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The Impact of Preeclampsia on Gene Expression at the Maternal-Fetal Interface

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Abstract

Preeclampsia (PE) impacts 8 million mother-infant pairs worldwide each year. This human pregnancy–specific disease characterized by hypertension and proteinuria accounts for significant maternal and neonatal morbidity and mortality. The current theory of the pathogenesis of PE as reviewed by Drs. Christopher Redman and Ian Sargent is thought to occur as a 2-stage process with poor placentation in the first half of pregnancy resulting in the maternal response in the second half of pregnancy. Our studies have focused on understanding the placental contribution to this serious disease by examining the gene expression profile of the *deciduas basalis* or basal plate, the region of the placenta involved in the "poor placentation". In this review we present summaries of our microarray datasets both of normal placentation and those gene expression changes resulting in the context of PE. Additionally, we have taken this opportunity to combine the data sets to provide a more comprehensive view of this region of the placenta. As defects in the basal plate are, in part, at the root of the disease process, we believe that understanding the pathobiology that occurs in this region will increase our ability to alter the development and/or course of PE.

Keywords

placenta; maternal-fetal interface; basal plate; preeclampsia

Normal Human Placental Development

Survival and growth of the fetus requires normal development of the placenta, which in humans involves the formation of a transient organ with both maternal and fetal contributions. During cytotrophoblast (CTB) differentiation, progenitors assume one of two

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fates. In floating villi, they fuse to form multinucleate syncytiotrophoblasts (STBs), whose primary functions are transport and hormone production. In anchoring villi, mononuclear CTBs acquire tumor-like properties that enable them to invade the decidua, the endometrium of pregnancy, and the adjacent third of the myometrium (interstitial invasion). They also breach the small uterine vessels they encounter, intercalating within the muscular walls and completely replacing the resident maternal endothelial lining (endovascular invasion). As a result, high-resistance spiral arterioles are transformed into low-resistance, high-capacitance vessels that divert uterine blood flow to the floating villi. This invasion process is most active during 10–20 wk of gestation. This region where maternal and fetal cells coexist is termed the basal plate or maternal-fetal interface, and its proper formation and function are required for normal pregnancy outcome (Fig. 1).

At a molecular level, many unusual processes occur in this area. For example, invasive CTBs execute a novel epithelial-to-mesenchymal transition that enables vascular mimicry, required for establishing blood flow to the placenta (1, 2). Perhaps most remarkably, the maternal immune system tolerates the invasion of the hemi-allogeneic fetal cells for the duration of pregnancy.

Over the past several decades, a great deal of information has been gained about placental development by taking a candidate molecule approach (3). For example, the fact that endovascular CTBs function as endothelial cells prompted investigators to study the role of vasculogenic/angiogenic molecules, including adhesion receptors, at the maternal-fetal interface (4, 5). As in many tumors, CTBs use matrix metalloproteinases for the purpose of invasion (6, 7). Interestingly, CTBs express several molecules involved in leukocyte function, including TOLL-like receptors, which are involved in the response to infections, and L-selectin, which mediates a novel type of rolling adhesion (8, 9).

However, there are also numerous examples of seemingly novel mechanisms that are unique to placental development. For example, trophoblasts in all locations lack major histocompatibility class (MHC) II expression, and upon allocation to the invasive pathway, CTBs upregulate HLA-G, a nonclassical MHC class I molecule, in the absence of HLA-A and B expression (10, 11). In addition, many lines of evidence suggest that CTBs have unusual responses to hypoxia, likely a reflection of the fact that the fetus resides in a physiologic state of lower oxygen tension (12). Furthermore, very little is known about gene expression during the embryonic and fetal stages of human development. Accordingly, unbiased analyses, such as microarray approaches, are also crucial for obtaining new insights into the mechanisms that are required for normal basal plate formation and function during pregnancy. Determining the gene expression profile of this critical region also provides an important foundation for investigations of the basal plate region in pathologic conditions such as preeclampsia.

Gene Expression Profiling at the Human Maternal-Fetal Interface Over Gestation

Basal plate biopsy specimens were obtained from 36 placentas (14–40 weeks) from women who had normal pregnancies. RNA was isolated, processed and hybridized to HG-U133A and HG-U133B Affymetrix GeneChips. Surprisingly, the expression of very few genes was modulated during the 14- to 24-week interval. In contrast, hundreds of genes, including those already known to be regulated over gestation, were modulated between mid-pregnancy (14–24 weeks) and term. These data allowed us to identify molecules that play potentially important roles in the formation of the maternal-fetal interface during the second trimester or in preparation of this area for parturition.

Our analysis revealed a total of 418 genes/expressed sequence tags that were differentially regulated between term and mid-gestation. A heat map of the top 25 up and down regulated genes is shown [Fig. 2; complete heat map and data (13)]. Based on GO annotations, the differentially expressed genes were involved in a variety of biological processes. At least one sixth were expressed sequence tags or hypothetical proteins and thus lacked annotations. Of the known differentially expressed genes, 17 were related to lipid metabolism, 10 were involved with formation or regulation of the extracellular matrix (ECM), 21 were immune effectors or modulators, 24 were transcription factors, and 6 had angiogenic/vasculogenic functions.

