8.3×10^{-4}	.0710
ILMN_1756937	NM_005668.3	ST8SIA4	ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 4, transcript variant 1	5	0.6470	8.3×10^{-4}	.0711

Løset. A transcriptional profile of the decidua in preeclampsia. Am J Obstet Gynecol 2011. *(continued)*

TABLE 2
Differentially expressed transcripts[a] *(continued)*

Illumina ID	GenBank ID	Symbol	Definition	Ch	Beta value[b]	*P* value[c]	FDR *P* value[d]
ILMN_1794534	NM_021827.3	CCDC81	Coiled-coil domain containing 81	11	−0.6588	8.4×10^{-4}	.0712
ILMN_1793615	NM_001014811.1	ME3	Malic enzyme 3, NADP(+)-dependent, mitochondrial, nuclear gene encoding mitochondrial protein, transcript variant 2	11	−0.6720	8.5×10^{-4}	.0716
ILMN_1885397	BM311228	Hs.503590	ig62e09y1 HR85 islet cDNA 5 sequence		−0.6606	8.5×10^{-4}	.0717
ILMN_1759375	NM_001083330.1	ZNF133	Zinc finger protein 133, transcript variant 2	20	−0.6532	8.5×10^{-4}	.0719
ILMN_1796851	XM_001131060.1	FOXL2	Forkhead box L2	3	−0.6417	8.6×10^{-4}	.0720
ILMN_1703105	NM_139178.2	ALKBH3	alkB, Alkylation repair homolog 3 (E coli)	11	−0.6275	8.5×10^{-4}	.0720
ILMN_1678710	NM_032439.1	PHYHIPL	Phytanoyl-CoA 2-hydroxylase interacting protein-like	10	0.6064	8.8×10^{-4}	.0733
ILMN_1758398	NM_000858.4	GUK1	Guanylate kinase 1	1	−0.6158	8.9×10^{-4}	.0735
ILMN_1796734	NM_003118.2	SPARC	Secreted protein, acidic, cysteine-rich (osteonectin)	5	−0.6468	8.8×10^{-4}	.0737
ILMN_1653856	NM_032873.3	STS-1	Cbl-interacting protein Sts-1	11	0.6288	8.9×10^{-4}	.0739
ILMN_1795251	NM_004684.3	SPARCL1	SPARC-like 1 (mast9, hevin)	4	−0.6301	9.0×10^{-4}	.0746
ILMN_1717206	NM_175060.1	CLEC14A	C-type lectin domain family 14, member A	14	−0.6376	9.2×10^{-4}	.0754
ILMN_1739496	NM_006902.3	PRRX1	Paired related homeobox 1, transcript variant pmx-1a	1	−0.6477	9.2×10^{-4}	.0754
ILMN_1718552	NM_006419.1	CXCL13	Chemokine (C-X-C motif) ligand 13 (B-cell chemoattractant)	4	−0.6037	9.2×10^{-4}	.0755
ILMN_1797191	NM_014656.1	KIAA0040	KIAA0040	1	0.6227	9.2×10^{-4}	.0757
ILMN_1737705	NM_015054.1	KIAA0701	KIAA0701 protein, transcript variant 1	12	0.6459	9.5×10^{-4}	.0770
ILMN_1682781	NM_003598.1	TEAD2	TEA domain family member 2	19	−0.6440	9.5×10^{-4}	.0771
ILMN_1673352	NM_006435.2	IFITM2	Interferon-induced transmembrane protein 2 (1-8D)	11	−0.6409	9.6×10^{-4}	.0772
ILMN_1750158	NM_007292.4	ACOX1	acyl-Coenzyme A oxidase 1, palmitoyl, transcript variant 2	17	0.6554	9.7×10^{-4}	.0772
ILMN_1657156	NM_207306.2	KIAA0495	KIAA0495	1	−0.6716	9.7×10^{-4}	.0773
ILMN_1787576	NM_004070.3	CLCNKA	Chloride channel Ka, transcript variant 1	1	−0.6609	9.6×10^{-4}	.0773
ILMN_1665449	NM_019055.4	ROBO4	Roundabout homolog 4, magic roundabout (Drosophila)	11	−0.6256	9.7×10^{-4}	.0773
ILMN_1796018	NM_004554.3	NFATC4	Nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 4	14	−0.6726	9.6×10^{-4}	.0774
ILMN_1765118	NM_003627.4	SLC43A1	Solute carrier family 43, member 1	11	−0.6174	9.9×10^{-4}	.0784

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(continued)

TABLE 2
Differentially expressed transcripts[a] *(continued)*

Illumina ID	GenBank ID	Symbol	Definition	Ch	Beta value[b]	*P* value[c]	FDR *P* value[d]
ILMN_1785424	NM_006720.3	ABLIM1	Actin-binding LIM protein 1, transcript variant 4	10	−0.6004	9.9×10^{-4}	.0784
ILMN_1701204	NM_005429.2	VEGFC	Vascular endothelial growth factor C	4	−0.6393	9.9×10^{-4}	.0784
ILMN_1769186	NM_001755.2	CBFB	Core-binding factor, beta subunit, transcript variant 2	16	0.6454	1.0×10^{-3}	.0785
ILMN_1651958	NM_000900.2	MGP	Matrix Gla protein	12	−0.6404	1.0×10^{-3}	.0786
ILMN_1770803	NM_004330.1	BNIP2	BCL2/adenovirus E1B 19 kDa interacting protein 2	15	0.6074	1.0×10^{-3}	.0786
ILMN_1720452	NM_001031855.1	LONRF3	LON peptidase N-terminal domain and ring finger 3, transcript variant 1	X	0.6477	1.0×10^{-3}	.0786
ILMN_1780349	NM_003292.2	TPR	Translocated promoter region (to activated MET oncogene)	1	−0.6328	1.0×10^{-3}	.0792
ILMN_1818018	DA321576	Hs.576997	DA321576 BRHIP3 cDNA clone BRHIP3014850 5 sequence		0.6451	1.0×10^{-3}	.0793
ILMN_1724424	NM_145239.1	PRRT2	Proline-rich transmembrane protein 2	16	−0.6496	1.0×10^{-3}	.0794
ILMN_1760849	NM_018092.3	NETO2	Neuropilin (NRP) and tolloid (TLL)-like 2	16	0.6201	1.0×10^{-3}	.0794
ILMN_1773742	NM_012328.1	DNAJB9	DnaJ (Hsp40) homolog, subfamily B, member 9	7	0.6452	1.0×10^{-3}	.0794
ILMN_1792529	NM_004783.2	TAOK2	TAO kinase 2, transcript variant 1	16	−0.6358	1.0×10^{-3}	.0796
ILMN_1740772	NM_133172.2	APBB3	Amyloid beta (A4) precursor protein-binding, family B, member 3, transcript variant 3	5	−0.6393	1.1×10^{-3}	.0796
ILMN_1737604	NM_018291.2	FLJ10986	Hypothetical protein FLJ10986	1	0.6581	1.0×10^{-3}	.0796
ILMN_1742272	NM_000537.2	REN	Renin	1	−0.6262	1.1×10^{-3}	.0798
ILMN_1806403	NM_016563.2	RASL12	RAS-like, family 12	15	−0.6338	1.1×10^{-3}	.0798
ILMN_1715647	NM_020335.1	VANGL2	Vang-like 2 (van gogh, Drosophila)	1	−0.6544	1.1×10^{-3}	.0800
ILMN_1655913	NM_005013.2	NUCB2	Nucleobindin 2	11	0.6408	1.1×10^{-3}	.0801
ILMN_1736080	NM_012432.2	SETDB1	SET domain, bifurcated 1	1	−0.6417	1.1×10^{-3}	.0805
ILMN_1663033	NM_138385.2	TMEM129	Transmembrane protein 129	4	−0.6319	1.1×10^{-3}	.0809
ILMN_1697585	NM_022496.3	ACTR6	ARP6 actin-related protein 6 homolog (yeast)	12	0.6055	1.1×10^{-3}	.0817
ILMN_1711124	NM_144724.1	MARVELD2	MARVEL domain containing 2, transcript variant 2	5	0.6463	1.1×10^{-3}	.0820
ILMN_1711919	NM_017988.4	SCYL2	SCY1-like 2 (S cerevisiae)	12	0.5937	1.1×10^{-3}	.0821
ILMN_1669142	NM_057175.3	NARG1	NMDA receptor regulated 1	4	0.6007	1.1×10^{-3}	.0822
ILMN_1678862	NM_173540.2	FUT11	Fucosyltransferase 11 (alpha [1,3] fucosyltransferase)	10	0.6192	1.1×10^{-3}	.0822
ILMN_1768393	NM_006938.2	SNRPD1	Small nuclear ribonucleoprotein D1 polypeptide 16 kDa	18	0.5974	1.1×10^{-3}	.0822

Løset. A transcriptional profile of the decidua in preeclampsia. Am J Obstet Gynecol 2011.

(continued)

TABLE 2
Differentially expressed transcripts[a] *(continued)*

Illumina ID	GenBank ID	Symbol	Definition	Ch	Beta value[b]	*P* value[c]	FDR *P* value[d]
ILMN_1782938	NM_018593.3	SLC16A10	Solute carrier family 16, member 10 (aromatic amino acid transporter)	6	0.5965	1.1×10^{-3}	.0822
ILMN_1899428	AW173494	Hs.483540	xj07f12x1 NCI_CGAP_Ut2 cDNA clone IMAGE:2656559 3 sequence		−0.6320	1.1×10^{-3}	.0825
ILMN_1748845	NM_002506.2	NGFB	Nerve growth factor, beta polypeptide	1	−0.6390	1.1×10^{-3}	.0832
ILMN_1767722	NM_203437.2	AFTPH	Aftiphilin, transcript variant 1	2	0.6445	1.2×10^{-3}	.0837
ILMN_1791545	NM_015515.3	KRT23	Keratin 23 (histone deacetylase inducible)	17	0.6391	1.2×10^{-3}	.0838
ILMN_1747183	NM_001099650.1	GLT8D3	Glycosyltransferase 8 domain containing 3, transcript variant 2	12	0.6353	1.2×10^{-3}	.0839
ILMN_1815666	NM_170665.2	ATP2A2	ATPase, Ca++ transporting, cardiac muscle, slow twitch 2, transcript variant 1	12	0.6340	1.2×10^{-3}	.0840
ILMN_1761425	NM_182487.2	OLFML2A	Olfactomedin-like 2A	9	−0.6347	1.2×10^{-3}	.0843
ILMN_1794825	NM_000382.2	ALDH3A2	Aldehyde dehydrogenase 3 family, member A2, transcript variant 2	17	−0.6101	1.2×10^{-3}	.0845
ILMN_1767459	NM_018082.4	POLR3B	Polymerase (RNA) III (DNA directed) polypeptide B	12	0.6220	1.2×10^{-3}	.0851
ILMN_1717905	NM_015726.2	WDR42A	WD repeat domain 42A	1	−0.6202	1.2×10^{-3}	.0853
ILMN_1682404	NM_006515.1	SETMAR	SET domain and mariner transposase fusion gene	3	−0.6306	1.2×10^{-3}	.0861
ILMN_1725338	NM_194284.2	CLDN23	Claudin 23	8	0.6369	1.2×10^{-3}	.0863
ILMN_1765371	NM_018032.3	LUC7L	LUC7-like (S cerevisiae), transcript variant 1	16	−0.6428	1.2×10^{-3}	.0863
ILMN_1756118	NM_014634.2	PPM1F	Protein phosphatase 1F (PP2C domain containing)	22	−0.5914	1.2×10^{-3}	.0864
ILMN_1793621	NM_001002262.1	ZFYVE27	Zinc finger, FYVE domain containing 27, transcript variant 3	10	0.6345	1.2×10^{-3}	.0865
ILMN_1654945	NM_153759.2	DNMT3A	DNA (cytosine-5-)-methyltransferase 3 alpha, transcript variant 2	2	0.6434	1.2×10^{-3}	.0866
ILMN_1754364	NM_001868.1	CPA1	Carboxypeptidase A1 (pancreatic)	7	0.6196	1.2×10^{-3}	.0866
ILMN_1663640	NM_000240.2	MAOA	Monoamine oxidase A, nuclear gene encoding mitochondrial protein	X	0.6353	1.3×10^{-3}	.0874
ILMN_1728581	NM_016210.2	C3orf18	Chromosome 3 open reading frame 18	3	−0.6159	1.3×10^{-3}	.0876
ILMN_1736834	NM_005414.2	SKIL	SKI-like oncogene	3	0.6206	1.3×10^{-3}	.0883
ILMN_1800731	NM_018328.3	MBD5	Methyl-CpG binding domain protein 5	2	−0.6235	1.3×10^{-3}	.0884

