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Altered Global Gene Expression in First Trimester Placentas of Women Destined to Develop Preeclampsia

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Abstract

Background—Preeclampsia is a pregnancy-specific disorder that remains a leading cause of maternal, fetal and neonatal morbidity and mortality, and is associated with risk for future cardiovascular disease. There are no reliable predictors, specific preventative measures or treatments other than delivery. A widely-held view is that the antecedents of preeclampsia lie with impaired placentation in early pregnancy. Accordingly, we hypothesized dysregulation of global gene expression in first trimester placentas of women who later manifested preeclampsia.

Methods—Surplus chorionic villus sampling (CVS) tissues were collected at 10–12 weeks gestation in 160 patients with singleton fetuses. Four patients developed preeclampsia, and their banked CVS specimens were matched to 8 control samples from patients with unaffected pregnancies. Affymetrix HG-U133 Plus 2.0 GeneChips were utilized for microarray analysis. Naïve Bayes prediction modeling and pathway analysis were conducted. qRT-PCR examined three of the dysregulated genes.

Results—Thirty-six differentially expressed genes were identified in the preeclampsia placentas. qRT-PCR verified the microarray analysis. Thirty-one genes were down-regulated. Many were related to inflammation/immunoregulation and cell motility. Decidual gene dysregulation was prominent. No evidence was found for alterations in hypoxia and oxidative stress regulated genes.

Conclusions—To our knowledge, this is the first study to show dysregulation of gene expression in the early placentas of women ~6 months before developing preeclampsia, thereby reinforcing a placental origin of the disorder. We hypothesize that placentation in preeclampsia is compromised in the first trimester by maternal and fetal immune dysregulation, abnormal decidualization, or both, thereby impairing trophoblast invasion. Several of the genes provide potential targets for the

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development of clinical biomarkers in maternal blood during the first trimester. Supplementary materials are available for this article via the publisher's online edition.

Keywords

Oligonucleotide; microarray; chorionic villus sampling; maternal-fetal interface; immune regulation; decidualization; cell motility; hypoxia inducible factor; oxidative stress

Preeclampsia is a pregnancy-specific disorder that leads among the causes of maternal and infant morbidity and mortality worldwide. Occurring in 4 to 8 % of all pregnancies, [1,2] this multi-systemic disorder contributes to 20% of maternal deaths in the United States alone. [3] Infants are at increased risk of growth restriction and the adverse effects of indicated preterm delivery, which is the only definitive treatment for preeclampsia. [4] Further burden is imposed by increased risk of cardiovascular disease later in life for women and offspring who survive preeclampsia. [5,6]

Prevention, early detection, and specific treatment of preeclampsia are hindered by the fact that the etiology remains unknown. [6] Current consensus implicates placental and endothelial dysfunction, inflammation and genetics in development of preeclampsia. [1,7,8] Extravillous trophoblasts in preeclamptic pregnancies fail to adequately remodel the maternal uterine spiral arteries, thereby compromising blood flow to the placenta. [1] This alteration reduces placental oxygen and nutrient supply, creating ischemia-hypoxia and oxidative stress associated with reperfusion injury. [9] This placental pathology leads to the elaboration of a variety of injurious agents into the maternal circulation producing excessive maternal systemic inflammation [10] and endothelial dysfunction, and results in potentially lethal sequelae, such as eclamptic seizure, stroke, pulmonary edema, renal failure and hemorrhage. [11,12]

A better understanding of the early pathophysiology at the molecular level is needed. Because preeclampsia resolves with removal of the placenta, [13] microarray studies have been conducted with second and third trimester placental tissue after delivery, in order to examine organ-specific, global genomic variations associated with preeclampsia. [14] Despite these efforts, the etiology remains elusive because prior microarray analyses in preeclampsia interrogated placentas after disease onset in later gestation, thereby precluding differentiation of cause and effect. [15]