Ingenuity Pathway Analysis (IPA) software was used to further evaluate the participation of the differentially expressed genes in metabolic and signaling pathways. Analysis of genes with at least a twofold change highlighted two metabolic pathways—folate biosynthesis and N-glycan degradation involving mannose-containing structures. With regard to signaling, eleven of the differentially expressed genes mapped to the Wnt-β-catenin pathway. We also used the IPA software to map networks of the differentially expressed genes. The largest network contained genes that were involved in cell motility, cell-to-cell signaling/interaction and tissue development.

We were also interested to find that genes encoding molecules that are involved in immune defense are highly regulated. For example, defensin alpha 1 was upregulated about threefold at the RNA level at term as compared with the second trimester. Production of this antimicrobial peptide is constitutive in some cells (e.g., neutrophils) and induced in others (e.g., monocytes and CD8 T lymphocytes) in response to proinflammatory mediators (14). The presence of defensins in human term placental tissue has been previously reported (15). Increased expression at term could occur in preparation for labor and placental separation, which increase the risk of infection. In contrast, the expression of another antimicrobial molecule, granulysin, which localizes to the cytolytic granules of T cells, natural killer (NK) cells (16) and certain dendritic cells (17), is downregulated at term. We speculate that the decreased granulysin expression we observed parallels the decrease in T cell and NK cell numbers at the maternal-fetal interface at term (18). The downregulation of Ly96 expression, another NK-cell-specific molecule, provides further support for this concept. Although the mechanisms that lead to the eventual disappearance of decidual leukocytes from the maternal-fetal interface are not known, the observed concurrent decrease in expression of chemotactic molecules, such as chemokine-like factor superfamily 6 (CKLFSF6) and secreted phosphoprotein 1 (SPP1), could be a related phenomenon.

A trophoblast-derived noncoding RNA (TncRNA) was one of the most interesting of the highly upregulated differentially expressed genes in the immune function category. This transcript, which directly suppresses MHC class II expression by interacting with the MHC IITA-PIII transactivator, likely accounts for the lack of trophoblast MHC class II expression (19). As such, this molecule could play an important role in promoting maternal immunotolerance of the hemi-allogeneic fetus. Why TncRNA expression increases at term is unclear, but this phenomenon could be related to the continuing need to suppress MHC class II expression in trophoblasts, particularly as they are shed into maternal blood at the time of delivery. In this regard, it is interesting to note that expression of carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1), which plays a role in regulating decidual immune responses, is also upregulated at term (20).

Additionally, our group has also been interested in the functions of the myriad angiogenic factors that are produced at the maternal-fetal interface (3, 21). In broad terms, we know that these molecules have at least three targets—the intrinsic placental vasculature, the maternal vasculature, and the CTB subpopulation that executes an unusual epithelial-to-endothelial

transition as the cells invade the uterine wall and remodel the maternal vasculature in this region. Thus, we anticipated that molecules involved in vasculogenesis/angiogenesis would be upregulated during the active phases of placentation, i.e., in the second trimester rather than at term, which is what we found. Consistent with our previously published work, the downregulated genes included angiopoietin (ANGPT)-2 (22).

As with every microarray analysis, we made a number of interesting observations that warrant additional follow-up. For example, the cluster analysis showed a striking co-downregulation at term of ANGPT-2 and microcephalin (MCPH1). Interestingly, ANGPT-2 and MCPH1 genes are transcribed from opposite strands of the same region (chromosome 8p23.1). Their tight coexpression suggests that transcription from this area could be silenced at term, perhaps by local chromatin modifications or the recruitment of inhibitory protein complexes to the same promoter element. It will be interesting to determine if the pattern of co-expression of ANGPT-2 and MCPH1 occurs in other tissues or is specific to our data set. It is known that MCPH1 controls brain size in humans by regulating the proliferative and, hence, differentiative capacity of neuroblasts, ultimately exerting its effects through cell cycle regulators (23, 24). Furthermore, during human evolution there is evidence that strong genetic selection has been exerted on MCPH1 (25). While the most obvious consequence is brain size, another interesting possibility is that placental form and function have been affected as well.

In summary, we found that gene expression patterns in the basal plate region change dramatically between the second trimester and term. Thus, it is important to control for this variable when studying the effect of pregnancy complications that occur during this timeframe. For our purposes, understanding the normal development and formation of the maternal-fetal interface is an important first step toward understanding PE-related changes.

Human Placental Development in Preeclampsia

PE, a pregnancy complication, is manifested by the onset of hypertension and proteinuria in the second half of pregnancy. PE is relatively common (4–8% of pregnancies), with potentially deadly consequences for the mother and/or her offspring. Currently, the only definitive treatment for this condition is delivery of the placenta, and therefore the infant, accounting for 15% of all preterm births in the U.S. Despite decades of research, a full understanding of the pathogenesis of PE remains elusive. Nevertheless, it is clear that the placenta plays a central role: the signs of PE can occur in molar pregnancies, which lack a fetus, and the disease resolves once the placenta is delivered.

During the last several years, a clearer picture of the pathogenesis of PE has begun to emerge. A two-stage model has been proposed in which the initiating event, poor placentation, is thought to occur early in gestation (26). This concept is supported by several studies that document the association between reduced blood flow to the placenta before 20 wk of gestation, as determined by color Doppler ultrasound evaluation of uterine artery blood flow, and a greatly increased risk of developing PE (27, 28). Anatomic examination shows that the specific area of the placenta most affected by this syndrome is the basal plate, the site of CTB invasion. Interstitial CTB invasion is often shallow, and endovascular invasion does not proceed beyond the terminal portions of the spiral arterioles. Thus, the maternal vessels do not undergo the complete spectrum of physiological changes that normally occur (*e.g.* loss of endothelial lining and musculoelastic tissue); the mean external diameter of the myometrial vessels is less than half that of equivalent vessels from uncomplicated pregnancies (29–31). In addition, fewer vessels show evidence of CTB invasion (32). Thus, their architecture precludes an adequate response to gestation-related fetal demands for increased blood flow.