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(continued)

TABLE 2
Differentially expressed transcripts[a] *(continued)*

Illumina ID	GenBank ID	Symbol	Definition	Ch	Beta value[b]	*P* value[c]	FDR *P* value[d]
ILMN_1805098	NM_000924.2	PDE1B	Phosphodiesterase 1B, calmodulin-dependent	12	−0.6434	1.3×10^{-3}	.0884
ILMN_1769764	NM_001039935.1	ANKRD55	Ankyrin repeat domain 55, transcript variant 2	5	0.6090	1.3×10^{-3}	.0885
ILMN_1814015	NM_004063.2	CDH17	Cadherin 17, LI cadherin (liver-intestine)	8	−0.6315	1.3×10^{-3}	.0885
ILMN_1802669	NM_021132.1	PPP3CB	Protein phosphatase 3 (formerly 2B), catalytic subunit, beta isoform	10	−0.6361	1.3×10^{-3}	.0888
ILMN_1800512	NM_002133.1	HMOX1	Heme oxygenase (decycling) 1	22	0.5922	1.3×10^{-3}	.0888
ILMN_1772731	NM_005326.4	HAGH	Hydroxyacylglutathione hydrolase, transcript variant 1	16	−0.6280	1.3×10^{-3}	.0890
ILMN_1756573	NM_020142.3	NDUFA4L2	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 4-like 2	12	−0.6186	1.3×10^{-3}	.0891
ILMN_1686464	NM_180991.4	SLCO4C1	Solute carrier organic anion transporter family, member 4C1	5	0.6381	1.3×10^{-3}	.0903
ILMN_1769083	NM_000847.3	GSTA3	Glutathione S-transferase A3	6	0.6117	1.3×10^{-3}	.0904
ILMN_1687410	NM_022776.3	OSBPL11	Oxysterol binding protein-like 11	3	0.6035	1.4×10^{-3}	.0918
ILMN_1651611	NM_000527.2	LDLR	Low-density lipoprotein receptor (familial hypercholesterolemia)	19	0.6122	1.4×10^{-3}	.0937
ILMN_1665123	NM_178177.2	NMNAT3	Nicotinamide nucleotide adenylyltransferase 3	3	−0.6152	1.4×10^{-3}	.0938
ILMN_1651370	NM_001014443.2	USP21	Ubiquitin-specific peptidase 21, transcript variant 3	1	−0.6228	1.4×10^{-3}	.0939
ILMN_1774110	NM_004067.2	CHN2	Chimerin (chimaerin) 2, transcript variant 2	7	0.6275	1.4×10^{-3}	.0950
ILMN_1730662	NM_001008745.1	LOC401431	Hypothetical gene LOC401431	7	−0.6048	1.4×10^{-3}	.0950
ILMN_1753554	NM_022763.2	FNDC3B	Fibronectin type III domain containing 3B	3	0.6167	1.4×10^{-3}	.0950
ILMN_1734254	NM_014106.2	ZNF770	Zinc finger protein 770	15	0.5801	1.4×10^{-3}	.0951
ILMN_1801889	NM_015011.1	MYO16	Myosin XVI	13	−0.6401	1.4×10^{-3}	.0951
ILMN_1703074	NM_001304.3	CPD	Carboxypeptidase D	17	0.6178	1.4×10^{-3}	.0952
ILMN_1885728	XM_001130020.1	KIAA1147	KIAA1147	7	0.6203	1.5×10^{-3}	.0952
ILMN_1652594	NM_024855.3	ACTR5	ARP5 actin-related protein 5 homolog (yeast)	20	−0.6197	1.4×10^{-3}	.0953
ILMN_1672287	NM_018657.3	MYNN	Myoneurin	3	0.6237	1.5×10^{-3}	.0954
ILMN_1680113	NM_004758.1	BZRAP1	Benzodiazepine receptor (peripheral)-associated protein 1	17	−0.6340	1.5×10^{-3}	.0966
ILMN_1660282	NM_022135.2	POPDC2	Popeye domain containing 2	3	−0.6316	1.5×10^{-3}	.0967
ILMN_1683441	NM_015261.2	NCAPD3	Non-SMC condensin II complex, subunit D3	11	−0.6169	1.5×10^{-3}	.0968
ILMN_1761486	NM_024808.2	C13orf34	Chromosome 13 open reading frame 34	13	0.6187	1.5×10^{-3}	.0970

Løset. A transcriptional profile of the decidua in preeclampsia. Am J Obstet Gynecol 2011. *(continued)*

TABLE 2
Differentially expressed transcripts[a] *(continued)*

Illumina ID	GenBank ID	Symbol	Definition	Ch	Beta value[b]	*P* value[c]	FDR *P* value[d]
ILMN_1894569	BX093121	Hs.571048	BX093121 Soares_placenta_ 8to9weeks_2NbHP8to9W cDNA clone IMAGp998K13561; IMAGE:257796 sequence		0.6106	1.5×10^{-3}	.0970
ILMN_1751086	NM_015459.3	DKFZP564J0863	DKFZP564J0863 protein	11	0.5780	1.5×10^{-3}	.0970
ILMN_1656386	NM_014822.1	SEC24D	SEC24-related gene family, member D (S cerevisiae)	4	0.6073	1.5×10^{-3}	.0971
ILMN_1760271	NM_194314.2	ZBTB41	Zinc finger and BTB domain containing 41	1	0.6038	1.5×10^{-3}	.0972
ILMN_1702683	NM_004733.2	SLC33A1	Solute carrier family 33 (acetyl-CoA transporter), member 1	3	0.6196	1.5×10^{-3}	.0972
ILMN_1878019	AL512695	Hs.278285	mRNA; cDNA DKFZp547G133 (from clone DKFZp547G133)		0.6348	1.5×10^{-3}	.0973
ILMN_1806487	NM_001002034.2	FAM109B	Family with sequence similarity 109, member B	22	−0.5735	1.5×10^{-3}	.0973
ILMN_1779748	NM_004192.2	ASMTL	Acetylserotonin O-methyltransferase-like	X,Y	−0.6146	1.5×10^{-3}	.0974
ILMN_1770084	NM_006283.1	TACC1	Transforming, acidic coiled-coil containing protein 1	8	−0.6145	1.6×10^{-3}	.0974
ILMN_1707534	NM_017544.2	NKRF	NF-kappaB repressing factor	X	0.6044	1.6×10^{-3}	.0974
ILMN_1678086	NM_138770.1	CCDC74A	Coiled-coil domain containing 74A	2	−0.6070	1.6×10^{-3}	.0975
ILMN_1669064	NM_001080493.2	HSZFP36	ZFP-36 for a zinc finger protein	19	0.6087	1.5×10^{-3}	.0975
ILMN_1810093	NM_005725.3	TSPAN2	Tetraspanin 2	1	−0.5941	1.5×10^{-3}	.0976
ILMN_1673522	NM_017947.1	MOCOS	Molybdenum cofactor sulfurase	18	0.6122	1.6×10^{-3}	.0976
ILMN_1764309	NM_000667.2	ADH1A	Alcohol dehydrogenase 1A (class I), alpha polypeptide	4	−0.6229	1.6×10^{-3}	.0977
ILMN_1795325	NM_001615.3	ACTG2	Actin, gamma 2, smooth muscle, enteric	2	−0.6151	1.6×10^{-3}	.0977
ILMN_1773814	NM_205853.2	MUSTN1	Musculoskeletal, embryonic nuclear protein 1	3	−0.6206	1.5×10^{-3}	.0977
ILMN_1703576	NM_012334.2	MYO10	Myosin X	5	0.6086	1.6×10^{-3}	.0977
ILMN_1780937	NM_025128.3	MUS81	MUS81 endonuclease homolog (S cerevisiae)	11	−0.6341	1.6×10^{-3}	.0977
ILMN_1757162	XM_945736.2	LOC654085	Similar to Glycine cleavage system H protein, mitochondrial precursor, transcript variant 2	19	0.6181	1.5×10^{-3}	.0977
ILMN_1832155	AK094744	Hs.167721	cDNA FLJ37425 fis, clone BRAWH2001530		−0.6007	1.6×10^{-3}	.0977
ILMN_1782688	NM_024838.4	THNSL1	Threonine synthase-like 1 (S cerevisiae)	10	0.6374	1.6×10^{-3}	.0977
ILMN_1757298	NM_018167.3	BTBD7	BTB (POZ) domain containing 7, transcript variant 2	14	0.6056	1.6×10^{-3}	.0978
ILMN_1798975	NM_005228.3	EGFR	Epidermal growth factor receptor (erythroblastic leukemia viral [v-erb-b] oncogene homolog, avian), transcript variant 1	7	0.6186	1.6×10^{-3}	.0978

Løset. A transcriptional profile of the decidua in preeclampsia. Am J Obstet Gynecol 2011. *(continued)*

TABLE 2
Differentially expressed transcripts[a] *(continued)*

Illumina ID	GenBank ID	Symbol	Definition	Ch	Beta value[b]	*P* value[c]	FDR *P* value[d]
ILMN_1775974	NM_019012.2	PLEKHA5	Pleckstrin homology domain containing, family A member 5	12	0.5995	1.6×10^{-3}	.0979
ILMN_1872404	AK055652	Hs.478682	cDNA FLJ31090 fis, clone IMR321000102		−0.6351	1.6×10^{-3}	.0979
ILMN_1808999	NM_153213.3	ARHGEF19	Rho guanine nucleotide exchange factor (GEF) 19	1	−0.6104	1.6×10^{-3}	.0979
ILMN_1733703	NM_018006.4	TRMU	tRNA 5-methylaminomethyl-2-thiouridylate methyltransferase, nuclear gene encoding mitochondrial protein, transcript variant 1	22	−0.6098	1.6×10^{-3}	.0980
ILMN_1795574	XM_928045.1	LOC644968	Hypothetical protein LOC644968	4	0.5916	1.6×10^{-3}	.0980
ILMN_1700994	NM_001039571.1	KREMEN1	Kringle containing transmembrane protein 1, transcript variant 4	22	0.6255	1.6×10^{-3}	.0980
ILMN_1737146	NM_014294.4	TRAM1	Translocation-associated membrane protein 1	8	0.6212	1.6×10^{-3}	.0980
ILMN_1809889	NM_173510.1	CCDC117	Coiled-coil domain containing 117	22	0.6109	1.6×10^{-3}	.0981
ILMN_1735909	NM_001033678.2	TRPT1	tRNA phosphotransferase 1, transcript variant 1	11	−0.6155	1.6×10^{-3}	.0982
ILMN_1670472	NM_014613.2	UBXD8	UBX domain containing 8	5	0.6387	1.7×10^{-3}	.0986
ILMN_1700633	NM_022060.2	ABHD4	Abhydrolase domain containing 4	14	−0.5964	1.7×10^{-3}	.0988
ILMN_1914072	BQ718005	Hs.562762	AGENCOURT_8100698 Lupski_sympathetic_trunk cDNA clone IMAGE:6190431 5 sequence		0.6098	1.7×10^{-3}	.0989
ILMN_1651642	NM_152742.1	GPC2	Glypican 2	7	−0.6187	1.7×10^{-3}	.0990
ILMN_1671046	NM_001541.2	HSPB2	Heat shock 27-kDa protein 2	11	−0.6162	1.7×10^{-3}	.0990
ILMN_1662578	NM_020156.1	C1GALT1	Core 1 synthase, glycoprotein-N-acetylgalactosamine 3-beta-galactosyltransferase, 1	7	0.5897	1.7×10^{-3}	.0990
ILMN_1693514	NM_001014795.1	ILK	Integrin-linked kinase, transcript variant 3	11	−0.6264	1.7×10^{-3}	.0992
ILMN_1800447	NM_001031835.1	PHKB	Phosphorylase kinase, beta, transcript variant 2	16	0.5895	1.7×10^{-3}	.0992
ILMN_1701933	NM_007308.1	SNCA	Synuclein, alpha (non-A4 component of amyloid precursor), transcript variant NACP112	4	−0.5903	1.7×10^{-3}	.0993
ILMN_1779547	NM_006665.2	HPSE	Heparanase	4	0.6297	1.7×10^{-3}	.0995
ILMN_1883624	DA589983	Hs.582952	DA589983 HLUNG2 cDNA clone HLUNG2011800 5 sequence		0.5802	1.7×10^{-3}	.0997
ILMN_1774717	NM_020182.3	TMEPAI	Transmembrane, prostate androgen-induced RNA, transcript variant 1	20	−0.5933	1.7×10^{-3}	.0998