Herein, we report global gene expression patterns contemporaneous with early placental development in the first trimester of preeclamptic and unaffected pregnancies to better seek clues to etiology and early circulating biomarkers of placental origin. In addition to pursuing the traditional discovery-based approach offered by microarray analysis, we tested a priori hypotheses about candidate genes and pathways. Based on our own previous work and the literature, we predicted over-expression of genes regulated by hypoxia inducible transcription factors (HIF) -1α and -2α [16–18] and oxidative stress, [19,20] as well as inflammation. [10]

Materials and methods

Samples

CVS—Samples were donated from 2001–2005 by women with singleton gestations who gave informed consent for research participation through procedures approved by the University of Pittsburgh and Magee-Women's Hospital Institutional Review Boards. Consent for the research was obtained by genetics counselors during the process of informed consent for the clinical chorionic villus sampling (CVS) procedure. The indication for the majority of

procedures in the cohort was advanced maternal age ≥ 35 years (AMA) and average age was 37.

A physician specialized in CVS (W. A. H.) obtained specimens for clinical cytogenetics by aspiration of tissue into a 20 cc syringe containing Amniomax solution for cytogenetics cell culture (Invitrogen, Carlsbad, CA). The solution was not expected to affect RNA in any way, although experiments to test this opinion have not been performed (T. Jackson, Invitrogen; personal communication, February 28, 2008). As part of the routine CVS procedure, the clinical specimen was poured from the syringe into a Petri dish to be assessed by the clinician for adequacy of amount aspirated (at least 25 mg of villi). No extra tissue was extracted for the research. If surplus tissue not needed for clinical analyses was available, villi grossly free of decidua and maternal blood were removed from the Amniomax in the Petri dish, placed in an Eppendorf tube, and snap-frozen in less than 10 minutes of CVS aspiration from the patient. Research specimens were stored at -80°C for analyses after birth outcomes became available. Frozen samples occupied approximately 5–30 μl in the Eppendorf tubes. Eighty percent of all 160 consented participants had surplus CVS tissue for the research study. The rate of preeclampsia in the cohort was $\sim 3\%$.

Samples for microarray study—Each of 4 CVS specimens from women who subsequently developed preeclampsia (PE) was matched based on parity, gestational age at CVS within 3 days, and race with 2 unaffected control (C) specimens for the microarray analysis. The sample size of 4 PE patients was determined by availability of samples in the CVS specimen bank meeting the study's diagnostic criteria and was the minimal number needed for statistical variance. PE was defined as new onset of hypertension and proteinuria after 20 weeks gestation with blood pressure ≥ 140 and/or 90 on at least 2 occasions at least 6 hours apart, and ≥ 300 mg of protein in a 24 hour urine.²¹ Hypertension was diagnosed prior to labor and administration of medications. Proteinuria was diagnosed within 72 hours of the hypertension. These women did not have underlying medical disorders or other obstetrical complications. Controls were defined as specimens from normotensive women with blood pressure $< 140/90$, no proteinuria, and without other pregnancy complications or underlying medical disorders. To our surprise, we obtained no samples from normotensive women delivering growth restricted babies for comparison.

Additional samples for replication—Additional samples (AS) were selected to evaluate gene expression levels by quantitative real time polymerase chain reaction (qRT-PCR) in a set of samples that had not undergone microarray analysis. This was done for purposes of replicating qRT-PCR results. We assumed that replication of the qRT-PCR levels would further corroborate the microarray results. Twenty-four stored specimens were selected from women without pregnancy complications or underlying medical disorders. Matching AS to the PE cases was prioritized by parity, gestational age at CVS and race. Parity was limited to ≤ 3 pregnancies and gestational age at CVS within 3 days.

Clinical Data Analysis—Demographic and clinical characteristics of participants were analyzed to examine underlying assumptions of group assignment. PE (N = 4) and C (N = 8) samples submitted for microarray procedures were compared using distributionally appropriate t-test or chi square with $p \leq 0.05$ set as level of significance.

