Published in final edited form as:

Am J Obstet Gynecol. 2008 November ; 199(5): 566.e1–566.11. doi:10.1016/j.ajog.2008.04.020.

Differential placental gene expression in preeclampsia

Daniel A. Enquobahrie, MD, PhD^{a,b,c}, Margaret Meller, PhD^c, Kenneth Rice, PhD^{a,d}, Bruce M. Psaty, MD, PhD^{a,b,e}, David S. Siscovick, MD, MPH^{a,b}, and Michelle A. Williams, ScD^{b,c}

^aCardiovascular Health Research Unit, University of Washington, Seattle, Washington

^bDepartment of Epidemiology, University of Washington, Seattle, Washington

^cCenter for Perinatal Studies, Swedish Medical Center, Seattle, Washington

^dDepartment of Biostatistics, University of Washington, Seattle, Washington

^eCenter for Health Studies, Group Health Washington, Seattle, Washington

Abstract

Objective—Candidate genes associated with preeclampsia have not been fully described. We conducted microarray and confirmatory QRT-PCR studies to investigate global placental gene expression in preeclampsia.

Study design—RNA was extracted from placental samples collected from 18 preeclampsia cases and 18 normotensive controls. Oligonucleotide probes representing 22,000 genes were used to measure gene expression in each sample. Differential gene expression was evaluated using Students T-test, fold change assessment and Significance Analysis of Microarrays (SAM). Functions and functional relationships of differentially expressed genes were evaluated.

Results—Genes (n=58) participating in immune system, inflammation, oxidative stress, signaling, growth and development pathways were differentially expressed in preeclampsia. These genes include previously described candidate genes (such as LEP), potential candidate genes with related functions (such as CYP11A) and novel genes (such as CDKN1C).

Conclusion—Expression of genes (both candidate and novel) with diverse functions is associated with preeclampsia risk, reflecting the complex pathogenesis.

Keywords

preeclampsia; microarray; placenta; global gene expression

© 2008 Mosby, Inc. All rights reserved.

Contact information for reprints and correspondence: Daniel A. Enquobahrie, MD, PhD, Harvard School of Public Health, Building 2, Room 322, 655 Huntington Avenue, Boston, MA 02115, Tel: 617 432 7153 Fax: 617 432 2435, E-mail: denquoba@hsph.harvard.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Condensation

In a global microarray gene expression study, candidate and novel genes with diverse cellular functions were differentially expressed in preeclamptic placenta reflecting its complex pathogenesis.

Introduction

Preeclampsia, a pregnancy-related vascular disorder, is characterized by failure of implantation and endothelial dysfunction that involves the placenta.^{1–4} A number of pathways involved in cellular growth, immune tolerance and other metabolic functions have been shown to be relevant in preeclampsia pathogenesis.^{1–3} While considerable progress has been made in identifying genes involved in these cellular functions, candidate genes associated with preeclampsia risk have not been fully described.³ Further, consensus has not been reached on significance of the role of identified candidate genes.^{3,5}

Placenta microarray studies in preeclampsia provide an opportunity to confirm or refute evidence for involvement of previously described candidate genes/pathways while identifying potential novel candidate genes/pathways. Thus far, 13 microarray studies investigated placental gene expression in preeclampsia.^{6–18} Six studies investigated global placental gene expressions.^{6,9–10,13,15,18} Except for leptin (LEP) and fms-related tyrosine kinase 1 (FLT1), most studies reported inconsistent lists of genes differentially expressed in preeclampsia.^{6–18} Most studies were conducted among a small number of study participants and none of these studies used statistical analytic techniques that take into account multiple hypothesis testing.

In a microarray study and a confirmatory quantitative real time polymerase chain reaction (QRT-PCR) study, we investigated global placental gene expressions among 18 preeclampsia cases and 18 controls. We identified differentially expressed genes in preeclampsia and applied network and path analysis to evaluate potential pathways involved in preeclampsia pathogenesis.

Materials and Methods

Study population and data collection

Study participants were selected among participants of the Omega study and a pilot Placenta MicroArray study.^{19–21} The study population for the Omega study, a cohort study designed to examine risk factors of preeclampsia and other pregnancy complications, comprised of women who initiated prenatal care before 16 weeks gestation and attended prenatal care clinics affiliated with Swedish Medical Center, Seattle, Washington. Participants of the Placenta MicroArray study, a case-control study designed to examine differential placental gene expressions associated with pregnancy complications, comprised of women who delivered at Swedish Medical Center. Preeclampsia was diagnosed when both pregnancy-induced hypertension (PIH) and proteinuria were present according to ACOG 2000 guidelines.²² PIH was defined as a sustained (≥ 2 measures 6 hours apart) blood pressure elevation ($>140/90$ mmHg) after 20 weeks of gestation. Proteinuria was defined as a sustained (≥ 2 measures 4 hours apart) presence of elevated protein in the urine (>30 mg/dL or $>1+$ on a urine dipstick). Controls were selected among those women who had normotensive pregnancies uncomplicated by proteinuria or gestational diabetes. Women who had history of chronic hypertension and/or pre-gestational diabetes as well as current non-singleton pregnancies were excluded.

Among eligible women, 18 cases (3 from Omega and 15 from Placenta MicroArray studies) and 18 controls (4 from Omega and 14 from Placenta MicroArray studies) consented, and provided placental samples at delivery. Medical records were used to obtain information on risk factors, pregnancy history and perinatal outcome. The Institutional Review Board of the Swedish Medical Center approved study protocols. All participants provided written informed consent.