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(continued)

TABLE 2
Differentially expressed transcripts[a] *(continued)*

Illumina ID	GenBank ID	Symbol	Definition	Ch	Beta value[b]	*P* value[c]	FDR *P* value[d]
ILMN_1789463	NM_021902.2	FXYD1	FXYD domain containing ion transport regulator 1 (phospholemman), transcript variant b	19	−0.6164	1.7×10^{-3}	.0999
ILMN_1651900	NM_002233.2	KCNA4	Potassium voltage-gated channel, shaker-related subfamily, member 4	11	0.6164	1.7×10^{-3}	.0999

FDR, false discovery rate; *mRNA*, messenger RNA.

[a] Gene expression in decidual tissue from preeclamptic and normal pregnancies has been compared; [b] Values are given in beta, measure of distance between group means, expressed in SD units–positive beta implies up-regulation and negative beta implies down-regulation in preeclamptic group compared with normal pregnant group; [c] $P < .05$, obtained with SOLAR; [d] FDR $P < .10$.

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may be influenced by gestational age,[23,24] it cannot be excluded that some of the differences observed between the preeclamptic and normal pregnancy groups are, in fact, gestational age related. Winn et al[23] compared global gene expression in basal plate (decidual) biopsies from normal pregnancies at mid-gestation (14-24 weeks) and at term (37-40 weeks) and found that 418 genes (of 39,000 transcripts examined) changed expression throughout gestation. This provides a useful dataset for comparison with the data obtained in this current study, albeit different profiling platforms were used. Winn et al[23] used the Affymetrix HG-U133 A&B chip for transcriptional profiling, whereas we used the Illumina HumanWG-6 v2 Expression BeadChip. By this, the number of possible comparisons was restricted to the 16,799 genes shared in both systems. Of the 455 transcripts found to be differentially expressed in this current study, 368 genes demonstrate no gestational age-influenced changes, according to the data of Winn et al.[23] It is therefore tempting to speculate that the differential expression of these 368 genes may be related to disease mechanisms at play in preeclampsia. Seventeen of our differentially expressed genes (TEMEM97, KIAA1598, SULT2B1, EGFR, FHL1, PLA2G7, SHANK3, NOTCH4, UBASH3B, ROBO4, NRARP, GPR116, IL6ST, LDLR, ANGPTL2, SRPRB, and KREMEN1) are reported to change expression with gestational age.[23] For 2 of these genes (SULT2B1 and EGFR), expression increases toward term.[23] Thus, isolated gestational age-related influences in the preeclampsia group would suggest a lower expression of SULT2B1 and EGFR, but both were up-regulated in our dataset. Similarly, the ANGPTL2 gene is down-regulated toward term,[23] but in contrast to what might be expected from gestational age–related changes, expression was lower in the preeclampsia group than in the normal pregnancy group. Based on this, we conclude that the differential expression of these 3 genes may also be ascribed to disease-related mechanisms. However, with regard to the remaining 14 genes in our dataset previously shown to exhibit gestational age-dependent changes in expression, conclusions are hampered by the fact that gestational age may have contributed to the differences observed between preeclamptic and normal pregnancies. To illustrate: expression of FHL1, SHANK3, NOTCH4, ROBO4, NRARP, and GPR116 increases toward term[23] and was down-regulated in the preeclampsia group, whereas TMEM97, KIAA1598, PLA2G7, UBASH3B, IL6ST, LDLR, SRPRB, and

TABLE 3
Results for selected genes from microarray and real-time quantitative polymerase chain reaction expression

		Microarray		RT-qPCR	
Gene symbol	Up/down	Beta value	*P* value[a]	Fold change	*P* value[b]
SLITRK4	↓	−1.04	4.59×10^{-8}	−1.98	< .0001 1.73 x 10-5
FZD4	↓	−0.91	4.05×10^{-7}	−1.35	.001 7.71 x 10-4
ANGPTL2	↓	−0.89	4.39×10^{-6}	−1.74	< .0001 4.79 x 10-5
PLA2G7	↑	0.83	1.58×10^{-5}	1.26	.068 6.79 x 10-2
MAN1A	↑	0.85	1.29×10^{-5}	1.30	.025 2.49 x 10-2
ARL5B	↑	0.91	4.46×10^{-7}	1.22	.017 1.66 x 10-2

RT-qPCR, real-time quantitative polymerase chain reaction.

[a] $P < .05$, obtained with SOLAR; [b] $P < .10$, obtained with *t* test statistics with software (SPSS, version 16; SPSS, Inc, Chicago, IL).

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TABLE 4
Canonical pathway analysis

Canonical pathway[a]	Genes	*P* value[b] Ingenuity Pathway Analysis	*P* value[c] Rotation Gene Set Enrichment Analysis
Tryptophan metabolism	ACMSD, ALDH3A2, ASMTL, CYP2E1, CYP2J2, INMT, KYNU, MAOA	5.51×10^{-4}	2.0×10^{-4}
Endoplasmic reticulum stress pathway	ATF6, DNAJC3, EIF2AK3, XBP1	5.81×10^{-4}	5.3×10^{-3}
Linoleic acid metabolism	CYP2E1, CYP2J2, PLA2G5, PLA2G2A, WISP2	3.91×10^{-3}	1.5×10^{-3}
Notch signaling	DTX3, HES1, NOTCH3, NOTCH4	6.72×10^{-3}	7.9×10^{-3}
Fatty acid metabolism	ACOX1, ACOX2, ADH1A, ALDH3A2, CYP2E1, CYP2J2	7.90×10^{-3}	10.0×10^{-5}
Arachidonic acid metabolism	CYP2E1, CYP2J2, PLA2G5, PLA2G2A, PTGIS, WISP2	8.66×10^{-3}	10.0×10^{-5}
NRF2-mediated oxidative stress response	ACTG2, DNAJB6, DNAJB9, DNAJB11, DNAJC3, EIF2AK3, GSTA3, HMOX1, UBE2K	9.99×10^{-3}	6.7×10^{-2}

[a] Ingenuity Pathway Analysis (www.ingenuity.com; Ingenuity Systems, Redwood City, CA) was used to bioinformatically identify canonical (ie, cell signaling and metabolic) pathways potentially involved in preeclampsia within our dataset; [b] *P* value obtained with Fisher's exact test; [c] *P* value obtained with use of *limma* package.

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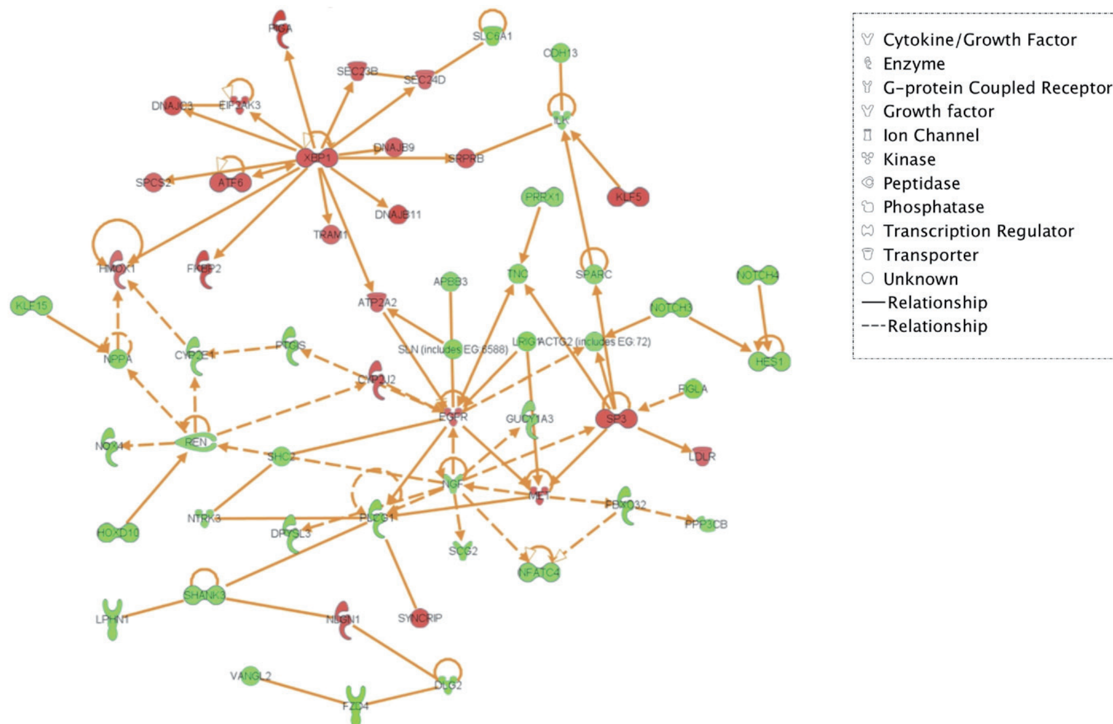
KREMEN1 expression decreases toward term[23] and was up-regulated in the preeclampsia group.

In genomewide transcriptional profiling, analysis of *groups* of genes is a strategy to increase power and reduce the dimensionality of the underlying statistical problem following multiple testing.[25] Further, it may be advantageous to put focus on canonical pathways and networks instead of single genes when the aim is to obtain insight in the pathophysiology of complex diseases, such as preeclampsia. The high interconnectivity of focus genes with other correlated genes within a biological network may imply functional and biological importance of these genes.[26,27] To be able to assess this in a comprehensive manner, we increased the FDR cutoff to 0.1 and consequently the number of genes included in the analysis. Using this approach, 7 significant canonical pathways were found to be represented by the differentially expressed genes identified in this current study (Table 4).

The most significant canonical pathway detected was tryptophan metabolism. The metabolism of tryptophan, through the kynurenine pathway, has previously been suggested to be involved in preeclampsia pathogenesis,[28,29] and, in accordance with this, the activity of the first enzyme of the kynurenine pathway, indoleamine 2,3 dioxygenase, has been reported to be reduced in placenta from preeclamptic pregnancies.[28] We found no disease-associated changes in indoleamine 2,3 dioxygenase expression, but the transcript encoding the enzyme kynureninase (KYNU) was up-regulated. KYNU metabolizes L-kynurenine, which suppresses T-cell proliferation and natural killer cells and influences immunotolerance to foreign antigens.[30] This implies that a consequence of KYNU up-regulation may be an increased inflammatory response (due to lack of L-kynurenine). An additional 7 genes were assigned to this canonical pathway (Table 4).