Additional samples (N = 24) selected for replication purposes were compared with PE and C groups using ANOVA and Bonferroni adjustment with $p \leq 0.05$ set as level of significance (SPSS 15.0, Carey, NC).

RNA extraction—Total RNA was extracted and microarray conducted at the University of Pittsburgh Genomics and Proteomics Core Laboratory (GPCL). Specialized methods were

applied for the particular tissue type, resulting in good RNA integrity (Agilent RIN ≥ 6.0 ; Supplement). Samples were stored at -80°C .

Microarray data collection

The Affymetrix GeneChip system (Affymetrix, Santa Clara, CA) was used for microarray analysis with HG-U133 Plus 2.0 GeneChips containing 53,613 probe sets. The GPCL conducted the analysis according to manufacturer's instructions (Supplement). Gene expression intensities were derived from the .cel files using dchip [22] and BRB-Array Tools. [23] The primary microarray data from these analyses are available at Gene Expression Omnibus, accession number GSE12767, <http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE12767>.

Finding Differentially Expressed Genes—Following data quality assurance/quality control (QA/QC) and normalization procedures (Supplement), differentially expressed genes were identified using the J5 test, Pooled Variance t-test (PVT) and Fold Change 3 ($\text{FC} = [\text{mean } 1 - \text{mean } 2] / \text{mean } 2$) as implemented in the online program for Cancer Gene Expression Data Analysis (caGEDA; Supplement). [24] QA/QC of sample #147 included many outliers. The genes found to be differentially expressed with the most efficient test, whether #147 was included or excluded, were considered further.

Prediction Modeling—Naïve Bayes prediction models were evaluated using leave-one-out cross-validation (LOOCV) in caGEDA [24] with the J5 test used for feature selection (detailed in Supplement). The models with lowest achieved classification error (ACE) found at the highest threshold (smallest number of genes) were preferred. Given the small data set size, and the possibility of optimism in estimates of the classifier prediction model's performance with LOOCV with small data sets, the prediction modeling was conducted primarily to further inform on feature selection (identification of differentially expressed genes) and to provide a preliminary assessment of the potential utility of CVS genomic biomarkers for the prediction of or early detection of preeclampsia.

Functional Analysis—Annotations were retrieved using a Batch Query given the Probe Set Identification numbers (Probe IDs) submitted to the Affymetrix NetAffx resource. [25] Probe IDs and J5 scores were also submitted to Ingenuity Pathways Analysis 5.5 bioinformatics software (IPA) [26] for an analysis of functions known to be related to the genes of interest. Investigation of functions and molecular networks serves to suggest plausible biological pathways involving the identified genes (Supplement).

Quantitative RT-PCR

Quantitative real-time polymerase chain reaction (qRT-PCR) was conducted on 3 genes differentially expressed in the microarray, LAIR2, CCK, and CTAG2, to validate the microarray results (Table 2 includes MIM numbers, gene names and symbols). Ribosomal protein large P0 (RPLP0; MIM 180510) was the most appropriate endogenous control for this tissue was determined by using the Endogenous Control Plate (Applied Biosystems [ABI]; Foster City, CA). qRT-PCR was conducted according to routine protocols using the ABIPRISM 7000 system (Supplement).

Data analysis—Raw cycle threshold (C_T) values were determined using SDS 1.1 software (ABI). Relative gene expression was determined using the comparative C_T method (Supplement). [27] Fold changes were analyzed by Kruskal-Wallis with significance set at $p \leq 0.05$. Analyses were carried out in Excel and SAS 9.1 (SAS Institute Inc., Cary, NC).