Placental sample collection

Placenta specimen were weighed, double bagged and transported in coolers to a dedicated placenta-processing lab. The chorionic plate and overlying membranes were removed. Tissue biopsies (~0.5 cm³ each) were obtained from 16 sites (8 maternal and 8 fetal) using systematic sampling technique to achieve uniformity and adequate sampling. Briefly, the placenta was laid flat with the fetal side facing up and mapped into four quadrants. Two samples were obtained from each quadrant; one medial (about 2 centimeters from the center) and one lateral (about 2 centimeters from the margin). The placenta was then turned over and eight corresponding samples were taken from the maternal side. For this analysis, biopsy samples taken from the maternal side consisting primarily of the villous tissue, utero-placental arteries and some decidua basalis, previously implicated in preeclampsia pathogenesis, were evaluated.¹⁻⁴ Biopsies were placed in cryotubes containing RNAlater (Qiagen Inc, Valencia, CA), at 10µl per 1 mg of tissue and stored at -80°C.²³

RNA extraction

A pooled sample (240 mg), for each placenta, composed of four 60mg tissue biopsies (two medial and two lateral) was obtained. Samples were homogenized using a Tissue Tearor (Biospec Products Inc., Bartlesville, OK) or Mini-Beadbeater 8 (Biospec Products Inc. Bartlesville, OK) in a lysis buffer from the RNeasy fibrous Midi Kit (Qiagen Inc, Valencia, CA) with added β-mercaptoethanol to disrupt any proteins that might be destroying nucleic acid. RNA was then extracted using a standardized protocol adapted from RNeasy Fibrous Tissue Midi Handbook (Qiagen, Inc., Valencia, CA). Total RNA concentration was calculated by determining absorbance at 260 nm (Spectramax Plus 384 spectrophotometer, Molecular Devices, Sunnyvale, CA) in 10 mM Tris-HCl. Evaluating the A260/A280 ratio monitored protein contamination. All samples had A260/A280 ratio greater than 1.8. They were aliquoted at 10µl for storage at -80°C. Samples were assessed for quality control (QC) using an Agilent 2100 Bioanalyzer capillary electrophoresis system (Agilent Technologies Inc, Palo Alto, CA) and a spectrophotometry scan. Samples were amplified using Ambion's MessageAmp I kit (Ambion Inc, Austin, TX) and subsequent aRNA was labeled with a fluorescent dye tag. All RNA samples, including reference RNAs, underwent a quality control check, and were labeled using the same standardized protocols.

Microarray experiment

Arrays were manufactured by depositing genome wide 70-mer oligonucleotide microarray probes (representing ~22,000 genes), from Operon's Human Genome Array Ready Oligo Set™ (AROS) version 2.1 (Operon Biotechnologies Inc, Huntsville, AL), onto Corning UltraGAP microarray slides (Corning, NY) using an OmniGrid 300 high-capacity microarray printer (Genomic Solutions, Ann Arbor, MI). Arrays were processed using GeneTAC hybridization station (Genomic Solutions, Ann Arbor, MI) and imaged using an Axon GenePix 4000B microarray scanner (Molecular Devices Corp., Sunnyvale, CA). Array images were quantified using GenePix Pro 6.0 image extraction software (Molecular Devices Corp., Sunnyvale, CA). Data were subsequently pre-processed through a custom-built quality-control filter (GDFilter) based on spot-level signal quality. Raw data (i.e., images) and pre-processed results were stored in an Iobion GeneTraffic relational database (Stratagene Corp., La Jolla, CA). Intra-array normalization was performed using a lowess algorithm to correct for intensity-dependent ratio biasing.²⁴ Microarray experiments were conducted at the Fred Hutchinson Cancer Research Center in Seattle, Washington.

Quantitative Real Time PCR (QRT-PCR) experiment

We conducted a confirmatory QRT-PCR experiment for selected differentially expressed genes in our microarray study. First strand cDNA was synthesized using the High Capacity

cDNA Archive Kit (Applied Biosystems, Foster City, CA). QRT-PCR was performed in duplicates using assays developed by Clontech (Clontech, Mountainview, CA). Further information on assays used in this study is available on request. Reactions were run on an ABI PRISM 7000 Real Time PCR machine (Applied Biosystems, Foster City, CA) using the default cycling conditions. Threshold cycle (Ct) values of the duplicates differing by greater than 0.5 times the standard deviation were re-tested. Ct value duplicates differing by less than 0.5 times the standard deviations were averaged for analysis. Raw measurements were normalized using the geometric mean of SDHA, TBP and YWHAZ genes as previously described by our group.²¹ The QRT-PCR experiment was conducted at the Center for Perinatal Studies, Swedish Medical Center, Seattle, WA.

Statistical Analysis

Analysis was conducted on natural log-transformed data. Genes with available information on less than 20% of samples in either test groups were dropped from further analysis. Differential gene expression among cases and controls was evaluated using Student's t-test (two sample, unequal variances). In addition, genes that met the following criteria in case-control comparisons constituted the final set of differentially expressed genes in preeclamptic placenta. These criteria were absolute fold change differences ≥ 1.5 and false discovery rates (FDR) $\leq 10\%$ in Significance Analysis of Microarrays (SAM)²⁵ analysis. A phylogenetic tree (heat map) of differentially expressed genes was constructed using Cluster and TreeView softwares that employ a hierarchical clustering approach based on Pearson's correlation coefficient.²⁶

In path analysis, relationships between differentially expressed genes were investigated using two independent tools; DAVID (Database for Annotation, Visualization and Integrated Discovery)²⁷ and Ingenuity Pathway Analysis (IPA) software (Ingenuity, Redwood City, CA). Gene-enrichment of annotation clusters (enrichment score) or networks (network score) was measured in DAVID or IPA respectively, using a modified Fischer's exact test to rank the biological significance of gene function clusters or networks in preeclampsia.