The second most significant canonical pathway identified was the ER stress pathway. Three genes (EIF2AK3, ATF6, and XBP1) included in the unfolded protein response, a coordinated adaptive response to ER stress, were up-regulated. ER stress has previously been suggested as one of the main sources for generation of placental oxidative stress.[31] Yung et al[32] have reported similar associations of the unfolded protein response signaling pathways to preeclampsia in placental tissue, but these findings are reported for the first time in decidual tissue. There is a close connection between oxidative stress and ER stress,[31,33] also indicated by the many direct relationships of the ER and oxidative stress–related genes in the generated network (Figure). The canonical pathway NRF2-mediated oxidative stress response was also among the significant pathways identified (Table 4). The nuclear factor NFR2 plays an essential role in the defense of oxidative stress by regulating the expression of antioxidant response elements.[34] In case of excessive oxidative stress, activation by reactive oxygen species, nitrogen oxide, and proinflammatory cytokines results in translocation of NRF2 to the nucleus. NRF2 binds to antioxidant response element sequences, leading to transcriptional activation of antioxidant genes (eg, glutathione and HMOX1). NRF2-mediated oxidative stress response included 9 genes, of which 3 genes have pre-

FIGURE
Network of preeclampsia-correlated genes



Ingenuity Pathway Analysis (www.ingenuity.com; Ingenuity Systems, Redwood City, CA) generated gene-gene product interaction network of preeclampsia-correlated genes. Genes or gene products are represented as nodes, and biological relationship between 2 nodes is represented as edge (*line*). All edges are supported by at least 1 published reference. *Solid edges* represent direct relationship, and *dashed edges* represent indirect relationship. Node color represents correlation of expression level with preeclampsia, and color intensity indicates degree of correlation (*red* is positive and *green* negative). Shape of each node represents functional class of gene product, as shown in *key*.

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viously been associated with preeclampsia (EIF2AK3,[32] GSTA3,[10] and HMOX1[21,22]). Several enzymes metabolize reactive oxygen species to exportable compounds, and in this study the transcripts encoding the antioxidant enzymes GSTA3, HMOX1, and UBE2K were up-regulated.

Three of the remaining significant canonical pathways generated by IPA represented metabolism of fatty acids: linoleic acid metabolism, fatty acid metabolism, and arachidonic acid metabolism. The genes included in these pathways were partly overlapping, as shown in Table 4. Decidual arterioles of preeclamptic women show atherosclerotic-like lesions,[35] suggesting an underlying atherogenic process of low-density lipoprotein lipid peroxidation.[36] Lipid peroxidation contributes to the development of preeclampsia,[37] and decidua basalis tissue from preeclamptic women has an increased content of lipid peroxides.[4] The first enzyme of the fatty acid β-oxidation pathway, acyl-coenzyme A oxidase (ACOX)1/palmitoyl-coA oxidase, donates electrons directly to molecular oxygen, thereby producing hydrogen peroxides. ACOX1 was found to be up-regulated, whereas ACOX2/branched chain ACOX, which is involved in the degradation of long branched fatty acids and bile acid intermediates in peroxisomes, was found to be down-regulated. Two genes involved in elimination of lipid peroxidation products were also down-regulated in the material: alcohol dehydrogenase 1a, which metabolizes a wide variety of substrates including lipid peroxidation products, and aldehydedehydrogenase 3 family member A2 isozymes, thought to play a major role in the detoxification of aldehydes generated by alcohol metabolism and lipid peroxidation. Increased generation or decreased elimination of lipid peroxidation products may be among the factors activating the maternal endothelium[38] and triggering systemic inflammation in preeclampsia.

Finally, the pathway analysis suggested a role of notch signaling, with inclusion of 4 down-regulated genes: DTX3, HES1, NOTCH 3, and NOTCH 4. Notch signaling is known to be involved in cell differentiation, proliferation, apopto-

sis,[39] and blood vessel formation,[40] processes neatly regulated in the placenta to maintain a normal pregnancy. Notch receptors are expressed on extravillous trophoblasts and are hypothesized to be involved in the differentiation and proliferation of both extravillous trophoblasts and endothelial cells.[41] Placental villi from preeclamptic pregnancies show down-regulation of notch pathway members.[42] Notch signaling in placenta has been suggested to play a role in the development of preeclampsia,[42,43] and the altered expression of DTX and HES1 in tissue from preeclamptic pregnancies compared with normal pregnancies is presented for the first time.

In summary, we have provided a comprehensive transcriptional profile of the decidua in preeclampsia. Our network analysis has demonstrated extensive connectivity between the differently expressed genes. Alteration of the expression level of 1 gene may influence the transcription of others included in the network. Due to this, it is difficult to pinpoint the genes having primary roles in perpetuating preeclampsia from our dataset. Some of our findings confirm and elaborate the current knowledge on the pathophysiology of preeclampsia, while others are novel. Further studies are warranted to replicate findings and confirm involvement of specific genes that have been identified. ■

ACKNOWLEDGMENTS

We thank all the delivering mothers whose participation made this work possible. We are grateful for the statistical expertise at Southwest Foundation for Biomedical Research, San Antonio, TX: Drs Jac C. Charlesworth and Thomas D. Dyer, and at the Norwegian University of Science and Technology, Trondheim, Norway: Drs Åsa Johansson and Jostein Johannesen at the FUGE bioinformatics platform. We thank Dr Linda T. Roten for her valuable advice and comments made during manuscript preparation.

REFERENCES

1. Redman CW, Sargent IL. Placental stress and pre-eclampsia: a revised view. Placenta 2009;30(Suppl):S38-42.
2. Hung TH, Burton GJ. Hypoxia and reoxygenation: a possible mechanism for placental oxidative stress in preeclampsia. Taiwan J Obstet Gynecol 2006;45:189-200.
3. Staff AC, Halvorsen B, Ranheim T, et al. Elevated level of free 8-iso-prostaglandin F2alpha in the decidua basalis of women with preeclampsia. Am J Obstet Gynecol 1999;181:1211-5.
4. Staff AC, Ranheim T, Khoury J, et al. Increased contents of phospholipids, cholesterol, and lipid peroxides in decidua basalis in women with preeclampsia. Am J Obstet Gynecol 1999;180:587-92.
5. Salonen Ros H, Lichtenstein P, Lipworth L, et al. Genetic effects on the liability of developing pre-eclampsia and gestational hypertension. Am J Med Genet 2000;91:256-60.
6. Moses EK, Fitzpatrick E, Freed KA, et al. Objective prioritization of positional candidate genes at a quantitative trait locus for pre-eclampsia on 2q22. Mol Hum Reprod 2006;12:505-12.
7. Lie RT, Rasmussen S, Brunborg H, et al. Fetal and maternal contributions to risk of pre-eclampsia: population based study. BMJ 1998;316:1343-7.
8. Schadt EE, Zhang B, Zhu J. Advances in systems biology are enhancing our understanding of disease and moving us closer to novel disease treatments. Genetica 2009;136:259-69.
9. Founds SA, Dorman JS, Conley YP. Microarray technology applied to the complex disorder of preeclampsia. J Obstet Gynecol Neonatal Nurs 2008;37:146-57.
10. Winn VD, Gormley M, Paquet AC, et al. Severe preeclampsia-related changes in gene expression at the maternal-fetal interface include sialic acid-binding immunoglobulin-like lectin-6 and pappalysin-2. Endocrinology 2009;150:452-62.
11. Herse F, Dechend R, Harsem NK, et al. Dysregulation of the circulating and tissue-based renin-angiotensin system in preeclampsia. Hypertension 2007;49:604-11.
12. Gifford RW, August PA, Cunningham G, et al. Report of the national high blood pressure education program working group on high blood pressure in pregnancy. Am J Obstet Gynecol 2000;183:S1-22.
13. Harsem NK, Staff AC, He L, et al. The decidual suction method: a new way of collecting decidual tissue for functional and morphological studies. Acta Obstet Gynecol Scand 2004;83:724-30.
14. Almasy L, Blangero J. Multipoint quantitative-trait linkage analysis in general pedigrees. Am J Hum Genet 1998;62:1198-211.
15. Goring HH, Curran JE, Johnson MP, et al. Discovery of expression QTLs using large-scale transcriptional profiling in human lymphocytes. Nat Genet 2007;39:1208-16.
16. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 2001;25:402-8.
17. Hellemans J, Mortier G, De Paepe A, et al. qBase relative quantification framework and software for management and automated analysis of real-time quantitative PCR data. Genome Biol 2007;8:R19.
18. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc 1995;57:289-300.
19. Gentleman RC, Carey VJ, Bates DM, et al. Bioconductor: open software development for computational biology and bioinformatics. Genome Biol 2004;5:R80.
20. Staff AC, Ranheim T, Halvorsen B. Augmented PLA2 activity in pre-eclamptic decidual tissue–a key player in the pathophysiology of 'acute atherosis' in pre-eclampsia? Placenta 2003;24:965-73.
21. Enquobahrie DA, Meller M, Rice K, et al. Differential placental gene expression in preeclampsia. Am J Obstet Gynecol 2008;199:566.e1-11.
22. Ahmed A, Rahman M, Zhang X, et al. Induction of placental heme oxygenase-1 is protective against TNFalpha-induced cytotoxicity and promotes vessel relaxation. Mol Med 2000;6:391-409.
23. Winn VD, Haimov-Kochman R, Paquet AC, et al. Gene expression profiling of the human maternal-fetal interface reveals dramatic changes between midgestation and term. Endocrinology 2007;148:1059-79.
24. Mikheev AM, Nabekura T, Kaddoumi A, et al. Profiling gene expression in human placentae of different gestational ages: an OPRU network and UW SCOR study. Reprod Sci 2008;15:866-77.
25. Ackermann M, Strimmer K. A general modular framework for gene set enrichment analysis. BMC Bioinformatics 2009;10:47.
26. Carlson MR, Zhang B, Fang Z, et al. Gene connectivity, function, and sequence conservation: predictions from modular yeast co-expression networks. BMC Genomics 2006;7:40.
27. Gawrieh S, Baye TM, Carless M, et al. Hepatic gene networks in morbidly obese patients with nonalcoholic fatty liver disease. Obes Surg 2010 May 16 [Epub ahead of print].
28. Kudo Y, Boyd CA, Sargent IL, et al. Decreased tryptophan catabolism by placental indoleamine 2,3-dioxygenase in preeclampsia. Am J Obstet Gynecol 2003;188:719-26.
29. Santoso DI, Rogers P, Wallace EM, et al. Localization of indoleamine 2,3-dioxygenase and 4-hydroxynonenal in normal and pre-eclamptic placentae. Placenta 2002;23:373-9.
30. Opitz CA, Wick W, Steinman L, et al. Tryptophan degradation in autoimmune diseases. Cell Mol Life Sci 2007;64:2542-63.
31. Burton GJ, Yung HW, Cindrova-Davies T, et al. Placental endoplasmic reticulum stress and oxidative stress in the pathophysiology of unexplained intrauterine growth restriction and early onset preeclampsia. Placenta 2009;30(Suppl):S43-8.
32. Yung HW, Calabrese S, Hynx D, et al. Evidence of placental translation inhibition and endoplasmic reticulum stress in the etiology of human intrauterine growth restriction. Am J Pathol 2008;173:451-62.
33. Cullinan SB, Diehl JA. Coordination of ER and oxidative stress signaling: the PERK/Nrf2 signaling pathway. Int J Biochem Cell Biol 2006;38:317-32.

34. Itoh K, Chiba T, Takahashi S, et al. An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. Biochem Biophys Res Commun 1997;236:313-22.
35. De Wolf F, Robertson WB, Brosens I. The ultrastructure of acute atherosis in hypertensive pregnancy. Am J Obstet Gynecol 1975;123:164-74.
36. Branch DW, Mitchell MD, Miller E, et al. Preeclampsia and serum antibodies to oxidized low-density lipoprotein. Lancet 1994;343:645-6.
37. Walsh SW. Maternal-placental interactions of oxidative stress and antioxidants in preeclampsia. Semin Reprod Endocrinol 1998;16:93-104.
38. Hubel CA, Roberts JM, Taylor RN, et al. Lipid peroxidation in pregnancy: new perspectives on preeclampsia. Am J Obstet Gynecol 1989;161:1025-34.
39. Artavanis-Tsakonas S, Rand MD, Lake RJ. Notch signaling: cell fate control and signal integration in development. Science 1999;284:770-6.
40. Liu ZJ, Shirakawa T, Li Y, et al. Regulation of Notch1 and Dll4 by vascular endothelial growth factor in arterial endothelial cells: implications for modulating arteriogenesis and angiogenesis. Mol Cell Biol 2003;23:14-25.
41. De Falco M, Cobellis L, Giraldi D, et al. Expression and distribution of notch protein members in human placenta throughout pregnancy. Placenta 2007;28:118-26.
42. Cobellis L, Mastrogiacomo A, Federico E, et al. Distribution of Notch protein members in normal and preeclampsia-complicated placentas. Cell Tissue Res 2007;330:527-34.
43. Sitras V, Paulssen RH, Gronaas H, et al. Differential placental gene expression in severe preeclampsia. Placenta 2009;30:424-33.