The second stage of PE is thought to be the maternal response to abnormal placentation. Systemic endothelial dysfunction appears to be an important common denominator (26, 33, 34). Recent data point to an imbalance in circulating factors with angiogenic/vasculogenic functions, such as soluble vascular endothelial growth factor receptor-1 (VEGFR-1, sFlt-1), placental growth factor, and the transforming growth factor-beta receptor endoglin (35–40).

Gene Expression at the Maternal-Fetal Interface Impacted by Preeclampsia

As in our studies of gestation-related change in gene expression at the maternal-fetal interface, we used an unbiased approach to analyze basal plate biopsies from pregnancies afflicted by PE. We focused on preeclampsia that presented in the preterm period (24–36 weeks) as this disease is thought to be more severe and also associated with the greatest morbidity. In this regard, we exploited our observation that preterm labor (PTL) without signs of inflammation is associated with normal CTB differentiation/invasion (41). Thus, basal plate specimens from these patients served as gestational age-matched controls.

The microarray analysis revealed 55 differentially expressed genes, of which the majority were not previously known to be dysregulated in PE [Fig. 3, (42)]. This list includes molecules that were previously reported to be present at higher than normal levels in maternal serum, chorionic villi and/or cord blood in pregnancies complicated by PE, a finding that gives added confidence to the novel genes that we identified as similarly regulated. However, even for these previously reported molecules, in most cases this was the first description of their increased expression in the basal plate region of the placenta.

For example, our data demonstrated increased leptin expression in the basal plate of PE placentas as compared to control tissue. Numerous investigators have reported a PE-associated increase in circulating levels of leptin (43–51), and a leptin gene polymorphism has been linked to an increased risk of developing this pregnancy complication (52). However, a clear picture of how an increase in leptin expression is linked to the pathophysiology of PE has yet to emerge. Interestingly, although the classic leptin receptors were not differentially expressed, we observed elevated levels of the mRNA that encodes Siglec-6, a transmembrane protein that also binds leptin. These findings suggest that this molecule may play an important role as a placental leptin receptor, and that increased Siglec-6 levels could contribute to the pathogenesis of PE. While the cloning strategy for Siglec-6 was based on its ability to interact with leptin, the other Siglec family members bind sialic acid-containing glycans. Siglec-6 has binding specificity for the sialyl-Tn epitope (Siaα2-6Gal-NAcα1-O-R, where R is a serine or threonine). Published data suggest that, in the placenta, leptin is a Siglec-6 ligand, but the endogenous binding partners have yet to be identified (53). Additionally, Siglec-6 expression has other interesting features. For example, in humans, it is restricted to the placenta and B-lymphocytes. In other species, including non-human primates, placental cells lack Siglec-6 expression, which B cells retain (54). The fact that Siglec-6 is expressed only in human placentas and not in non-human primate placentas (54) is intriguing, as PE is thought to be a uniquely human disease; spontaneous PE has not been reported in other animals, even non-human primates (55).

A PE-associated increase in the expression of pappalysin (PAPP-A2) was another novel observation that emerged from our work. PAPP-A2, which has 46% sequence identity with PAPP-A, is a metalloproteinase that cleaves insulin-like growth factor (IGF) binding protein-5 (IGFBP-5) (56). In a fibroblast model, an increase in IGFBP-5 proteolysis attenuates IGF-I stimulatory effects on cell migration (57). If CTBs respond in an analogous manner, then the observed PE-associated increase in PAPP-A2 levels could inhibit CTB invasion by mechanisms that include an increase in IGFBP-5 proteolysis. These two interesting novel observations, enhanced Siglec-6 and PAPP-A2 expression, were validated

at both the RNA and the protein level. *In toto*, these results suggest fundamental alterations in important biological processes including pathways that are regulated by leptin and IGF signals.

The results of this work highlight the complex pathophysiology of PE and the many pathways it impacts. Understanding the pregnancy-related functions of the differentially expressed genes in PE will likely lead to a better understanding of the pathogenesis of this human-specific condition, the crucial first step in the rational design of treatments (both preventative and therapeutic) that address the causes, rather than the consequences, of this pregnancy complication.

Combined Analysis of Gene Expression Profiles at the Human Maternal-Fetal Interface

To develop a more global view of the maternal-fetal interface we combined the gestational age and preeclampsia microarray datasets. The sample characteristics are summarized in Fig. 4. Intensity measures from the Affymetrix U133 A and B chips for each basal plate biopsy were stored as CEL files and probe level normalization performed using robust multichip averaging. The two classes, preeclampsia and “other” (second trimester, PTL and term), were compared using the linear analysis of microarrays (limma; Bioconductor) and Comparative Marker Selection (GenePattern). Genes considered significant had a Bonferonni-corrected $p<0.01$ and an absolute fold change of ≥ 1.5. This analysis resulted in the identification of 33 genes as increased and 89 genes as decreased in PE. A heat map of the top 25 up and down regulated genes is presented (Fig. 5; complete heat map Supplemental Fig. S1).