Finally, we investigated the correlation between microarray expression results and QRT-PCR expression results for selected genes from our differentially expressed genes list using Spearman's correlation coefficient among cases and controls separately. We also evaluated differences in placental gene expressions among cases and controls for the selected genes based on QRT-PCR measurements. We used Student's T-test and fold-change analysis to compare whether results were generally consistent with those obtained from microarray experiments.

Results

Maternal age and race/ethnicity distribution of preeclampsia cases and controls was comparable (Table 1). Cases delivered at earlier gestational age, had higher cesarean delivery rates and pre-pregnancy BMI compared with normotensive controls. Expression was measured for 21,250 genes represented in the platform (96.6%). Placental expressions of 15,725 genes, for which information was available for at least 80% of cases and 80% of controls, were further analyzed.

A volcano plot of Students' T-test p-values comparing gene expressions among cases and controls (Y-axis) against fold change differences (X-axis) reveals signal in the data, as there were many more genes with extreme p-values than would have been expected by chance (Figure 1). For example, there were more than 20 genes with T-test p-values below 1/15725; if no differential expression was present, only one computed p-value would be expected to exceed this threshold.

A total of 1,164 genes had expressions that were different between preeclampsia cases and controls in Student's T-test comparison (p -value < 0.05) (Figure 2). Of these, expressions of 58 genes, (56 up regulated and 2 down-regulated) had absolute fold change differences greater than 1.5 and $FDR \leq 10\%$ (in SAM analysis) when comparing preeclampsia cases and controls. These 58 genes constituted our list of differentially expressed genes (Table 2). This list of differentially expressed genes in preeclampsia includes genes with significant *a priori* interest for involvement in preeclampsia pathogenesis (e.g. LEP, FLT1, INHA and F2R). Other potential candidate genes with limited prior evidence for direct involvement in preeclampsia but which are known to regulate functional pathways of potential importance in preeclampsia pathogenesis were also identified (e.g. CYP11A, FCGR2B, HMOX1, PSG6, CDKN1C and TPBG).

The heat map shows correlation analysis of samples and selected differentially expressed genes (Figure 3) resulted in hierarchical clustering of most cases (14 of 18) and controls (14 of 18). In path analysis using DAVID, genes differentially expressed in our study belonged to cluster of genes involved in reproductive physiology, immune responses, cytokines and to a lesser extent to genes involved in negative cell function regulation and cell cycle (Table 3). Assessment using IPA showed that networks involving cellular development, particularly of the hematological, lymphatic, connective tissue and immune systems as well as inflammatory disease were particularly enriched by genes in our set of differentially expressed genes (Table 4). Two networks that were significantly enriched (scores of 30 and 28) are shown in Figure 4. In addition to genes already identified in our study as differentially expressed, some genes such as TGFBI and TNFRSF1B from network 1 and Akt and P38MAPK from network 2 play central roles in these networks.

Our confirmatory QRT-PCR study on selected genes (LEP, FLT1, CYP11A, F2R, FCGR2B, CDO1, PROCR, TPBG and NR4A2), differentially expressed in preeclamptic placenta, showed significant correlation between microarray and PCR expression measurements for most genes among cases and controls (Table 5); and, a similar pattern of expression difference between cases and controls (Figure 5). However, fold-change differences and Student's T-test p -values comparing PCR gene expression measurements in cases and controls were not as pronounced as those observed in the microarray study.

Comment

In this microarray study of expression of over 15,000 genes in placenta, 58 genes were differentially expressed in preeclampsia cases compared with controls. Genes with previously described roles in preeclampsia as well as potential candidates that participate in putative pathways were identified. Identified genes participate in diverse cellular functions reflecting involvement of several pathways in preeclampsia pathogenesis.

Previous studies have documented the diverse differential placental gene expression pattern in preeclampsia.^{6–18} For instance, Reimer et al identified 59 genes that function as transcription factors/signaling molecules, immunological factors, neuromediators, oncogenic factors, protease inhibitors, hormones and growth factor-binding proteins.¹⁸ Of note, in their study, LEP was up-regulated 43.6-fold among preeclampsia cases compared with controls. The profound contribution of leptin to preeclampsia pathology has been well documented in previous studies.^{18,28–30} Pang et al have reported differentially expressed genes in preeclampsia with functions that span cell cycle apoptosis, immune-activation and cytokine receptor/kinase regulation.¹⁴ Supporting evidence from another microarray study also implicate altered expression of genes involved in oxygen metabolite imbalance, abnormal trophoblast invasion, disorders of lipoprotein metabolism and signal transduction in the pathogenesis of preeclampsia.⁹

Common regulatory mechanisms may be responsible for differential expression of genes with related functions. For instance, increased expression of angiogenesis related genes can result from a physiologic adaptive response to reduced oxygenation following abnormal placentation and aberrant remodeling of maternal spiral arteries in preeclampsia.^{6–7,11–12,31} Evidence for function of the HIF-1 α protein that corresponded with production of angiogenesis-related proteins (VEGF receptors FLT1 and FLK1), tyrosine kinase as well as expression of oxygen-regulated genes has been reported.^{12,32–33} These genes contain similar functional hypoxia response elements (HRE) in their promoter sequence.³²

Pathway interactions, through gene expression regulation, is one area that has been explored well in preeclampsia.¹⁴ Regulatory pathways that were extensively studied include the NF κ B and the NRF2 (transcription factor for ARE, antioxidant response element genes) signaling pathways.³⁴ Others include p38 MAPK, NH2-terminal JNK/SAPK, AGE, RAGE and PKC.³⁴ We provided supporting evidence for some of these defined relationships in our networks.