Paper IV

Placenta 32 (2011) 823–829

Contents lists available at SciVerse ScienceDirect

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Increased endoplasmic reticulum stress in decidual tissue from pregnancies complicated by fetal growth restriction with and without pre-eclampsia

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A R T I C L E I N F O

Article history:
Accepted 14 August 2011

Keywords:
Pre-eclampsia
Fetal growth restriction
Endoplasmic reticulum stress
Gene expression
Decidua basalis

A B S T R A C T

Objectives: Endoplasmic reticulum (ER) stress has been implicated in both pre-eclampsia (PE) and fetal growth restriction (FGR), and is characterised by activation of three signalling branches: 1) PERK-pEIF2α, 2) ATF6 and 3) splicing of XBP1(U) into XBP1(S). To evaluate the contribution of ER stress in the pathogenesis of PE relative to FGR, we compared levels of ER stress markers in decidual tissue from pregnancies complicated by PE and/or FGR.
Study design: Whole-genome transcriptional profiling was performed on decidual tissue from women with PE ($n = 13$), FGR ($n = 9$), PE+FGR ($n = 24$) and controls ($n = 58$), and used for pathway and targeted transcriptional analyses of ER stress markers. The expression and cellular localisation of ER stress markers was assesses by Western blot and immunofluorescence analyses.
Results: Increased ER stress was observed in FGR and PE+FGR, including both the PERK-pEIF2α and ATF6 signalling branches, whereas ER stress was less evident in isolated PE. However, these cases demonstrated elevated levels of XBP1(U) protein. ATF6 and XBP1 immunoreactivity was detected in most (>80%) extravillous trophoblasts, decidual cells and macrophages. No difference in the proportion of immunopositive cells or staining pattern was observed between study groups.
Conclusions: Increased PERK-pEIF2α and ATF6 signalling have been associated with decreased cellular proliferation and may contribute to the impaired placental growth characterising pregnancies with FGR and PE+FGR. XBP1(U) has been proposed as a negative regulator of ER stress, and increased levels in PE may reflect a protective mechanism against the detrimental effects of ER stress.
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1. Introduction

Impaired spiral artery remodelling is a prominent feature of the decidua basalis in pregnancies complicated by pre-eclampsia (PE) and/or fetal growth restriction (FGR) [1]. The incompletely remodelled spiral arteries retain their musculo-elastic structure, which renders the vessels more responsive to vasoactive stimuli. Subsequent fluctuation in uteroplacental blood supply is proposed to cause ischaemia-reperfusion insults and oxidative stress [2]. Several studies have shown that decidual tissue is an important source of oxidative stress [3,4], and increased levels of oxidative stress have been reported in pregnancies complicated by PE and FGR [3,5,6]. Furthermore, ischaemia-reperfusion insults can induce endoplasmic reticulum (ER) stress in trophoblast-like cell lines [7].

The ER is an eukaryotic organelle involved in protein folding and maturation, lipid synthesis and calcium homeostasis [8], as well as sensing, coordinating and mediating stress responses [9,10]. Conditions that interfere with ER functions are collectively called ER stress. Stimuli such as accumulation of unfolded proteins, nutrient deprivation and oxidative stress can induce ER stress and activate the unfolded protein response (UPR) [8,9]. The UPR is a cellular self-defence mechanism, aiming to alleviate ER stress and re-establish homeostasis [8]. Three ER transmembrane sensors,

Abbrevations: ER, endoplasmic reticulum; UPR, unfolded protein response; PERK, PKR-like ER kinase; IRE1, inositol-requiring enzyme 1; ATF6, activating transcription factor 6; EIF2α, eukaryotic translation initiation factor 2α; XBP1, X-box binding protein 1.

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doi:10.1016/j.placenta.2011.08.005

Table 1
Primary and secondary antibodies used for Western blot and immunohistochemical analyses.

	Species	Manufacturer	Catalogue no.	Dilution Western blot	Dilution immunofluorescence
Primary antibodies					
XBP1	Rabbit anti-human	Santa Cruz Biotechnology, CA	sc-7160	1:500	1:5
ATF6	Mouse anti-human	Imgenex, San Diego, CA	IMG-273	1:200	1:300
pEIF2α	Rabbit anti-human	Cell Signalling Technology, Danvers, MA	#9721	1:1500	
EIF2α	Rabbit anti-human	Cell Signalling Technology, Danvers, MA	#9722	1:3000	
β-actin	Mouse anti-human	Abcam, Cambridge, UK	ab6276-100	1:30,000	
CK7	Mouse anti-human	Dako, Glostrup, Denmark	M7018		1:750
CK7	Rabbit anti-human	Abcam, Cambridge, UK	ab52870		1:50
Vimentin	Mouse anti-human	Dako, Glostrup, Denmark	M0725		1:200
Prolactin	Rabbit anti-human	Dako, Glostrup, Denmark	A0569		1:60
CD68	Mouse anti-human	Dako, Glostrup, Denmark	M0814		1:1000
CD68	Rabbit anti-human	Santa Cruz Biotechnology, CA	sc-9139		1:5
Secondary antibodies					
IRDye 680	Goat anti-rabbit	Li-Cor Biosciences, Cambridge, UK	#926-32221	1:5000	
IRDye 800CW	Donkey anti-mouse	Li-Cor Biosciences, Cambridge, UK	#926-32212	1:5000	
FITC[a]	Goat anti-mouse	Dako, Glostrup, Denmark	F0479		1:10
TRITC[b]	Swine anti-rabbit	Dako, Glostrup, Denmark	R0156		1:10

[a] Fluorescein iso-thiocyanate.
[b] Tetramethyl rhodamine iso-thiocyanate.

PKR-like ER kinase (PERK), inositol-requiring enzyme 1 (IRE1) and activating transcription factor 6 (ATF6), regulate the UPR through their respective signalling cascades [9]. Activated PERK phosphorylates the eukaryotic translation initiation factor 2α (EIF2α), which leads to attenuation of mRNA translation and a reduced ER workload [8]. IRE1 splices the constitutively expressed (unspliced) mRNA of the X-box binding protein 1, *XBP1(U)*, into a spliced isoform, *XBP1(S)* [11]. Both *XBP1(U)* and *XBP1(S)* mRNA are translated into transcription factors, but XBP1(S) has higher transcriptional activator activity [11]. The transcription factor ATF6 is activated by proteolytic cleavage in the Golgi compartment [12]. With partly overlapping functions, XBP1 and ATF6 initiate transcription of genes that aim to increase protein folding capacity and degradation of misfolded proteins in ER [8]. These multiple signalling pathways allow for diversity in responses to ER stress, from minor homeostatic adjustments to oxidative stress [13] and activation of inflammatory pathways [10]. Additionally, if the cell fails to combat ER stress, the UPR can trigger apoptosis to eliminate damaged cells [9], but the manner in which the UPR switches from a protective to an apoptotic role is complex and not fully understood [14].

Increased levels of ER stress have been detected in FGR and PE+FGR placentas, where ER stress was associated with decreased cellular proliferation and apoptosis, and proposed as an important cause for the reduced placental growth characterising these phenotypes [15,16]. We recently performed whole-genome transcriptional profiling of decidual tissue from pre-eclamptic and normal pregnancies, identifying upregulation of several transcripts involved in ER stress in PE [17]. Thus, current data indicate that ER stress is involved in the pathogenesis of both PE and FGR, but whether the degree of ER stress differs between these pregnancy complications is unknown. Emerging observations indicate that PE and/or FGR may represent more or less severe stages on a continuous spectrum of responses to impaired placentation, where ischaemia-reperfusion insults and oxidative stress following impaired spiral artery remodelling appear to be common pathophysiological events [16,18]. However, as the clinical outcomes differ, disparate phenomena must take place at some point during the pathogenesis. Given the multiple responses ER stress may elicit, differential activation of UPR signalling branches might explain some of the differences in clinical outcome. The present study aimed to compare the degree of ER stress in pregnancies complicated by PE and/or FGR by analysing transcriptional- and protein expression of key mediators in each branch of the ER stress response.

2. Materials and methods

2.1. Study subjects

Women with pregnancies complicated by PE and/or FGR (cases) and women with uncomplicated pregnancies (controls) were recruited at Trondheim University Hospital (Norway) and Haukeland University Hospital (Bergen, Norway) from 2002 to 2006. PE was defined as persistent hypertension (blood pressure of ≥140 mmHg systolic or 90 mmHg diastolic), plus proteinuria (≥0.3 g in a 24 h urine collection or ≥ 1+ according to a dipstick test), developing after 20 weeks of gestation [19]. PE was sub-classified as severe in accordance with criteria recommended by Sibai et al. [20]. FGR was defined as birthweight <2.5 percentile adjusted for gestational age and sex according to a Scandinavian normogram [21], in addition to at least one of the following criteria: 1) reduced fundal height in serial measurements; 2) serial ultrasound biometry identifying failure to grow along a consistent percentile; or 3) abnormal umbilical artery waveform. Severe FGR was defined as birthweigh <1.7 percentile [22]. Cases diagnosed with PE or FGR before gestational week 34 were classified as early onset. Exclusively healthy women with no prior pregnancy complications were included as controls. Pregnancies with chromosomal aberrations, fetal and placental structural abnormalities or suspected perinatal infections were excluded from both study groups. Cases had caesarean section (CS) performed due to medical indications, whereas controls were undergoing CS for reasons considered irrelevant to the aim of this study (i.e. breech presentation, previous CS or maternal request). Only singleton pregnancies delivered by CS without labour activity were included. The study was approved by the Norwegian Regional Committee for Medical Research Ethics (REK no. 054-02) and informed consent was obtained from all participants.

2.2. Decidual tissue

Decidua basalis tissue was collected by vacuum suction immediately after separation of the placenta from the placental bed during CS [3,23]. The vacuum suction procedure was performed in less than 1 min. Collected tissue was flushed with saline to remove excessive blood. Decidual tissue was placed in RNAlater (for microarray analyses), 10% neutral-buffered formalin and paraffin embedded (for immunohistochemical analyses) and snap frozen in liquid nitrogen (for Western blot analyses) within 15 min of tissue collection.

2.3. Illumina microarray analysis

Total RNA extracted from decidual tissue was used for synthesis of biotin labelled anti-sense RNA (aRNA) which was hybridised to Illumina HumanWG-6 v2 Expression BeadChips (Illumina Inc., San Diego, CA) as previously described [17]. Microarray expression data were preprocessed and analysed using Sequential Oligogenic Linkage Analysis Routines (SOLAR) [24] as previously described [25]. Microarray were performed in accordance with the Minimum Information About a Microarray Experiment (MIAME) guidelines [26]. Experimental data have been submitted to ArrayExpress (www.ebi.ac.uk/arrayexpress/) under accession no. E-TABM-682. The microarray dataset was validated by quantitative real-time polymerase chain reaction (qRT-PCR) analyses for six of the most differentially expressed transcripts as previously described [17].

Table 2
Clinical characteristics of the study subjects included in the Microarray analysis ($n = 104$).