When considering this combined analysis there a few clinical features to keep in mind. First, the second trimester and term samples were from non-labored deliveries while all the PTL and the majority of the PE subjects were from labored deliveries. Interestingly, labor had relatively little impact. Also the clinical outcome for the second trimester samples was not known. Given the ~5% incidence of PE, it is likely that 1–2 samples included in the analysis were from pregnancies that would have been impacted by PE. It is tempting to consider the few samples that clearly have different expression patterns that correspond to PE-specific changes (e.g., leptin, FSTL3, coagulation factor 5 and semaphoring 6D) as being in this category.

Several interesting patterns emerge from this analysis. Some genes that are up re regulated in PE and term samples as compared to second trimester and PTL. These included corticotropin releasing hormone (CRH), fatty acid binding protein 4 (FABP4) and lipoprotein lipase (LPL). Interestingly, these are also the genes that tightly clustered in the gestational age dataset discussed above suggesting co-regulation. Conversely, matrix metallopeptidase 12 (MMP12) and several of the collagens are examples of genes that were down regulated in both PE and term samples. This analysis provides a glimpse at what may be the molecular profile of premature placental aging in PE. However, it is also clear that this is not the only process involved in PE, as some genes are distinctly dysregulated by this disease process as compared to all other samples regardless of gestational age or condition. Examples of these PE-specific genes are leptin, Flt-1 (the parent molecule of sFlt-1), PAPPA2 and inhibin A (INHA), which are up regulated in PE and laminin alpha 2 (LAMA2), secreted phosphoprotein 1 (SPP1) and ankyrin repeated and SOCS box-containing 2 (ASB2), which are down regulated in PE. Interestingly, although there is a strong correlation of elevated leptin and sFlt-1 with PE, these molecules are clearly not elevated in several of the subjects despite a clear clinical diagnosis of PE upon review of the clinical data. This is additional evidence that PE is a syndrome and may be the end result of

divergent pathogenesis pathways. In fact, we observed substantial variability in gene expression for both the PTL and PE groups as compared to the ostensibly normal samples, a reflection of the heterogeneity of the underlying pathologies. Our data suggest that molecular signatures could be used to better classify these disease entities.

A third group of genes were distinctly expressed in the second trimester samples. These include ANGPT2, chemokine (C-X-C motif) ligand 14 (CXCL14) and (hyaluronoan and proteoglycan link protein 1 (HAPLN1). These may be genes with critical activities during the active process of CTB interstitial and endovascular invasion, which forms the maternal-fetal interface.

Further analyses using IPA showed that the most differentially expressed genes mapped to the PPAR (as was seen with the gestational age dataset) and the neuronal guidance pathways. The latter result was particularly interesting given the importance of neuronal guidance molecules, EPH and Ephrins, in CTB invasion and vascular remodeling (58). These differences may be related at the molecular level to the impaired invasion observed in the basal plate regions of PE placentas.

In summary, the maternal-fetal interface is a remarkable chimeric tissue that holds the answers to many interesting biological questions regarding invasion, vasculogenesis/angiogenesis and immunotolerance. It may also hold the answer to understanding the early pathogenesis of PE. Our studies provide a global analysis of the expression patterns of genes that are involved in these and other processes. Further, the results of these studies provide reference data sets of gene expression profiles against which changes that occur in a variety of other pregnancy complications such as recurrent miscarriage, intrauterine growth restriction and placenta accreta can be compared. Now we are in a position to differentiate changes in gene expression that occur in these conditions with the hope of providing sufficient insight to develop ways to impact the clinical course of a variety of obstetrical complications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The field of obstetrics and particularly preeclampsia owes a great deal to the life work of Prof. Chris Redman. His contributions will continue to guide research toward finding diagnostic markers, predictive markers as well as preventative and curative strategies for the treatment of preeclampsia. His work will benefit the health of mothers and children around the world and inspire generations of researchers to come.

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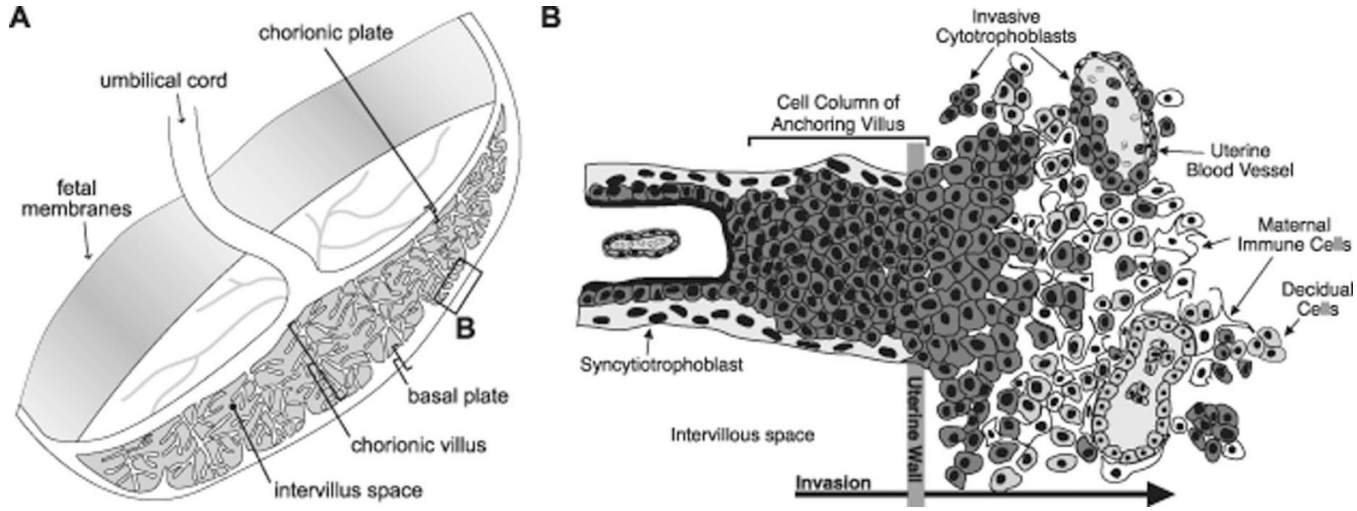