Results

Clinical data

All women in the sample for microarray analysis (total N = 12) were of AMA (35–44), White, and had normal fetal karyotype on CVS. PE (N = 4) and C (N = 8) groups did not differ in maternal age ($p = 0.34$; Table 1). Eleven of 12 samples were from nulliparous women and one C was a multiparous participant in her second pregnancy; groups did not differ by parity ($p = 1.00$). Although not significantly different, body mass index (BMI) at the time of CVS showed a trend toward higher PE BMI 29.9 ± 4.2 versus C 24.5 ± 4.0 (mean $\pm$ SD; $p = 0.06$). Gestational age at CVS of both PE and C groups ranged from 10.7–12.4 weeks and did not differ between groups ($p = 0.78$). PE and C groups did not differ in gestational age at delivery ($p = 0.11$), birthweight ($p = 0.10$), or infant sex ($p = 1.00$). One C participant reported smoking; all others were self-reported nonsmokers. Groups did not differ in smoking ($p = 1.00$).

The 4 PE participants met inclusion criteria for hypertension and proteinuria. Three were hyperuricemic for gestational age (Table 1). Systolic and diastolic blood pressures at less than 20 weeks showed no preexisting hypertension. Participant #147 reached a severe blood pressure above 160/110 and was delivered by cesarean section. Participant #19 had low platelets, high creatinine, normal liver enzymes, and was also delivered by cesarean section. Two PE cases (#21 and #147) delivered preterm, prior to 37 weeks' gestation. PE #21 was obese (BMI ≥ 30) and her infant was growth restricted, below 10th percentile for gestational age, induced with pitocin and delivered vaginally.

Participants in the AS group ranged in age from 34–45 years (mean 38.2 years) and were 10.4–13.0 weeks gestation (mean 11.5 weeks) with BMI 19.5–36.4 (mean 25.6) at CVS. Seven were nulliparous and 17 were multiparous women with uncomplicated pregnancies and unknown CVS gene expression patterns. All were White women and had normal fetal karyotype on CVS. All were self-reported nonsmokers. It is unknown whether each multiparous woman conceived with the same partner for all of her pregnancies. The AS group did not differ from PE or C by maternal age ($p = 0.59$), gestational age at CVS ($p = 0.66$), or BMI ($p = 0.19$) at CVS.

Microarray data

Oligonucleotide microarray analysis of CVS specimens produced global gene expression patterns in early pregnancies destined for preeclamptic versus unaffected outcomes. Four PE compared with 8 C specimens resulted in a set of 36 differentially expressed genes.

The QA/QC correlogram scatterplots comparing signal intensity across the microarrays included and excluded #147 (Figure 1-Supplement). High-expression outliers found in #147 resulted in all subsequent analyses including and excluding #147. Robust Multi-array Average (RMA) normalization in RMA-BRB-Array Tools [23] resulted in a coefficient of variation of 0.001.

Expression levels were assessed using the J5 test, PVT and FC as implemented in caGEDA. [24] The various tests for identifying differentially expressed genes were compared using the "Efficiency Analysis" option, which splits the sample into two data sets, ranks the genes on each test, and identifies which test exhibits the highest congruence among the top-ranked genes. The J5 test using the raw Perfect Match data only (Supplement) led to the most efficient and internally consistent set of differentially expressed genes, exhibiting 40% consistency in the set of genes found to be differentially expressed between PE and C (Figure 2-Supplement). This contrasts with less than 5% internal consistency explained by PVT and approximately 10% explained by FC. Fold change and the t-test are known to lead to high false-positive rates, especially with small sample number. [24] Efficiency analysis in J5 identified 36 genes of interest in the overlap of gene sets with and without #147.

Prediction modeling was explored with Naïve Bayes models using LOOCV (Figure 3-Supplement). Modeling was conducted with and without sample #147. The modeling that included #147 exhibited perfect accuracy, equaling sensitivity and specificity (SN=SP=ACC=1.0). The model with a J5 score of 8, which excluded #147, led to the lowest ACE for potential genomic biomarkers of preeclampsia with 90% accuracy.