Global gene expression studies may lead to candidate gene discoveries or direct identification of chromosomal regions that are potentially associated with disease. Initial evidence for potential involvement of FLT1 in preeclampsia pathogenesis in particular and cardiovascular diseases in general was brought about by findings from microarray studies conducted in preeclamptic placenta.^{6–7,11–12} Comparison of previously identified chromosomal regions (in chromosomes 2,4,9,7 and 11)³ associated with preeclampsia and locations of our differentially expressed genes suggest similarities between a cluster at 11p15.5 previously identified in preeclampsia and CDKN1C, a gene that has been investigated in preeclampsia mice model (BPH/5).^{3,35} These findings implicate CDKN1C as a potential candidate preeclampsia gene that warrants further investigation.

Potential candidate genes previously reported by other microarray studies were considerably different while general agreement exists between previous reports of functions of cluster of genes differentially expressed in preeclamptic placenta. Discordance between specific genes that were up or down regulated in our study compared with other studies may be related to differences in microarray platform used, gene representation (presence/absence of probes), experimental variability and data analysis approaches.³⁶ However, consensus on comparable biological themes may be achieved when data across disparate platforms and laboratories are analyzed using GO (gene ontology) nodes (analysis of collection of genes).^{34,36} Further, use of statistical analytic methods, such as applying filters or multiple testing adjustments, that reduce the number of false positives will likely help minimize inconsistencies.

Several caveats should be considered when interpreting results from our study. We did not match cases and controls on gestational age or mode of delivery. We preferred not to match on these variables to avoid over-representation of complicated pregnancies leading to preterm and/or surgical deliveries among our controls. We reasoned that gene expression profiles of non-preeclamptic pregnancies that delivered preterm or by C-section may be altered in ways that are similar to our case group and result in non-representation of uncomplicated term pregnancies. We did, however, complete sensitivity analyses to assess the extent to which placental gene expressions differ by gestational age or mode of delivery by performing analyses within strata of term/preterm delivery and mode of delivery (vaginal/C-section). Results from these analyses indicted no material differences in gene expression according to gestational age and mode of delivery. We also repeated analyses after excluding three preeclampsia cases with a concomitant gestational diabetes diagnosis. Inferences from this post hoc analysis were not different from those drawn from our analysis of all 18 preeclampsia cases.

Concordance between expression measurements of our microarray experiment and the confirmatory QRT-PCR experiment results were generally similar and comparable to previous reports.³⁷ Absence of complete concordance between microarray and QRT-PCR expression measurements can result from differences in experiment setup including differences in probe sequences and target location. Further, production of mRNA transcripts of multiple isoforms by splicing or other post-transcriptional processing can result in result discrepancies. Some limitations of our study include the fact that observed gene expression differences may be a result of the disease process (or treatment effect) instead of disease onset and/or pathogenesis. Also, as a dynamic event, a single measure of RNA expression that is dependent on half-life, rate of initiation to degradation, stability and other factors may not adequately describe the full picture of placental gene expression in preeclampsia.

Our study has a number of strengths. It is the largest placenta microarray study in preeclampsia to date, to our knowledge. We conducted the study among well-characterized study population. We used multiple criteria to identify our set of differentially expressed genes including a SAM based filter that takes into account multiple testing and minimizes false discovery. We also applied multiple tools to conduct path analysis and identify potential candidate genes and networks that may be significant in preeclampsia pathogenesis.

In summary, in this microarray study, a number of genes participating in immune, inflammatory, oxidative stress, growth and development were differentially expressed among preeclamptic placenta compared with normotensive placenta. Further, potential signal pathways were identified. Future studies to replicate our findings and confirm involvement of specific genes that have been identified are warranted. Understanding molecular events associated with preeclampsia may provide the basis for designing new prevention and treatment strategies to improve reproductive outcomes.

Acknowledgments

This research was supported in part by an award from the National Institutes of Health (HD/HL R01-32562). The authors are indebted to the participants of the Omega study and the Placenta MicroArray study for their cooperation. They are also grateful for the technical expertise of The Center for Perinatal Studies, Swedish Medical Center staff; and the staff of the Genomics Resource at the Fred Hutchinson Cancer Research Center.

References

1. Baumwell S, Karumanchi SA. Pre-Eclampsia: Clinical Manifestations and Molecular Mechanisms. *Nephron Clin Pract* 2007;106:c72–c81. [PubMed: 17570933]
2. Mutter WP, Karumanchi SA. Molecular mechanisms of preeclampsia. *Microvasc Res.* 2007 May 6; [Epub ahead of print]
3. Chappell S, Morgan L. Searching for genetic clues to the causes of pre-eclampsia. *Clinical Science* 2006;110:443–458.
4. Redman CW. Current topic: pre-eclampsia and the placenta. *Placenta* 1991;12:301–308. [PubMed: 1946241]
5. Goddard KAB, Tromp G, Romero R, et al. Candidate-gene association study of mothers with pre-eclampsia, and their infants, analyzing 775 SNPs in 190 genes. *Hum Hered* 2007;63:1–16. [PubMed: 17179726]
6. 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;28:487–497. [PubMed: 16860862]
7. Herse, f; Dechend, R.; Harsem, NK., et al. Dysregulation of the circulating and tissue-based rennin-angiotensin system in preeclampsia. *Hypertension* 2007;49:604–611. [PubMed: 17261642]
8. Han JY, Kim YS, Cho GJ, et al. Altered gene expression of caspase-10, death receptor-3 and IGFBP-3 in preeclamptic placentas. *Mol Cells* 2006;22:168–174. [PubMed: 17085968]