	PE ($n = 13$)	FGR ($n = 9$)	PE+FGR ($n = 24$)	Controls ($n = 58$)
Maternal age (y)	30.1 ± 5.4	32.7 ± 6.4	29.7 ± 5.5	32.4 ± 5.1
Systolic BP (mmHg)	153.1 ± 17.5**	128.3 ± 14.7*	151.0 ± 14.9**	116.2 ± 10.4
Diastolic BP (mmHg)	94.7 ± 8.6**	74.4 ± 8.3	96.2 ± 12.1**	69.9 ± 8.8
BMI (kg/m^2)[a]	29.2 ± 6.3	28.5 ± 7.5	26.4 ± 6.1	26.0 ± 4.5
Primipara	8/13	4/9	16/24	29/55[b]
GA (weeks)	34.8 ± 2.7**	31.9 ± 5.0**	30.3 ± 3.5**	38.7 ± 0.8
Birthweight (g)	2363.9 ± 509.7**	1224.7 ± 672.4**	1118.2 ± 470.1**	3608.3 ± 491.9

Data are presented as means ± SD, $^{*}p < 0.05$ and $^{**}p < 0.001$ comparing pre-eclampsia (PE), fetal growth restriction (FGR) and PE+FGR to controls; BP, blood pressure.
[a] BMI, Body Mass Index measured at first antenatal care visit.
[b] No info available for three of the controls; GA, gestational age.

2.4. Pathway and targeted transcriptional analyses of ER stress markers

We recently performed whole-genome transcriptional profiling of decidual tissue from pre-eclamptic and normal pregnancies, identifying upregulation of several transcripts involved in ER stress in PE. In the previous study, we solely focused on *PE associated* transcriptional changes, i.e. using only a subset of the total microarray dataset used in this work [17]. To further explore the role of ER stress in the pathogenesis of *both* PE and FGR, we performed pathway analyses on a group of 20 ER stress related transcripts (as annotated by Ingenuity Pathway Analysis) (Supplementary Table 1) in cases and controls. Pathway analyses were performed using Rotation Gene Set Tests (ROAST) and Rotation Gene Set Enrichment Analysis (ROMER), implemented in the *limma* package [27] available via the Bioconductor Project (www.bioconductor.org). ROAST was used to test whether any of the transcripts in the pathway were differentially expressed [28] and ROMER was used to test whether the subset of transcripts in the pathway was more differentially expressed than any other subset of transcripts in the total dataset [29]. As a second step in our approach, three central ER stress marker from each branch of the UPR (ATF6, XBP1 and PERK), known to be upregulated by ER stress [30,31], were selected for a targeted transcriptional comparison to test if there was any differential UPR activation between cases with PE, FGR, PE+FGR and controls.

2.5. Western blotting

Decidual tissue was homogenised in lysis buffer (Active Motif, Rixensart, Belgium) using a rotor-stator homogeniser (Ultra-Turrax T25, Janke & Kunkel IKA Labortechnik, Staufen, Germany). Total protein extracts were prepared using a Nuclear extract kit (#40010, Active Motif), following manufacturer's instructions. Homogenised decidual tissue was lysed on ice for 30 min and cell debris pelleted at 14,000 g at 4 °C for 20 min. The supernatant was recovered and equal amounts (100 μg) of protein were separated on precast 10% denaturing NuPAGE gels (Invitrogen Life Technologies, Carlsbad, CA) and transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA). Membranes were blocked in Odyssey blocking buffer (Li-Cor Biosciences, Cambridge, UK) for 1 h at room temperature (RT) before incubating with primary antibodies against pEIF2α, EIF2α, XBP1 and ATF6. The antibody against XBP1 detected both spliced and unspliced variants of XBP1 [32,33], and the antibody against ATF6 detected both cleaved and uncleaved ATF6 [33,34]. Blots were reprobed with β-actin as a loading control. Primary antibodies were diluted in Odyssey blocking buffer (Li-Cor Biosciences) and hybridised to the membranes overnight at 4 °C. Membranes were washed 3 × 10 min in tris-buffered saline (TBS) and incubated with fluorescently labelled secondary antibodies diluted in Odyssey blocking buffer (Li-Cor Biosciences) for 1 h at RT. Specifications for the primary and secondary antibodies are listed in Table 1. Membranes were scanned using the Odyssey Infrared Imaging System (Li-Cor Biosciences). Specific bands for pEIF2α (37 kDa), EIF2α (37 kDa), XBP1(U) (31 kDa), XBP1(S) (54 kDA), ATF6 (50 kDA) and β-actin (42 kDa) were detected in all samples. Band intensities were determined from two or three scans, normalised relative to loading control and quantified by densitometric analysis using Odyssey imaging software v3.0 (Li-Cor Biosciences).

2.6. Immunohisotchemical analyses

Cellular localisation and expression of ATF6 and XBP1 in decidual tissue was assesses using the same antibodies against XBP1 and ATF6 as used in Western blot experiments, in combination with antibodies against cytokeratin 7 (CK7) to detect extravillous trophoblasts (EVTs), vimentin and prolactin to detect decidual cells (DeCs), and CD68 to detect macrophages (Mφs). Double immunofluorescence staining was performed manually after deparaffination in xylen, rehydration and heat-induced antigen retrieval in TBS. Protein Block (X0909, Dako, Glostrup, Denmark) was added for 10 min to inhibit non-specific binding, and slides were incubated in a primary antibody mixture overnight at 4 °C. The slides were incubated with appropriate species-specific secondary antibodies for 30 min in a dark chamber. Slides were examined using a fluorescent microscope (Nikon Eclipse 90i with CytoVision 3.7 software, Applied Imaging, New Milton Hampshire, UK) at magnification ×600. Decidual glands, tonsil tissue and pituitary glands were used as positive controls for CK7, vimentin/CD68 and prolactin, respectively. For negative controls, primary antibodies were substituted with isotype-matched rabbit- (#011-000-120, Jackson ImmunoResearch, PA) and mouse (#400102, BioLegend, CA) immunoglobulins. Specifications for the primary and secondary antibodies are listed in Table 1. The proportion of XBP1 and ATF6 immunopositive EVTs, DeCs and Mφs was counted in five randomly selected fields on each slide, and calculated as the number of positive cells among the total number of CK7, vimentin/prolactin or CD68 positive cells, respectively.

2.7. Statistical analyses

Nonparametric data were analysed using Mann–Whitney *U* test, whereas parametric data were analysed using the Students *t*-test. Fisher's exact test and χ^2 test were used for categorical data, and Spearman's rank correlation was used for correlation analyses. The significance threshold was set to 0.05. All analyses were performed using SPSS v15 (SPSS, Chicago, IL).

3. Results

3.1. Clinical characteristics of the study subjects

Clinical characteristics of the study subjects included in the microarray analysis are presented in Table 2. A total of 104 samples were included (PE, $n = 13$; FGR, $n = 9$; PE+FGR, $n = 24$ and controls, $n = 58$). As expected, elevated blood pressure was observed in pregnancies with PE and PE+FGR, and lower gestational age and birthweight were observed in all case groups (Table 2).

A total of 30 samples (PE, $n = 7$; FGR, $n = 7$; PE+FGR, $n = 8$ and controls, $n = 8$) were included in Western blot analysis (Table 3).

Table 3
Clinical characteristics of the study subjects included in the Western blot analysis ($n = 30$).

	PE ($n = 7$)	FGR ($n = 7$)	PE+FGR ($n = 8$)	Controls ($n = 8$)
Birthweight (g)	2137.1 ± 259.6**	1363.1 ± 753.8**	1105.3 ± 329.1**	3576.3 ± 507.1
Placenta weight (g)	447.8 ± 30.3*	269.2 ± 115.0*	261.3 ± 55.9**	657.9 ± 163.9
Placenta Weight Ratio[a]	1.0 ± 0.3	0.5 ± 0.2*	0.7 ± 0.1*	0.9 ± 0.1
Severe PE or FGR	5/7	6/7	8/8	
Early onset < 34 weeks	7/7	5/7	8/8	
Mean uterine artery score[b]	2.4**	2.2*	3.5**	0

Data are presented as means ± SD; $^{*}p < 0.05$, $^{**}p < 0.001$ comparing pre-eclampsia (PE), fetal growth restriction (FGR) and PE+FGR to controls.
[a] Placenta Weight Ratio was calculated as observed/expected placenta weight according to gestational age and sex according to a Norwegian normogram [42].
[b] Uterine artery score is based on pulsatility index and notching in Aa.uterinae [43]; Blood pressure, maternal age, gestational age, Body Mass Index and parity did not differ statistically from that of women included in the microarray analysis (Table 2).

Twenty-four of these were also included in the microarray study population, whereas the remaining six were selected from our total study population, but not included in microarray analyses due to low RNA quality. As ER stress probably arises secondary to ischaemia-reperfusion insults, we selected cases with clinical characteristics suggesting vascular malperfusion, which is closely associated with abnormal uterine artery Doppler findings and more common in early onset, severe PE [35] and severe FGR [36]. Thus, only cases with severe clinical characteristics were included, i.e. cases with uterine artery score ≥ 1 and/or early onset PE or FGR (Table 3). The mean uterine artery score was higher in all case groups compared to controls, whereas the placental weight ratio was lower in cases with FGR and PE+FGR as compared to controls (Table 3). Sixteen decidual samples selected from the Western blot study population were included in immunohistochemical analyses (PE, $n = 4$; FGR, $n = 4$; PE+FGR, $n = 4$ and controls, $n = 4$). The clinical characteristics of these samples did not differ from those included in Western blot analyses.

3.2. Pathway and targeted transcriptional analyses of ER stress markers

Pathway analyses (ROAST and ROMER) showed that the ER stress pathway was upregulated in cases with FGR and PE+FGR, whereas in PE, a trend towards upregulation was observed (Table 4). The targeted comparison of transcript data for *XBP1*, *ATF6* and *PERK* demonstrated that cases with FGR had increased expression of *ATF6*, cases with PE+FGR had increased expression of *XBP1*, *ATF6* and *PERK*, whereas *XBP1* was increased in PE (Table 4). The Illumina probe interrogating *XBP1* (ILMN_1710675) detected both the spliced and unspliced *XBP1* variants. No differences between the case groups were observed for any of these three transcripts *(data not shown)*.

3.3. Western blot analyses

Cases with FGR and PE+FGR showed increased phosphorylation of EIF2α ($p < 0.05$), increased ratio of pEIF2α/EIF2α ($p < 0.05$) and increased levels of ATF6 ($p < 0.01$) (Fig. 1A–C). Although cases with PE had high mean levels of pEIF2α/EIF2α and ATF6, large variations within this group rendered the results non-significant in comparison to controls ($p = 0.09$ and 0.43, respectively), as indicated by the larger standard deviation bars for the PE group in Fig. 1B–C. No differences in protein levels of the spliced form of XBP1, XBP1(S), were observed between any of the case groups compared to controls. However, increased levels of the unspliced form of XBP1, XBP1(U), and a decreased ratio of XBP1(S)/XBP1(U) were observed in isolated PE ($p < 0.05$ and 0.01, respectively) (Fig. 1A and C). The levels of XBP1(U) was higher in PE compared to that of FGR, and the ratio of XBP1(U)/XBP1(S) was lower in PE compared to FGR (all p's < 0.05). No differences in protein levels of pEIF2α/EIF2α, ATF6 or XBP1(S) were observed between case groups.