Figure 1. Diagram of the human maternal-fetal interface

(A) Representation of the human placenta after delivery. The placental surface that was adjacent to the uterine wall is termed the basal plate. The boxed area denotes the region biopsied for these studies. (**B**) View of the basal plate at the cellular level. This chimeric region of the placenta is composed of both maternal and fetal cells: extravillous (invasive) cytotrophoblasts (dark grey), decidual cells (light grey), remodeled vasculature (both invasive CTBs and maternal endothelium) and maternal immune cells (white). (Reproduced with permission from Endocrinology (13)).

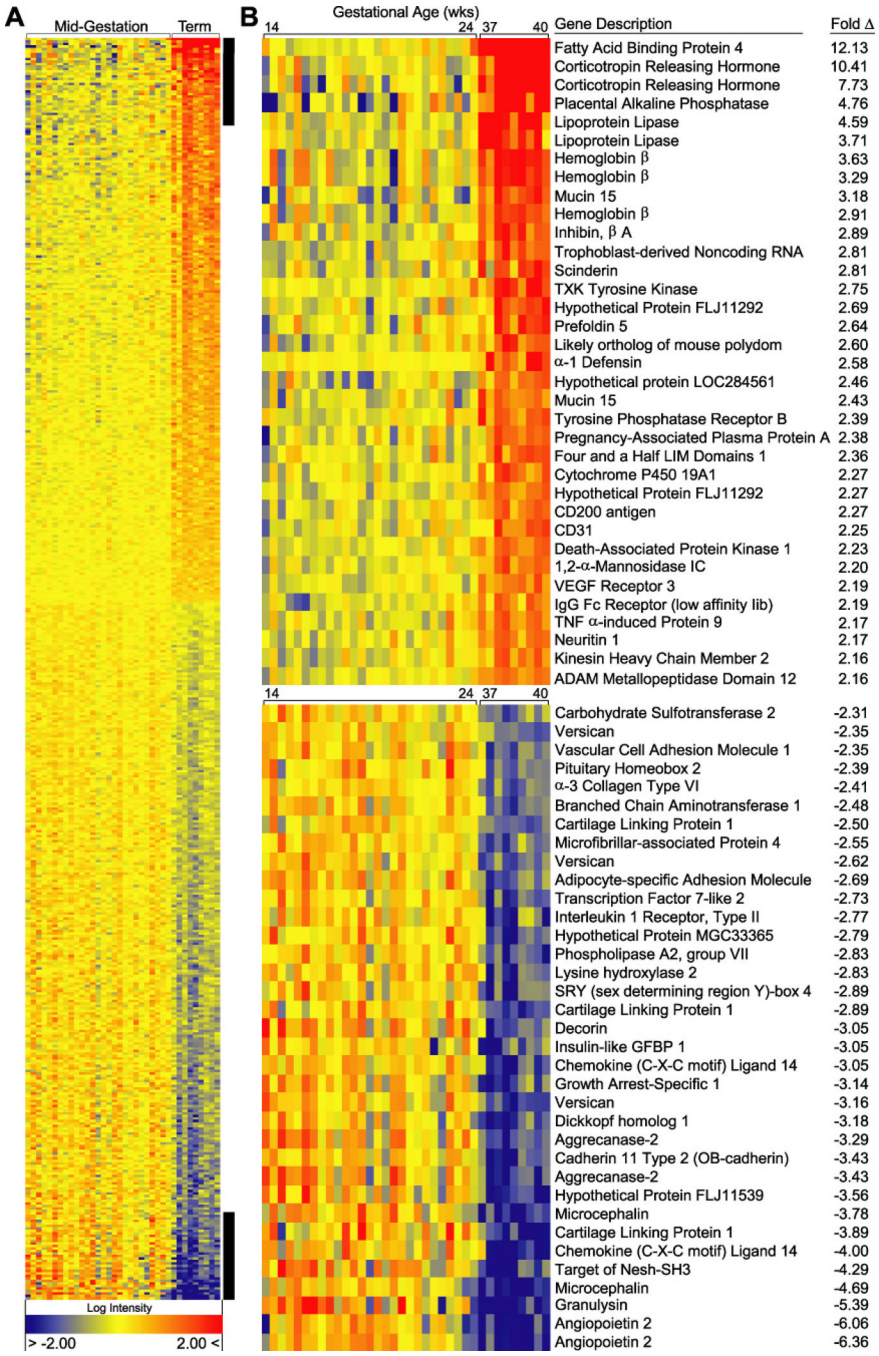
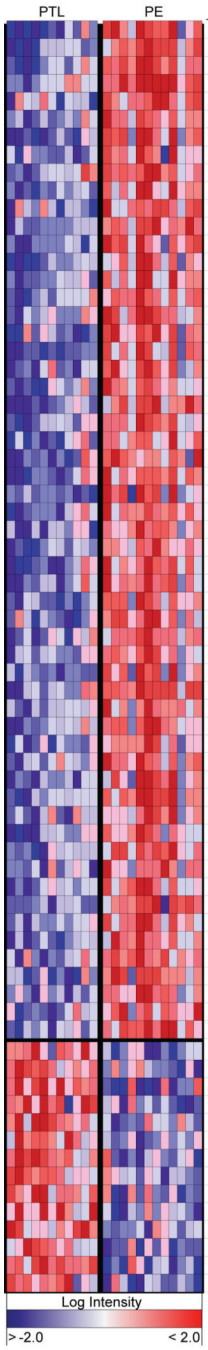