9. Zhou R, Zhu Q, Wang Y, Ren Y, et al. Genomewide oligonucleotide microarray analysis on placentae of preeclamptic pregnancies. *Gynecologic and Obstetric Investigation* 2006;62:108–114. [PubMed: 16651850]
10. Hansson SR, Chen Y, Brodzski J, et al. Gene expression profiling of human placentas from preeclamptic and normotensive pregnancies. *Mol Hum Reprod* 2006;12:169–179. [PubMed: 16556680]
11. Gack S, Marme A, Marme F, Wrobel G, et al. Preeclampsia: increased expression of soluble ADAM 12. *J Mol Med* 2005;83:887–896. [PubMed: 16247621]
12. Soleymanlou N, Jurisica I, Nevo O, et al. Molecular evidence of placental hypoxia in preeclampsia. *J Clin Endocrinol Metab* 2005;90:4299–4308. [PubMed: 15840747]
13. Heikkila A, Tuomisto T, Hakkinen S, Keski-Nisula L, Heinonen S, Yla-herttua S. Tumor suppressor and growth regulatory genes are overexpressed in severe early-onset preeclampsia- an array study on case-specific human preeclamptic placental tissue. *Acta Obstet Gynecol Scand* 2005;84:679–689. [PubMed: 15954879]
14. Pang Z, Xing F. Comparative profiling of metabolism-related gene expression in pre-eclamptic and normal pregnancies. *Arch Gynecol Obstet* 2004;269:91–95. [PubMed: 14648175]
15. Tsoi SC, Cale JM, Bird IM, Kay HH. cDNA microarray analysis of gene expression profiles in human placenta: up-regulation of the transcript encoding muscle subunit of glycogen phosphorylase in preeclampsia. *J Soc Gynecol Investig* 2003;10:496–502.
16. Pang ZJ, Xing FQ. Comparative study on the expression of cytokine--receptor genes in normal and preeclamptic human placentas using DNA microarrays. *J Perinat Med* 2003;31:153–162. [PubMed: 12747232]
17. Pang ZJ, Xing FQ. Expression profile of trophoblast invasion-associated genes in the pre-eclamptic placenta. *Br J Biomed Sci* 2003;60:97–101. [PubMed: 12866918]
18. Reimer T, Koczan D, Gerber B, Richter D, Thiesen HJ, Friese K. Microarray analysis of differentially expressed genes in placental tissue of pre-eclampsia: up-regulation of obesity-related genes. *Molecular Human Reproduction* 2002;8:674–680. [PubMed: 12087083]
19. Meller M, Qiu C, Kuske BT, Abetew DF, Muy-Rivera M, Williams MA. Adipocytokine expression in placentas from pre-eclamptic and chronic hypertensive patients. *Gynecol Endocrinol* 2006;22:267–273. [PubMed: 16785148]
20. Meller M, Qiu C, Vadachkoria S, Abetew DF, Luthy DA, Williams MA. Changes in placental adipocytokine gene expression associated with gestational diabetes mellitus. *Physiol Res* 2006;55:501–512. [PubMed: 16343040]
21. Meller M, Vadachkoria S, Luthy DA, Williams MA. Evaluation of housekeeping genes in placental comparative expression studies. *Placenta* 2005;26:601–607. [PubMed: 16085039]
22. Report of the National High Blood Pressure Education Program Working Group report on high blood pressure in pregnancy. *Am J Obstet Gynecol* 2000;183:S1–S22.
23. Mutter GL, Zahrieh D, Liu C, et al. Comparison of frozen and RNALater solid tissue storage methods for use in RNA expression microarrays. *BMC Genomics*. 2004 Nov 10;published online
24. Yang YH, Dudoit S, Luu P, et al. Normalization for cDNA microarray data: a robust composite method addressing single and multiple slide systematic variation. *Nucleic Acids Res* 2002;20:e15. [PubMed: 11842121]
25. Tusher VG, Tibshirani R, Chu G. Significance analysis of microarrays applied to the ionizing radiation response. 1998;9:5116–5121.
26. Eisen MB, Spellman PT, Brown PO, Botstein D. Cluster analysis and display of genome-wide expression patterns. *Proc Natl Acad Sci USA* 1998;95:14863–14868. [PubMed: 9843981]
27. Dennis, Glynn, Jr; Sherman, Brad T.; Hosack, Douglas A., et al. DAVID: Database for Annotation, Visualization, and Integrated Discovery. *Genome Biology* 2003;4:P3. [PubMed: 12734009]
28. Henson MC, Castracane VD. Leptin in pregnancy: an update. *Biol Reprod* 2006;74:218–229. [PubMed: 16267210]
29. Ning Y, Williams MA, Muy-Rivera M, Leisenring WM, Luthy DA. Relationship of maternal plasma leptin and risk of pre-eclampsia: a prospective study. *J Matern Fetal Neonatal Med* 2004;15:186–192. [PubMed: 15280145]