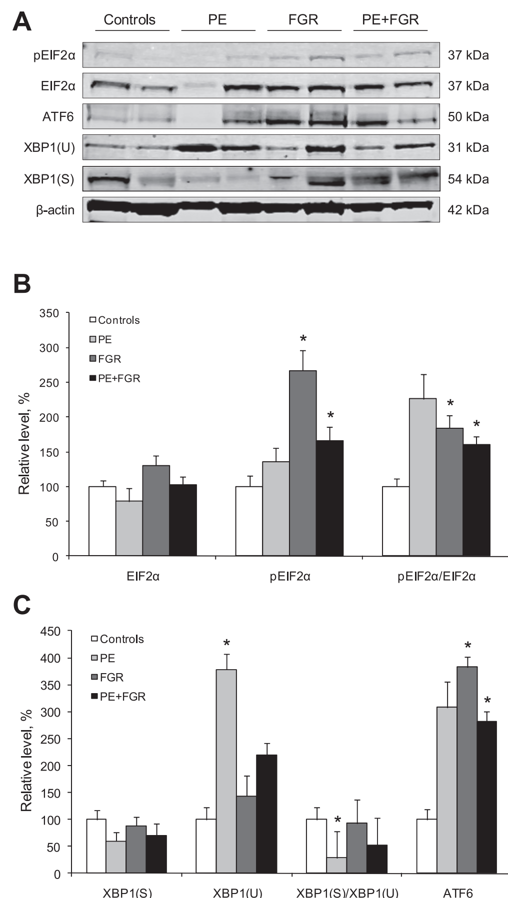

Fig. 1. Western blot analyses of endoplasmic reticulum (ER) stress markers in decidual tissue from pregnancies complicated by pre-eclampsia (PE, $n = 7$), fetal growth restriction (FGR, $n = 7$), PE+FGR ($n = 8$) and controls ($n = 8$). (A) A representative Western blot of phosphorylated and total EIF2α, ATF6, spliced (S) and unspliced (U) forms of XBP1 and loading control (β-actin). (B and C) Densitometry of bands expressed relative to controls (100%), showing increased levels of pEIF2α and ATF6 in pregnancies complicated by FGR and PE+FGR. Increased levels of XBP1(U) and a decreased XBP1(S)/XBP1(U) ratio were observed in pregnancies complicated by PE. Phosphorylation status of EIF2α is presented as the ratio between phosphorylated and total level of EIF2α. No significant differences in EIF2α were observed between any of the study groups. Data are presented as means ± SE, $^{*}p < 0.05$ as compared to controls.

Table 4
Pathway and targeted transcriptional analyses of ER stress related transcripts.

	PE vs. controls	FGR vs. controls	PE+FGR vs. controls
Pathway analysis[a]			
ROAST	$p = 0.06$ (NS)	↑, $p < 0.05$	↑, $p < 0.001$
ROMER	$p = 0.09$ (NS)	↑, $p < 0.05$	↑, $p < 0.05$
Microarray analysis[b]			
XBP1	↑, $p < 0.01$	$p = 0.07$ (NS)	↑, $p < 0.05$
ATF6	$p = 0.22$ (NS)	↑, $p < 0.05$	↑, $p < 0.001$
PERK	$p = 0.09$ (NS)	$p = 0.55$ (NS)	↑, $p < 0.05$

[a] Rotation Gene Set Tests (ROAST) and Rotation Gene Set Enrichment Analysis (ROMER) pathway analyses of 20 transcripts involved in the endoplasmic reticulum (ER) stress response (Supplementary Table 1).
[b] A targeted comparison of transcription data for ER stress markers from each branch of ER stress response; NS, not significant; ↑, upregulation.

3.4. Immunohistochemical analyses

Both cytoplasmic and nuclear immunoreactivity for XBP1 and ATF6 was observed in most (>80%) EVTs, DeCs and Mφs (Fig. 2 A,A′-R,R′). No differences in the proportion of XBP1 or ATF6 immunopositive cells or staining pattern were observed between the study groups. In general, XBP1 and ATF6 immunoreactivity was present in most cells in the decidual tissue, in contrast to tonsil tissue and pituitary glands, in which only a few cells were positive (*not shown*). Decidual glands displayed intense nuclear immunoreactivity for XBP1 in both cases and controls (Fig. 2S). No differences in staining

intensity of XBP1 and ATF6 were observed between the study groups in any of the evaluated cell types.

3.5. Correlation analyses

Correlation analyses demonstrated a negative correlation between the pEIF2α/EIF2α ratio and the placental weight ratio ($r_s = -0.561$, $p < 0.05$). A similar tendency was observed for ATF6 ($r_s = -0.352$, $p = 0.072$). None of the ER stress markers were correlated with gestational age.

4. Discussion

In this work, we have shown that decidual ER stress is increased in pregnancies complicated by FGR and PE+FGR. Initially, this was demonstrated by pathway analyses, showing upregulation of the ER stress pathway in FGR and PE+FGR. In addition, targeted transcriptional and protein analyses showed upregulation of the PERK-pEIF2α and ATF6 signalling branches of the UPR. In PE, none of these branches were significantly altered However, a marked increase in XBP1(U) was observed. These results indicate divergent activation of the UPR in FGR and PE+FGR compared to isolated PE.

Consistent with our finding of increased pEIF2α in deciduas from FGR and PE+FGR, increased placental levels of pEIF2α have previously been reported in these cases [15]. In trophoblast-like cell lines, increased levels of pEIF2α were associated with reduced proliferation through suppression of protein synthesis and decreased survival [15]. The net effect of reduced proliferation and cell survival was proposed as a cause for reduced placental growth in pregnancies with FGR and PE+FGR [15,16], which are characterised by decreased placental villous tissue volume and surface area [37]. Of relevance, we observed that the pEIF2α/EIF2α ratio was negatively correlated with placental weight ratio, with a similar tendency for ATF6, suggesting an association between ATF6 and PERK-pEIF2α signalling and reduced placental weight.

Cases with isolated PE only showed a trend towards upregulation of the ER stress response. However, we observed increased levels of *XBP1* mRNA and XBP1(U) protein in isolated PE. It was recently shown that XBP1(U), the constitutively expressed form of XBP1, accumulates in the recovery phase of ER stress [38] and is

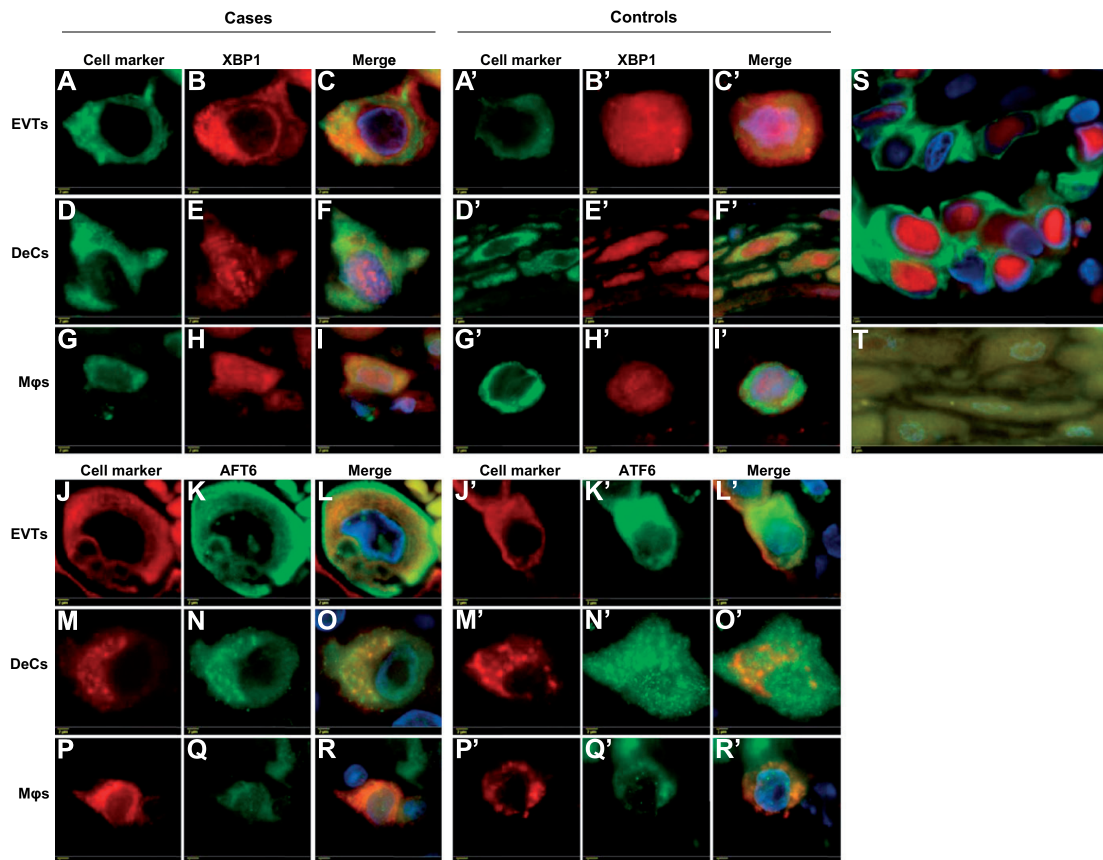

Fig. 2. Immunofluorescensce analyses of XBP1 and ATF6 in extravillous trophoblasts (EVTs), decidual cells (DeCs) and macrophages (Mφs), identified using antibodies against cytokeratin 7, vimentin/prolactin and CD68, respectively. Nuclei were counterstained with 4′-6-diamidino-2-phenylindole (DAPI) (blue signal). Cytoplasmic and nuclear immunoreactivity of XBP1 (red signal) was observed in EVTs, DeCs and Mφs in both cases with PE and/or FGR (A–I) and controls (A′–I′). A similar staining pattern was observed for ATF6 (green signal) for the same cell types in cases with PE and/or FGR (J–R) and controls (J′-R′). In decidual glands, strong nuclear immunoreactivity for XBP1 (red signal) was observed (S). No positive staining was found in negative controls where primary antibodies were substituted with isotype-matched immunoglobulins of the appropriate species (T). Magnification ×600 in D′-F′, S and T, otherwise ×1000. Scale bar 2 μm.

able to inhibit XBP1(S) and ATF6, implying that XBP1(U) functions as a negative regulator during ER stress [39,40]. This correlates well with our finding of the highest level of XBP1(U) and the lowest level of XBP1(S) in PE, and that ATF6 was not significantly increased in these cases. Based on this, it is tempting to speculate that IRE1-XBP1 signalling via upregulation of XBP1(U) may protect against or decrease the effects of ER stress in PE, which could be reflected by the normal placental weigh ratio observed in this group. Correspondingly, reduced placental weight in FGR and PE+FGR, but not in isolated PE, was recently reported in a Norwegian cohort of 317,688 pregnancies [41].

Both nuclear and cytoplasmic immunoreactivity for XBP1 and ATF6 was observed in EVTs, DeCs and Mφs, but no differences in the proportion of immunoreactive cells or staining pattern was observed between the study groups. No differences in cytoplasmic staining intensity for XBP1, representing XBP1(U), or nuclear staining intensity of ATF6, representing cleaved ATF6, were detected in any of these cell types between the study groups. In general, nuclear staining intensity was difficult to assess due to photobleaching. Thus, no determination of the cellular source of increased levels of XBP1(U) or cleaved ATF6 in decidual tissue, as detected by Western blot, could be made by immunofluorescence analysis. However, we cannot exclude that other cell types in the decidua, that were not evaluated in the present work may have contributed to the increased ATF6 and XBP1(U) levels.

In summary, we found that decidua basalis is a source of ER stress, and that ER stress is increased in pregnancies complicated by FGR and PE+FGR. In PE, we found increased levels of XBP1(U), which may be a protective mechanism against the detrimental effects of ER stress. This could explain some of the observed clinical differences in between PE and FGR. However, future studies are warranted to test this hypothesis and elucidate the implications of our findings.

Acknowledgements

We would like to thank Mette Langaas for her contribution to the pathway analyses and Linda T. Roten for her valuable comments made during manuscript preparation. We would like to thank Kristine Pettersen, Anne Gøril Lundemo, Caroline H.H. Pettersen and Svanhild A. Schønberg at the Department of Cancer Research and Molecular Medicine, Faculty of Medicine, Norwegian University of Science and Technology (NTNU), for their technical support and help in optimising the Western blot protocols. We are deeply grateful to Toril Rolfseng at Department of Laboratory Medicine, Children's and Women's Health, NTNU, for performing the immunofluorescence staining. This study was supported by grants from NTNU (IAL, ML, SBM, IPE), functional genomics (FUGE) mid-Norway (RA), Haukeland University Hospital, Bergen, Norway (LB), and in part by grants from Southwest Foundation Forum (MPJ), National Institutes of Health (NIH) Grant R01 HD049847 (EKM) and Research Facilities Improvement Program Grant C06 RR017515 from the National Center for Resources, NIH. The funding sources had no involvement in study design, data collection and analyses, or in the preparation and submission of this article.