Figure 2. Heat map of the most highly up regulated (upper panel) and down regulated (lower panel) differentially expressed genes in the basal plate region at term in normal pregnancy

The normalized log intensity values were centered to the median value of each probe set and colored on a range of −2 to +2. Red denotes up regulated, yellow denotes intermediate, and blue denotes down regulated expression levels as compared with the median value. Columns contain data from a single basal plate specimen, and rows correspond to a single probe set. Samples are arranged from left to right, ordered by increasing gestational age. Rows are ranked by fold change (mean term value [*n* = 9] divided by mean midgestation value [*n* = 27]). (Reproduced with permission from Endocrinology (13))



Affymetrix ID	Symbol	Gene Description	Fold Δ
207092_at	LEP	Leptin	11.79
205629_s_at	CRH	Corticotropin releasing hormone	6.36
210511_s_at	INHBA	Inhibin, b A	3.89
205630_at	CRH	Corticotropin releasing hormone	3.43
205387_s_at	CGB	Chorionic gonadotropin b	3.32
221200_at	NA	PP3227 mRNA	3.29
222033_s_at	FLT1	Fms-related tyrosine kinase 1 (VEGF receptor)	2.87
227140_at	NA	Predicted: Inhibin bA, transcript variant 2	2.81
210287_s_at	FLT1	Fms-related tyrosine kinase 1 (VEGF receptor)	2.81
210796_x_at	SIGLEC6	Sialic acid binding Ig-like lectin 6	2.73
214471_x_at	LHB	luteinizing hormone beta polypeptide	2.62
203140_at	BCL6	B-cell CLL/lymphoma 6 (zinc finger protein 51)	2.60
210141_s_at	INHA	Inhibin a	2.60
204926_at	INHBA	Inhibin, b A	2.57
206520_x_at	SIGLEC6	Sialic acid binding Ig-like lectin 6	2.50
211918_x_at	PAPPA2	Pappalysin 2	2.46
228237_at	PAPPA2	Pappalysin 2	2.38
201809_s_at	ENG	Endoglin (Osler-Rendu-Weber syndrome 1)	1.97
219888_at	SPAG4	Sperm associated antigen 4	1.92
225467_s_at	RDH13	Retinol dehydrogenase 13 (all-trans/9-cis)	1.91
222646_s_at	ERO1L	ERO1-like (S. cerevisiae)	1.91
215990_s_at	BCL6	B-cell CLL/lymphoma 6 (zinc finger protein 51)	1.84
203087_s_at	KIF2A	Kinesin heavy chain member 2A	1.84
210732_s_at	LGALS8	Lectin, galactoside-binding, soluble, 8 (galectin 8)	1.83
219911_s_at	SLCO4A1	Solute carrier organic anion transporter family, member 4A1	1.82
221665_s_at	EPS8L1	EPS8-like 1	1.79
213236_at	SASH1	SAM and SH3 domain containing 1	1.77
218779_x_at	EPS8L1	EPS8-like 1	1.75
214396_s_at	MBD2	Methyl-CpG binding domain protein 2	1.71
228758_at	BCL6	B-cell CLL/lymphoma 6 (zinc finger protein 51)	1.69
91826_at	EPS8L1	EGF receptor kinase substrate 8-like protein	1.68
221655_x_at	EPS8L1	EGF receptor kinase substrate 8-like protein	1.66
215812_s_at	SLC6A8	Solute carrier family 6 (Creatine transporter 1)	1.65
210589_s_at	GBA	Glucosidase, b	1.64
201185_at	HTRA1	HtrA serine peptidase 1	1.61
224817_at	SH3PXD2A	SH3 and PX domains 2A	1.60
41644_at	SASH1	SAM and SH3 domain containing 1	1.58
213332_at	PAPPA2	Pappalysin 2	1.57
240055_at	NANOG	Nanog homeobox	1.56
44702_at	SYDE1	Synapse defective 1, Rho GTPase, homolog 1 (C. elegans)	1.55
209093_s_at	GBA	Glucosidase, b	1.54
204368_at	SLCO2A1	Solute carrier organic anion transporter family, member 2A1	1.53
201819_at	SCARB1	Scavenger receptor class B, member 1	1.53
214180_at	MAN1C1	Mannosidase, alpha, class 1C, member 1	1.49
219764_at	FZD10	Frizzled homolog 10 (Drosophila)	1.48
207169_x_at	DDR1	Discoidin domain receptor family, member 1	1.46
219542_at	NEK11	NIMA (never in mitosis gene a)- related kinase 11	1.46
228740_at	NA	CDNA clone IMAGE:5276765	1.42
210749_x_at	DDR1	Discoidin domain receptor family, member 1	1.39
202734_at	TRIP10	Thyroid hormone receptor interactor 10	1.39
1007_s_at	DDR1	Discoidin domain receptor family, member 1	1.37
204254_s_at	VDR	Vitamin D (1,25- dihydroxyvitamin D3) receptor	1.34
208200_at	IL1A	Interleukin 1, alpha	1.32
205325_at	PHYHIP	Phytanoyl-CoA 2-hydroxylase interacting protein	1.32
206662_at	GLRX	Glutaredoxin (thioltransferase)	1.29
205977_s_at	EPHA1	EPH receptor A1	1.26
238682_at	CCDC96	Coiled-coil domain containing 96	1.17
227231_at	KIAA1211	KIAA1211 protein	-1.27
221485_at	B4GALT5	UDP-Gal:bGlcNAc b 1,4- galactosyltransferase, polypeptide 5	-1.27
201236_s_at	BTG2	BTG family, member 2	-1.36
238497_at	TMEM136	Transmembrane protein 136	-1.40
237134_at	NA	cDNA clone IMAGE:2042048	-1.44
205952_at	KCNK3	Potassium channel, subfamily K, member 3	-1.44
231029_at	F5	Coagulation factor V (proaccelerin, labile factor)	-1.44
228950_s_at	GPR177	G protein-coupled receptor 177	-1.45
222102_at	GSTA3	Glutathione S-transferase A3	-1.48
203184_at	FBN2	Fibrillin 2 (congenital contractural arachnodactyly)	-1.54
220889_s_at	CA10	Carbonic anhydrase X	-1.57
227915_at	ASB2	Ankyrin repeat and SOCS box-containing 2	-1.66
205829_at	HSD17B1	Hydroxysteroid (17-b) dehydrogenase 1	-1.84
220092_s_at	ANTXR1/TEM8	Anthrax toxin receptor 1 / Tumor Endothelial Marker 8 (TEM8)	-1.87