The Affymetrix probe identification numbers (probe IDs) submitted to NetAffx resulted in annotations for gene names and Gene Ontology (GO) Molecular Function Description for the 36 genes of interest (Table 2). Five genes of interest were up-regulated and 31 were down-regulated. At this time, 7 are mapped genes with unknown functions and 4 are unmapped with unknown function. NetAffx identified GO Pathway information for only 3 of the 36 genes of interest.

IPA identified known pathways in biological functions and diseases associated with the differentially expressed genes (Supplement). Two Networks (interacting pathways) were developed from the 36 dysregulated genes imputed with others annotated in the Ingenuity Pathways Knowledge Base (Figure 1). Top Functions and Diseases associated with Network 1 involving 10 of the 36 genes of interest were Cancer, Respiratory Disease, and Cellular Movement. Top Functions and Diseases associated with Network 2, involving 7 genes of interest, were Inflammatory Disease, Cellular Movement, and Hematological System Development and Function. MMP12 was the only gene shared between Networks 1 and 2.

A total of 72 Function and Disease categories involving the genes of interest were identified in IPA. The top 10 functional categories were most significant and included more than one of the 36 genes (Table 3). Categories in each of the 2 Networks (Figure 1) included subcategories of pathway information that indicate potential biological functions of the incorporated genes. Lower level functions within Cancer were cancer, neoplasia, tumorigenesis, ovarian cancer, gonadal tumor, mitosis, cell rounding, invasion, apoptosis, adhesion, migration, attachment, and detachment. Respiratory Disease was the 28th most significant Function and Disease category (not in Table 3) which included FN1, MMP12, CCK, and EPAS1 (-log p-value = 1.63E-03–2.72E-02). Lower level functions within Respiratory Disease included primary pulmonary hypertension, lung tumor, lung cancer, adhesion, neonatal surfactant, and emphysema. Cellular Movement included migration of all types of leukocytes, blood, intestinal, embryonic, neuronal, bone marrow, gonadal and cancer cells, cell movement, immobilization, scattering, and invasion. Inflammatory Disease was the 62nd most significant Function and Disease category (not in Table 3) which included F11R, S100A8, and MMP12 (-log p-value = 8.24E-03–2.42E-02) and lower level functions related to juvenile rheumatoid arthritis and emphysema. Hematological System Development and Function included migration and cell movement of all types of leukocytes, cell spreading, adhesion, immobilization, replacement, and proliferation.

qRT-PCR data

Relative quantitation of gene expression in LAIR2, CCK, and CTAG2 compared to a calibrator sample was conducted by qRT-PCR in PE, C, and AS samples (Supplement). There were no differences between C and AS groups in any of the 3 genes. LAIR2 was significantly down-regulated by a median fold change of 0.21 (range 0.02–0.41) in PE, compared to median fold change 1.17 (0.44–127.56) in C, and 4.99 (0.51–315.17) in AS ($p = 0.0004$).

Although the trend for CCK fold change was in the same direction as the microarray over-expression, the qRT-PCR differences by Kruskal-Wallis were not significant ($p = 0.60$). However, the median fold change in CCK was 25.37 (range 1.01–51.45) in PE, compared to 2.19 (0.46–30.38) in C, and 5.77 (0.07–26.63) in AS. The raw microarray expression level and

qRT-PCR fold change in each PE sample were 4223.4 and 51.45 in #19, 732 and 1.72 in #21, 7339.4 and 49.01 in #58, 59.7 and 1.01 in # 147.

The trend in CTAG2 fold change was toward over-expression in PE, similar to the microarray results, but the differences were not significant ($p = 0.39$). qRT-PCR for CTAG2 resulted in 11 of 36 samples as “undetermined.” The values for endogenous control RPLPO were consistent among all 36 samples for each of the 3 genes in qRT-PCR with an average of 24.25 ± 0.12 in CTAG2. The median fold change in CTAG2 was 5.78 (range 0.28–11.27) in PE, compared to 0.04 (0.01–1.00) in C, and 0.90 (0.02–12.64) in AS.