Appendix. Supplementary material

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.placenta.2011.08.005.

References

[1] Khong TY, De Wolf F, Robertson WB, Brosens I. Inadequate maternal vascular response to placentation in pregnancies complicated by pre-eclampsia and by small-for-gestational age infants. Br J Obstet Gynaecol 1986;93:1049–59.
[2] Hung TH, Skepper JN, Burton GJ. In vitro ischemia-reperfusion injury in term human placenta as a model for oxidative stress in pathological pregnancies. Am J Pathol 2001;159:1031–43.
[3] Staff AC, Ranheim T, Khoury J, Henriksen T. Increased contents of phospholipids, cholesterol, and lipid peroxides in decidua basalis in women with preeclampsia. Am J Obstet Gynecol 1999;180:587–92.
[4] Zusterzeel PL, Rutten H, Roelofs HM, Peters WH, Steegers EA. Protein carbonyls in decidua and placenta of pre-eclamptic women as markers for oxidative stress. Placenta 2001;22:213–9.
[5] Takagi Y, Nikaido T, Toki T, Kita N, Kanai M, Ashida T, et al. Levels of oxidative stress and redox-related molecules in the placenta in preeclampsia and fetal growth restriction. Virchows Arch 2004;444:49–55.
[6] Hubel CA. Oxidative stress in the pathogenesis of preeclampsia. Proc Soc Exp Biol Med 1999;222:222–35.
[7] Yung HW, Korolchuk S, Tolkovsky AM, Charnock-Jones DS, Burton GJ. Endoplasmic reticulum stress exacerbates ischemia-reperfusion-induced apoptosis through attenuation of Akt protein synthesis in human choriocarcinoma cells. FASEB J 2007;21:872–84.
[8] Kim I, Xu W, Reed JC. Cell death and endoplasmic reticulum stress: disease relevance and therapeutic opportunities. Nat Rev Drug Discov 2008;7:1013–30.
[9] Kaufman RJ, Scheuner D, Schroder M, Shen X, Lee K, Liu CY, et al. The unfolded protein response in nutrient sensing and differentiation. Nat Rev Mol Cell Biol 2002;3:411–21.
[10] Zhang K, Kaufman RJ. From endoplasmic-reticulum stress to the inflammatory response. Nature 2008;454:455–62.
[11] Yoshida H, Matsui T, Yamamoto A, Okada T, Mori K. XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor. Cell 2001;107:881–91.
[12] Haze K, Yoshida H, Yanagi H, Yura T, Mori K. Mammalian transcription factor ATF6 is synthesized as a transmembrane protein and activated by proteolysis in response to endoplasmic reticulum stress. Mol Biol Cell 1999;10:3787–99.
[13] Austin RC. The unfolded protein response in health and disease. Antioxid Redox Signal 2009;11:2279–87.
[14] Xu C, Bailly-Maitre B, Reed JC. Endoplasmic reticulum stress: cell life and death decisions. J Clin Invest 2005;115:2656–64.
[15] Yung HW, Calabrese S, Hynx D, Hemmings BA, Cetin I, Charnock-Jones DS, et al. Evidence of placental translation inhibition and endoplasmic reticulum stress in the etiology of human intrauterine growth restriction. Am J Pathol 2008;173:451–62.
[16] Burton GJ, Yung HW, Cindrova-Davies T, Charnock-Jones DS. Placental endoplasmic reticulum stress and oxidative stress in the pathophysiology of unexplained intrauterine growth restriction and early onset preeclampsia. Placenta 2009;30(A):S43–8.
[17] Løset M, Mundal SB, Johnson MP, Fenstad MH, Freed KA, Lian IA, et al. A transcriptional profile of the decidua in preeclampsia. Am J Obstet Gynecol; 2010 Oct 8 [Epub ahead of print].
[18] Redman CW, Sargent IL. Immunology of pre-eclampsia. Am J Reprod Immunol 2010;63:534–43.
[19] Report of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy. Am J Obstet Gynecol 2000;183:S1–S22.
[20] Sibai B, Dekker G, Kupferminc M. Pre-eclampsia. Lancet 2005;365:785–99.
[21] Marsal K, Persson PH, Larsen T, Lilja H, Selbing A, Sultan B. Intrauterine growth curves based on ultrasonically estimated foetal weights. Acta Paediatr 1996; 85:843–8.
[22] Kramer MS, Olivier M, McLean FH, Willis DM, Usher RH. Impact of intrauterine growth retardation and body proportionality on fetal and neonatal outcome. Pediatrics 1990;86:707–13.
[23] Harsem NK, Staff AC, He L, Roald B. The decidual suction method: a new way of collecting decidual tissue for functional and morphological studies. Acta Obstet Gynecol Scand 2004;83:724–30.
[24] Almasy L, Blangero J. Multipoint quantitative-trait linkage analysis in general pedigrees. Am J Hum Genet 1998;62:1198–211.
[25] Goring HH, Curran JE, Johnson MP, Dyer TD, Charlesworth J, Cole SA, et al. Discovery of expression QTLs using large-scale transcriptional profiling in human lymphocytes. Nat Genet 2007;39:1208–16.
[26] Brazma A, Hingamp P, Quackenbush J, Sherlock G, Spellman P, Stoeckert C, et al. Minimum information about a microarray experiment (MIAME)-toward standards for microarray data. Nat Genet 2001;29:365–71.
[27] Smyth GK. Limma: linear models for microarray data. In: Gentleman R, Carey V, Dudoit S, Irizarry R, Huber W, editors. Bioinformatics and computational biology solutions using R and bioconductor. New York: Springer; 2005. p. 397–420.
[28] Goeman JJ, Buhlmann P. Analyzing gene expression data in terms of gene sets: methodological issues. Bioinformatics 2007;23:980–7.
[29] Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A 2005;102: 15545–50.
[30] Jakobsen CH, Storvold GL, Bremseth H, Follestad T, Sand K, Mack M, et al. DHA induces ER stress and growth arrest in human colon cancer cells: associations with cholesterol and calcium homeostasis. J Lipid Res 2008;49: 2089–100.
[31] Namba T, Ishihara T, Tanaka K, Hoshino T, Mizushima T. Transcriptional activation of ATF6 by endoplasmic reticulum stressors. Biochem Biophys Res Commun 2007;355:543–8.

[32] Balague C, Mozos A, Martinez D, Hernandez L, Colomo L, Mate JL, et al. Activation of the endoplasmic reticulum stress-associated transcription factor x box-binding protein-1 occurs in a subset of normal germinal-center B cells and in aggressive B-cell lymphomas with prognostic implications. Am J Pathol 2009;174(6):2337–46.
[33] van Kollenburg B, van Dijk J, Garbern J, Thomas AA, Scheper GC, Powers JM, et al. Glia-specific activation of all pathways of the unfolded protein response in vanishing white matter disease. J Neuropathol Exp Neurol 2006;65(7):707–15.
[34] Kikuchi H, Almer G, Yamashita S, Guégan C, Nagai M, Xu Z, et al. Spinal cord endoplasmic reticulum stress associated with a microsomal accumulation of mutant superoxide dismutase-1 in an ALS model. Proc Natl Acad Sci U S A 2006;103(15):6025–30.
[35] Crispi F, Dominguez C, Llurba E, Martin-Gallan P, Cabero L, Gratacos E. Placental angiogenic growth factors and uterine artery Doppler findings for characterization of different subsets in preeclampsia and in isolated intrauterine growth restriction. Am J Obstet Gynecol 2006;195:201–7.
[36] Viero S, Chaddha V, Alkazaleh F, Simchen MJ, Malik A, Kelly E, et al. Prognostic value of placental ultrasound in pregnancies complicated by absent end-diastolic flow velocity in the umbilical arteries. Placenta 2004;25:735–41.
[37] Mayhew TM, Manwani R, Ohadike C, Wijesekara J, Baker PN. The placenta in pre-eclampsia and intrauterine growth restriction: studies on exchange surface areas, diffusion distances and villous membrane diffusive conductances. Placenta 2007;28:233–8.
[38] Yoshida H, Oku M, Suzuki M, Mori K. pXBP1(U) encoded in XBP1 pre-mRNA negatively regulates unfolded protein response activator pXBP1(S) in mammalian ER stress response. J Cell Biol 2006;172:565–75.
[39] Feng-Jin G, Lin EA, Liu P, Lin J, Liu C. XBP1U inhibits the XBP1S-mediated upregulation of the iNOS gene expression in mammalian ER stress response. Cell Signal 2010;22:1818–28.
[40] Yoshida H, Uemura A, Mori K. pXBP1(U), a negative regulator of the unfolded protein response activator pXBP1(S), targets ATF6 but not ATF4 in proteasome-mediated degradation. Cell Struct Funct 2009;34:1–10.
[41] Eskild A, Vatten LJ. Do pregnancies with pre-eclampsia have smaller placentas? A population study of 317 688 pregnancies with and without growth restriction in the offspring. BJOG 2010;117:1521–6.
[42] Thompson JM, Irgens LM, Skjaerven R, Rasmussen S. Placenta weight percentile curves for singleton deliveries. BJOG 2007;114:715–20.
[43] Hernandez-Andrade E, Brodszki J, Lingman G, Gudmundsson S, Molin J, Marsal K. Uterine artery score and perinatal outcome. Ultrasound Obstet Gynecol 2002;19:438–42.

Dissertations at the Faculty of Medicine, NTNU

1977

1. Knut Joachim Berg: EFFECT OF ACETYLSALICYLIC ACID ON RENAL FUNCTION
2. Karl Erik Viken and Arne Ødegaard: STUDIES ON HUMAN MONOCYTES CULTURED *IN VITRO*

1978

3. Karel Bjørn Cyvin: CONGENITAL DISLOCATION OF THE HIP JOINT.
4. Alf O. Brubakk: METHODS FOR STUDYING FLOW DYNAMICS IN THE LEFT VENTRICLE AND THE AORTA IN MAN.

1979

5. Geirmund Unsgaard: CYTOSTATIC AND IMMUNOREGULATORY ABILITIES OF HUMAN BLOOD MONOCYTES CULTURED IN VITRO

1980

6. Størker Jørstad: URAEMIC TOXINS
7. Arne Olav Jenssen: SOME RHEOLOGICAL, CHEMICAL AND STRUCTURAL PROPERTIES OF MUCOID SPUTUM FROM PATIENTS WITH CHRONIC OBSTRUCTIVE BRONCHITIS

1981

8. Jens Hammerstrøm: CYTOSTATIC AND CYTOLYTIC ACTIVITY OF HUMAN MONOCYTES AND EFFUSION MACROPHAGES AGAINST TUMOR CELLS *IN VITRO*

1983

9. Tore Syversen: EFFECTS OF METHYLMERCURY ON RAT BRAIN PROTEIN.
10. Torbjørn Iversen: SQUAMOUS CELL CARCINOMA OF THE VULVA.

1984

11. Tor-Erik Widerøe: ASPECTS OF CONTINUOUS AMBULATORY PERITONEAL DIALYSIS.
12. Anton Hole: ALTERATIONS OF MONOCYTE AND LYMPHOCYTE FUNCTIONS IN REALTION TO SURGERY UNDER EPIDURAL OR GENERAL ANAESTHESIA.
13. Terje Terjesen: FRACTURE HEALING AND STRESS-PROTECTION AFTER METAL PLATE FIXATION AND EXTERNAL FIXATION.
14. Carsten Saunte: CLUSTER HEADACHE SYNDROME.
15. Inggard Lereim: TRAFFIC ACCIDENTS AND THEIR CONSEQUENCES.
16. Bjørn Magne Eggen: STUDIES IN CYTOTOXICITY IN HUMAN ADHERENT MONONUCLEAR BLOOD CELLS.
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1985

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1987

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1988

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2000

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159. xxxxxxxxx (blind number)
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