Figure 3. Heat map of differentially expressed genes in basal plates of PE placentas as compared to controls

The normalized log intensity values for 71 differentially expressed probe sets were centered to the median value of each probe set and colored on a range of −2.5 to +2.5. Red denotes upregulated and blue denotes downregulated expression levels as compared with the median value. Columns contain data from a single basal plate specimen, and rows correspond to a single probe set. Samples are arranged from left to right, ordered by increasing gestational age within each category. Rows are ranked by fold change (mean PE value [n = 12] divided by mean PTL value [n = 11]). (Reproduced with permission from Endocrinology (42)).

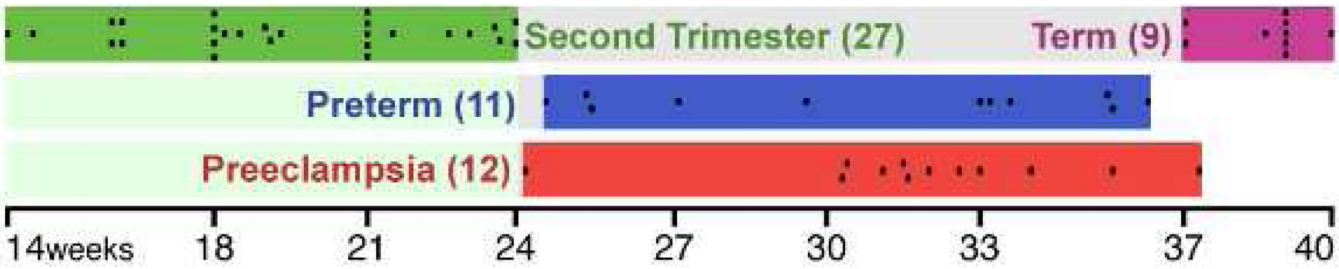
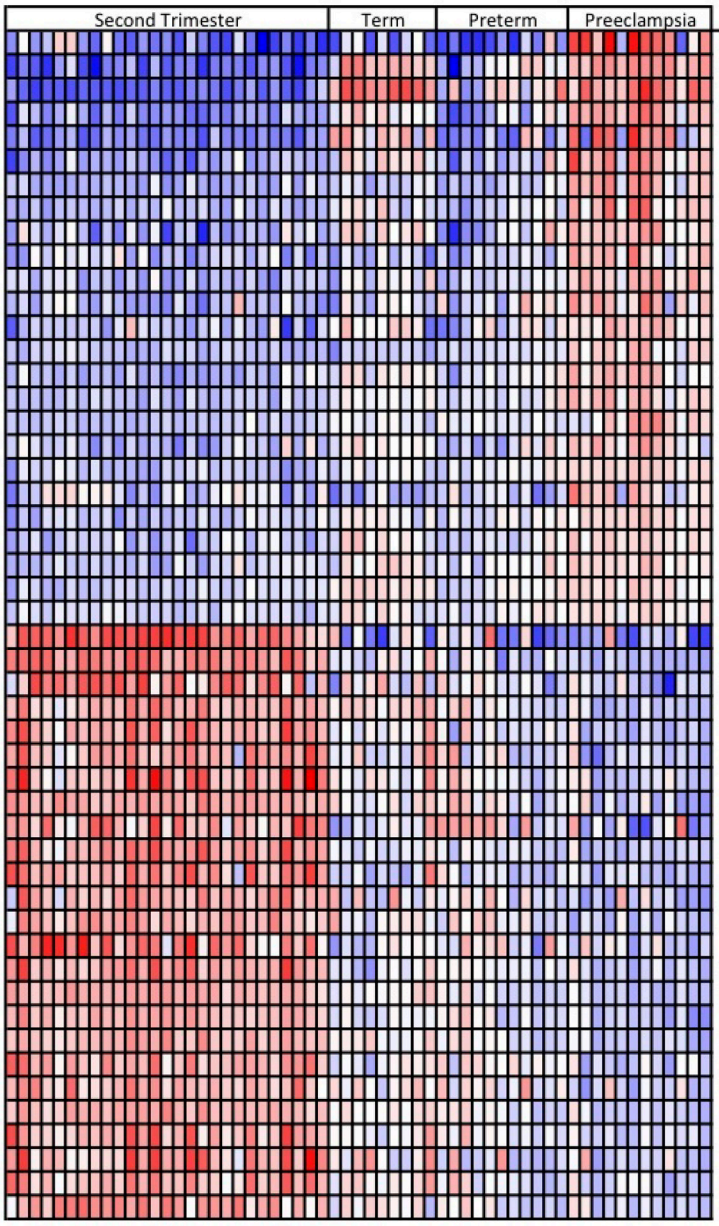