DISCUSSION

To our knowledge, this microarray analysis of surplus CVS specimens produced the earliest differential global gene expression of placentas in pregnancies destined for preeclampsia. Utilization of first trimester snap-frozen tissues with known pregnancy outcomes, oligonucleotide genechips, and subsequent prediction modeling distinguish our study from previous placental microarray investigations in preeclampsia. [14] qRT-PCR of LAIR2 supported the microarray results. qRT-PCR expression levels in the additional unaffected samples (AS) replicated C group results, further verifying the microarray. qRT-PCR of CCK and CTAG2 trended in over- and under-expression consistent with the microarray results, and the lack of statistical differences are likely due to sample sizes. The variation in CTAG2 qRT-PCR expression patterns may indicate methylation in nonexpressors, denoted as “undetermined” among all 3 groups of samples. [28] CCK was up-regulated 3.1 fold in placentas of preeclamptic pregnancies at 29–32 weeks in a previous study. [29]

The IPA analyses suggested potential pathways in which some of the 36 genes probably function. Ten genes were not incorporated into any of the Function and Disease pathways produced by IPA, nor were they located in current literature searches in conjunction with preeclampsia: CTAG2, MUC15, OXGR1, SCARA5, MAGEB6, TNRC9, TMC4, DEPDC7, RUFY3, and LAIR2. We suggest functional groupings, other than immune/inflammation, integrating these with all other genes of interest (Table 4). The IPA findings were synthesized with hand searches of the literature to inform our interpretation of the dysregulated genes in relation to preeclampsia.

Overall, our results directly support the concept of the placental origins of the disorder [30] and allow for targeted investigation of placental derived biomarkers in *early* pregnancy. Assessment of cause rather than effect of preeclampsia is likely to have been more discernable in these first trimester placental tissues. The findings in this study suggest that impaired placentation in preeclampsia may be associated with an overall deficiency rather than an excess of gene expression, insofar as 31 of the 36 genes of interest were down-regulated. Preconceptional testing of susceptibility to preeclampsia could be developed from variants of the genes of interest. In addition, several produce secreted protein (Figure 1), such that measurement of one or a combination of these biomarker proteins in maternal blood in the first trimester may prove to be a predictive screening test for preeclampsia.

Genes expressed in the CVS specimens can be interpreted as maternal and fetal. Innate immune responses at the maternal-fetal interface are likely to be represented. Remarkably, 12 of the 36 genes, 7 not previously associated with preeclampsia, are involved in immune dysregulation (Table 2). All of the immunoregulatory genes except S100A8 were down-regulated, implicating deficient, blocked, or impaired function. LAIR2, HPS3, and SART3 are immune-related genes (Table 2) that were not incorporated by IPA into the immune pathway (Figure 1; Table 3).

The immune dysregulated cells may be trophoblasts, which are fetoplacental epithelial cells [31] that act as a pregnancy-specific component of the innate immune system. [32] By day 14 post conception, cytotrophoblasts (CTBs) have breached the chorionic basement membrane, switching from a proliferative to an invasive phenotype as extravillous trophoblasts (EVTs). [33] Cellular Movement functions in IPA Networks 1 and 2, including inflammation, migration, and invasion, are known to be involved in CTB placental processes (Figure 1). The EVT forms cell columns contacting maternal immune cells in the decidua. [34] From these columns, EVTs invade the uterine wall and remodel the maternal spiral arteries by displacing smooth muscle and endothelial cells. [35] Normal trophoblast development differs from cancer in that proliferation ceases during invasion. [33] Various genes associated with both of these processes were down-regulated in preeclampsia (Table 2). In the current analysis, no notable differential expression existed between PE and C in EVT epithelial integrins [36,37] or human leukocyte antigens [38] identified in other studies as dysregulated in CTBs of later gestation. Alternatively, some of the differentially expressed immunoregulatory genes may suggest abnormalities of fetoplacental Hofbauer cells, which are macrophages that populate the villous core. [34]