30. Muy-Rivera M, Ning Y, Frederick IO, Vadachkoria S, Luthy DA, Williams MA. Leptin, soluble leptin receptor and leptin gene polymorphism in relation to preeclampsia risk. *Physiol Res* 2005;54:167–174. [PubMed: 15544427]
31. Muy-Rivera M, Vadachkoria S, Woelk GB, Qiu C, Mahomed K, Williams MA. Maternal plasma VEGF, sVEGF-R1, and PlGF concentrations in preeclamptic and normotensive pregnant Zimbabwean women. *Physiol Res* 2005;54:611–622. [PubMed: 15717861]
32. Rajakumar A, Brandon HM, Daftary A, Ness R, Conrad KP. Evidence for the functional activity of hypoxia-inducible transcription factors overexpressed in preeclamptic placentae. *Placenta* 2004;25:763–769. [PubMed: 15451190]
33. Kunsch C, Medford RM. Oxidative stress as a regulator of gene expression in the vasculature. *Circ Res* 1999;85:753–766. [PubMed: 10521248]
34. Hubel CA. Oxidative stress in the pathogenesis of preeclampsia. *Proc Soc Exp Biol Med* 1999;222:22–35.
35. Knox KS, Baker JC. Genome-wide expression profiling of placentas in the p57Kip2 model of pre-eclampsia. *Mol Hum Reprod* 2007;13:251–263. [PubMed: 17289831]
36. Toxicogenomics Research Consortium. Standardizing global gene expression analysis between laboratories and across platforms. *Nature Methods* 2005;2:351–356. [PubMed: 15846362]
37. Romero T, Tromp G. High-dimensional biology in obstetrics and gynecology: functional genomics in microarray studies. *Am J Obstet Gynecol* 2006;195:360–363. [PubMed: 16890547]

Figure 1. Volcano plot of placental gene expression

Distribution of Students' t-test p-value (y-axis: $-\ln[p\text{-value}]$) and fold change (x-axis: $\ln[\text{fold change}]$) results comparing placental gene expressions of preeclampsia cases and controls. All genes are shown in blue while those genes that are differentially expressed in evaluations using at least two of the following: Student's T-test p-value > 0.05 , absolute fold change ≥ 1.5 and/or false discovery rate in SAM analysis $< 10\%$, are shown in purple.

Figure 2. Venn diagram summary of distribution of differentially expressed genes

Circles represent numbers of differentially expressed genes comparing preclampsic placenta with normotensive placenta using Students' t-test p-value < 0.05 (light blue), fold change > 1.5 (light purple) and SAM false discovery rate < 10% (light green). Numbers within circles represent total number of genes and numbers of either up-regulated (↑) or down-regulated (↓) genes. The intersections of the circles represent the number of genes differentially expressed using two or greater than two criteria as defined above.

Figure 3. Heat map illustration of phylogenetic tree of samples and selected differentially selected genes

The genes (rows) and participants (columns) were grouped according to level and nature of gene expression and subjected to hierarchical tree clustering. The color code for signal strength in the classification scheme is as follows; induced genes are indicated by shades of red while repressed genes are indicated by shades of green. Gray represents absent data.

Figure 4. Pathway networks identified using Ingenuity Pathway Analysis

The networks were generated through the use of Ingenuity Pathways Analysis (Ingenuity® Systems, www.ingenuity.com). Each gene identifier was mapped to its corresponding gene object in the Ingenuity Pathways Knowledge Base (IPKB) and overlaid onto a global molecular network developed from information contained in the IPKB. Colored genes are genes in our set of differentially expressed genes (pink=upregulated and green=down regulated).

Figure 5. Comparison of microarray and QRT-PCR expression measurements

Box plots describing distributions of microarray and QRT-PCR expression measurements of selected genes among cases and controls.

Table 1

Characteristics of Study Population

Characteristics	Preeclampsia Cases (n=18)	Normotensive Controls (n=18)
Age, years	32.6	30.0
Race, %		
White	61.1	66.7
Black	5.6	11.1
Hispanic	16.7	16.7
Asian	16.7	5.6
Gestational age at delivery, weeks	35.8	38.9
Mode of delivery, %		
Cesarean	55.6	38.9
Vaginal	44.4	61.1
Labor, %	66.7	72.2
Nulliparous, %	72.2	55.6
Pre-pregnancy BMI, kg/m ²	27.0	25.3
Infant birth weight, kg	2.6	3.3
Low birth weight (<2.5kg)	44.4	5.6
Preterm Delivery (<37 weeks), %	50.0	5.6

Abbreviations: BMI: body mass index; kg: kilogram.