Figure 4. Gestational Timeline of Basal Plate Biopsies Used in the Composite Analysis

The microarray data from human basal plate biopsies were used in a combined analysis. Each black box represents one individual placenta and is listed by gestational age and condition.



Symbol	Title	Δ
LEP	leptin	8.2
CRH	corticotropin releasing hormone	5.1
FABP4	fatty acid binding protein 4, adipocyte	4.9
INHBA	inhibin, beta A	4.0
LPL	lipoprotein lipase	3.6
	not annotated; Affymetrix ID: 221200_at	3.6
FLT1	fms-related tyrosine kinase 1 (VEGF receptor)	3.1
SIGLEC6	sialic acid binding Ig-like lectin 6	2.9
	not annotated; Affymetrix ID: 227140_at	2.7
INHA	inhibin, alpha	2.7
BCL6	B-cell CLL/lymphoma 6	2.6
BHLHE40	basic helix-loop-helix family, member e40	2.4
PSG11	pregnancy specific beta-1-glycoprotein 11	2.3
SPAG4	sperm associated antigen 4	2.2
LIMCH1	LIM and calponin homology domains 1	2.2
PPL	periplakin	2.2
LTF	lactotransferrin	2.1
PLIN2	perilipin 2	2.1
PAPPA2	pappalysin 2	2.1
FSTL3	follistatin-like 3 (secreted glycoprotein)	2.0
HTRA1	HtrA serine peptidase 1	1.9
EPS8L1	EPS8-like 1	1.9
DUSP1	dual specificity phosphatase 1	1.8
EFHD1	EF-hand domain family, member D1	1.6
SYDE1	synapse defective 1, Rho GTPase, homolog 1 (C. elegans)	1.6
ANGPT2	angiopoietin 2	-4.0
CXCL14	chemokine (C-X-C motif) ligand 14	-3.3
COL6A2	collagen, type VI, alpha 2	-2.7
LAMA2	laminin, alpha 2	-2.6
	not annotated; Affymetrix ID: 228750_at	-2.7
SLC26A2	solute carrier family 26 (sulfate transporter), member 2	-2.6
	not annotated; Affymetrix ID: 230130_at	-2.6
SPP1	secreted phosphoprotein 1	-2.5
MMP12	matrix metallopeptidase 12 (macrophage elastase)	-2.5
HAPLN1	hyaluronan and proteoglycan link protein 1	-2.5
ATP1B1	ATPase, Na+/K+ transporting, beta 1 polypeptide	-2.5
ABCB1	ATP-binding cassette, sub-family B1	-2.4
ASB2	ankyrin repeat and SOCS box-containing 2	-2.4
	not annotated; Affymetrix ID: 229554_at	-2.4
C3orf58	chromosome 3 open reading frame 58	-2.4
COL1A2	collagen, type I, alpha 2	-2.4
COL6A3	collagen, type VI, alpha 3	-2.4
COL6A1	collagen, type VI, alpha 1	-2.3
COL21A1	collagen, type XXI, alpha 1	-2.3
AGPAT5	1-acylglycerol-3-phosphate O-acyltransferase 5	-2.3
COL3A1	collagen, type III, alpha 1	-2.3
COL14A1	collagen, type XIV, alpha 1	-2.3
	not annotated; Affymetrix ID: 227897_at	-2.3
PLAGL1	pleiomorphic adenoma gene-like 1	-2.2
COL1A1	collagen, type I, alpha 1	-2.2

Figure 5. Heat map of the most highly up regulated and down regulated differentially expressed genes in basal plates of PE placentas as compared to the second trimester, term and preterm labor samples

The normalized log intensity values for the differentially expressed probe sets were centered to the median value of each probe set and colored on a range of −2.5 to +2.5. Red denotes up regulated and blue denotes down regulated expression levels as compared with the median value. Columns contain data from a single basal plate specimen, and rows correspond to a single probe set. Samples within each catergory are arranged from left to right, ordered by increasing gestational age. Rows are ranked by fold change. The complete heat map is shown in supplemental Fig. S1.