Table 2

List of selected differentially* expressed genes in preeclamptic placenta

Gene symbol	Gene name	Gene Ontology (Molecular Function)	Location	Fold Change*	P-value*	FDR*
LEP	Leptin	Growth factor activity, hormone activity, protein binding	7q31.3	5.52	0.0020	0
FLT1	Fms-related tyrosine kinase 1	ATP binding, identical protein binding, kinase activity, nucleotide binding, protein kinase activity, protein-tyrosine kinase activity, receptor activity, transferase activity, transmembrane receptor protein tyrosine kinase activity, vascular endothelial growth factor receptor activity	13q12	2.76	0.0005	0
PCDHA3	Protocadherin alpha 3	Calcium ion binding	5q31	2.68	0.0007	0
CYP11A	Cytochrome P450, subfamily X1A	Cholesterol monooxygenase activity, heme binding, iron ion binding, metal ion binding, monooxygenase activity, oxidoreductase activity	15q23-24	2.26	0.0077	2.21
F2R	Coagulation factor II (thrombin) receptor	G-protein coupled receptor activity, purinergic nucleotide receptor activity, G-protein coupled, receptor activity, rhodopsin-like receptor activity, signal transducer activity, thrombin receptor activity	5q13	2.16	0.0008	2.21
FCGR2B	Fc fragment of IgG, low affinity IIb, receptor for (CD32)	IgG binding, protein binding, receptor binding, receptor signaling protein activity	1q23	2.16	0.0051	2.21
IL9	Interleukin 9	Cytokine activity, growth factor activity, hematopoietin/interferon-class, cytokine receptor binding, interleukin-9 receptor binding	5q31.1	2.15	0.0069	2.21
CDO1	Cysteine dioxygenase, type I	Cysteine dioxygenase activity, electron carrier activity, iron ion binding, metal ion binding, oxidoreductase activity	5q22-23	2.14	0.0019	0
VGLL1	Vestigial like 1	Transcription regulator activity	Xq26.3	2.10	0.0163	6.79
EBI3	Epstein-Barr virus induced gene 3	Cytokine activity	19p13.3	2.07	0.0127	5.90
INSL4	Insulin-like 4 (placenta)	Hormone activity, insulin-like growth factor receptor binding	9p24	2.05	0.0087	4.12
PROCR	Protein C receptor, endothelial (EPCR)	Protein-C-terminus binding, receptor activity	20q11.2	1.99	0.0043	2.21
FCGR1A	Fc fragment of IgG, high affinity Ia, receptor (CD64)	IgG binding, IgG receptor activity, receptor activity	1q21.2-21.3	1.91	0.0009	0
PMM2	Phosphomannomutase 2	Catalytic activity, isomerase activity, phosphomannomutase activity	16p13.3-13.2	1.90	0.0152	4.12

Gene symbol	Gene name	Gene Ontology (Molecular Function)	Location	Fold Change*	P-value*	FDR*
INHA	Inhibin, alpha	Growth factor activity, hormone activity	2q33-36	1.88	0.0002	0
FSTL3	Follistatin-like 3 (secreted glycoprotein)	Activin binding, activin inhibitor activity	19p13	1.86	0.0220	5.90
CPVL	Carboxypeptidase, vitellogenic-like	Carboxypeptidase activity	7p15-14	1.84	0.0029	2.21
CLIC3	Chloride intracellular channel 3	Chloride channel activity, chloride ion binding, ion channel activity, voltage-gated chloride channel activity, voltage-gated ion channel activity	9q34.3	1.84	0.0019	0
BCORL1	Hypothetical protein FLJ11362	DNA binding, transcription factor activity	Xq25-26.1	1.82	0.0069	2.21
BCL6	B-cell CLL/lymphoma 6 (zinc finger protein 51)	Chromatin binding, DNA binding, metal ion binding, nucleic acid binding, protein binding, sequence-specific DNA binding, transcriptional repressor activity, zinc ion binding	3q27	1.78	0.0154	9.13
CSF2RA	Colony stimulating factor 2 receptor, alpha, low-affinity	Hematopoietin/interferon-class cytokine receptor activity, receptor activity	Xp22.32 and Yp11.3	1.78	0.0015	0
HMOX1	Heme oxygenase (decycling) 1	Heme oxygenase activity, iron ion binding, metal ion binding, oxidoreductase activity	22q13.1	1.75	0.0002	0
TPBG	Trophoblast glycoprotein	Protein binding	6q14-15	1.74	0.0070	4.12
PSG6	Pregnancy specific beta-1-glycoprotein 6	Pregnancy	19q13.2	1.72	0.0086	5.66
GJA5	Gap junction protein, alpha 5, 40kDa	Connexon channel activity, gap-junction forming channel activity	1q21.1	1.67	0.0047	4.12
RRP9	RRP9, small subunit processome component	RNA binding	3p21.1	1.64	0.0114	6.79
SEMA4B	Semaphorin 4B	Receptor activity	15q25	1.63	0.0000	0
HEXB	Hexosaminidase B	Beta-N-acetylhexosaminidase activity, catalytic activity, cation binding, hydrolase activity, protein heterodimerization activity	5q13	1.60	0.0120	5.03
CDKN1C	Cyclin-dependent kinase inhibitor 1C	Cyclin-dependent protein kinase inhibitor, kinase activity, protein binding, protein-kinase inhibitor activity	11p15.5	1.59	0.0051	4.12
MXI1	MAX interactor 1	DNA binding, protein binding, transcription regulator activity, transcriptional repressor activity	10q24-25	1.56	0.0026	0
NXPH1	Neurexophilin 1	Receptor binding	7p22	1.52	0.0012	0
TMEM8	Transmembrane protein 8	Protein binding	16p13.3	1.51	0.0090	5.66

Gene symbol	Gene name	Gene Ontology (Molecular Function)	Location	Fold Change [*]	P-value [*]	FDR [*]
IFIT3	Interferon-induced protein with tetrapeptide repeats 3	Binding	10q4	1.51	0.0003	0
MGC11324	Lung cancer metastasis-associated protein	Acyltransferase activity	4q21.23	-1.92	0.0004	5.90
NR4A2	Nuclear receptor subfamily 4, group A, member 2	Ligand-dependent nuclear receptor activity, metal ion binding, protein binding, receptor activity, sequence-specific DNA binding, steroid hormone receptor activity, transcription factor activity, zinc ion binding	2q22-23	-2.01	0.0001	0

^{*} Selected genes differentially expressed in preeclamptic placenta in order of fold change values, P-value: Students' t-test p-value, FDR: false discovery rate in SAM analysis.

Table 3

DAVID mapping of genes differentially expressed in preeclamptic placenta

Gene List	Enrichment Score	Cluster
PSG6, INHA, FLT1, INSL4	2.57	Reproductive organismal physiological process, reproductive physiological process, reproduction
FCGR1A, FCGR2B, IL9, INHA, EBI3, NR4A2, PROCR, IFTT4, BCL6	2.24	Immune response, defense response
FCGR1A, FCGR2B, PSG6, SIGLEC6, FLT1	2.02	Domain: Ig-like C2-type 2, domain:Ig-like C2-type 1, Immunoglobulin, Immunoglobulin C2 type, Immunoglobulin subtype, IG, Immunoglobulin-like
PSG6, INSL4	1.88	Pregnancy, physiologic interaction between organisms, interaction between organisms
IL9, INHA, EBI3	1.66	Regulation of cytokine biosynthesis, cytokine biosynthesis, regulation of cytokine production, cytokine metabolism, cytokine production, regulation of immune response, regulation of protein biosynthesis, regulation of cellular biosynthesis, regulation of biosynthesis, regulation of organismal physiological process, regulation of protein metabolism, cytokine activity
CDKN1C, INHA, MXI1, HRASLS3, BCL6	1.38	Negative regulation of cellular physiological process, negative regulation of physiological process, negative regulation of cellular process, negative regulation of biological process.
CDKN1C, INHA, HRASLC3, F2R	1.22	Cycle, regulation of cell cycle, cell cycle

* Genbank accession numbers were mapped using functional annotation clustering in the DAVID 2007 pathway analysis tool. For each group, the processes or functions are tabulated with the gene list and enrichment score. Enrichment score is calculated as the geometric mean (in log scale) of members' p-values in a corresponding annotation cluster. Clusters shown here are those with enrichment scores > 1.0.

Table 4

Gene clusters identified using Ingenuity Path Analysis in preclampsia placenta.

Genes in Network	Score	Focus genes	Functions
AKT2, ASS1, AZGP1, beta-estradiol, CASP14, CDO1 , CTSH , CYP24A1, FSTL3 , GAL, GCLC, HEXB , HRASLS3 , IFIT3 , IFNG, IGFBP4, IGSF1, INHA , MBP, MOG, MXI1 , MYOD1, NPY1R, NXPH1 , PCSK1, PMM2 , prostaglandin E2, PYGM , SLCO2A1 , TCF12 , TEAD2, TGFB1, TNFRSF11B, VGLL1 , ZFP36	30	14	Cellular Development, Hematological System Development and Function, Immune and Lymphatic System Development and Function
ADRB3, Akt, ASS1, BCL6 , BSG, CDKN1C, CPT1A, CSF2RA , DLL4, EBI3 , F2R , FCGR1A , FCGR2B , FLT1 , GCLC, HMOX1 , IL9 , IL5RA, IL9R, LEP , Mapk, NR4A2 , P38 MAPK, PDE3B, PMCH, PRKAA1, RETN, RUNX1T1 , SHC2 (includes EG:25759), SLC25A5, STAT5a/b, UCN, Vegf, Vegf Receptor , VEGFB (includes EG:7423)	28	13	Cell Death, Cellular Growth and Proliferation, Inflammatory Disease
4-androstene-3,17-dione, ABCB11, ABCC3, ASS1, carbon monoxide, CYP11A1 , CYP2E1, CYP7A1, FCGR2B , GCLC, GHRL, GRN, Gsk3, HMGCR, HSD11B1, IGFBP4, IL27, IL10RA, IL1B, LAD1 , Nos, NR4A2 , NR4A3, ORM2, PGF, PROCR , PRTN3, RPSA, SCARB1, SEPP1, TFAP2C, TG, THBD, TNF, TNFRSF11B	8	5	Cellular Growth and Proliferation, Small Molecule Biochemistry, Connective Tissue Development and Function

* The networks were generated through the use of Ingenuity Pathways Analysis (Ingenuity® Systems, www.ingenuity.com). Each gene identifier was mapped to its corresponding gene object in the Ingenuity Pathways Knowledge Base (IPKB). These genes were overlaid onto a global molecular network developed from information contained in the IPKB. Network enrichment is then assessed using a network score (negative log of p-values of Fisher tests). Focus genes (in bold) are genes identified in our list of differentially expressed genes. Networks shown here are those with network scores > 3.0.

Table 5
Correlation between microarray and QRT-PCR^{*} expression measurements

	Microarray		QRT-PCR		Spearman Rank Correlation [∞]	
	T-test (p-value) [‡]	Fold change	T-test (p-value)	Fold change	Controls	Cases
LEP	0.00	5.52	0.01	3.82	0.41(0.09)	0.93(0.00)
FLT1	0.00	2.76	0.00	1.88	0.36(0.16)	0.92(0.00)
CYP11A	0.01	2.26	0.13	1.13	0.33(0.18)	0.67(0.00)
F2R	0.00	2.16	0.47	1.38	0.56(0.02)	0.47(0.06)
FCGR2B	0.01	2.16	0.46	1.12	0.83(0.000)	0.55(0.02)
CDO1	0.00	2.14	0.02	1.15	0.03(0.92)	0.35(0.17)
PROCR	0.00	1.99	0.02	1.52	0.44(0.07)	0.86(0.00)
TPBG	0.01	1.74	0.00	1.64	0.54(0.02)	0.78(0.00)
NR4A2	0.00	0.50	0.03	0.57	0.52(0.04)	0.67(0.00)

* QRT-PCR conducted using Clontech gene assays.

‡ Students' T-test p-values comparing expression measurement among cases and controls.

∞ Spearman rank correlation (p-values) between microarray and QRT-PCR gene expression measurements.