The maternal innate immune system predominates at this stage of placental development with 70% of decidual leukocytes consisting of natural killer cells (NK), 20–25% macrophages and about 2% dendritic cells. [39] Approximately 10% of decidual immune cells at this time are adaptive system T lymphocytes; no B cells are present. [40] Thus, some of the immunoregulatory genes of interest could also be of maternal origin. Finally, one cannot exclude the potential contribution of circulating fetal or maternal immune cells in the placenta. [33]

Surprisingly, a number of differentially expressed genes may be found in decidual stroma, including MUC15, [41] IGFBP1, [42] and PAEP. [43] Although the goal of CVS is to obtain chorionic tissue for fetal genetic diagnosis, maternal decidual tissue is invariably present, as corroborated by our microarray analysis. Decidual tissue likely derives from placental septae projecting upwards from the basal plate towards the chorionic plate that contains an admixture of decidual cells, EVTs, and occasional trophoblast giant cells. [34] On balance, the results suggest that preeclampsia may be associated with impaired decidualization. Whether this is etiological or secondary to suboptimal interaction with and stimulation by trophoblasts or maternal immune cells, or both is currently unknown. An alternative explanation, albeit less likely, is that there are fewer of these septae in early preeclampsia placentas, thus decreasing decidual tissue and consequently decidual gene expression in these CVS specimens.

In order to examine hypotheses concerning hypoxia inducible transcription factors and oxidative stress, a secondary analysis of expression fold changes was conducted with the caveat that a high rate of false positives could be expected (Table 1-Supplement). [24] A previous study, showing that gene expression of first trimester villous explant cultures incubated under 3% oxygen mimicked gene expression of various preeclamptic placentas at delivery, suggested that hypoxia may be involved in the pathogenesis [44], but is not necessarily informative of etiology. Indeed, the concept that the placenta is hypoxic or over-expresses HIF- α protein during early gestation, thereby impairing trophoblast invasion in preeclampsia, [16] is not corroborated by our microarray analysis of CVS tissue. We interrogated 26 genes proven to be HIF target genes [45] and several have been shown to be over-represented in placentas delivered from preeclamptic women (Table 2-Supplement). [46] Only IGFBP1, WT1 and TH genes showed differential expression in one probe. Moreover, IGFBP1 and TH are typically up- and not down-regulated by hypoxia. [47, 48] Interestingly, EPAS1 or HIF2 α (Table 2) expression was markedly decreased, but did not consistently correlate with putative specific HIF α target genes (Table 3-Supplement), [49] suggesting adequate HIF2 α protein levels, transcriptional activity or compensation despite markedly reduced HIF2 α mRNA expression.

We found no difference in HIF1 α expression between preeclamptic and control samples by our microarray study.

Nor were we able to support the differential expression of oxidative stress regulated genes at this early stage of preeclamptic pregnancy. [19,20] We interrogated fold changes in 11 genes previously shown to be regulated by oxidative stress (Table 4-Supplement), [50] and expression differences were nonsignificant. In fact, blood flow and oxygen delivery to the intervillous space begins around 10–12 weeks of gestation, [19] but expression profiles of the hypoxia (Table 2-Supplement) and oxidative stress (Table 4-Supplement) regulated genes do not support the concept of an undue delay or acceleration of this crucial physiological event, respectively. Thus, ischemia-hypoxia and oxidative stress due to reperfusion injury are likely to be later events in preeclampsia.

Noteworthy is that 17 of the 36 genes identified by the Naïve Bayes prediction model and J5 test were among the 152 identified by 2-fold FC analysis. Thus, there is considerable intersection of the two analytical approaches. The finding of aberrant decidualization in early placentas of preeclampsia revealed by the prediction modeling is bolstered by the FC analysis, insofar as FSTL3 (FC -2.56) [50] and prolactin (FC -7.86) [52] are down-regulated (Table 1-Supplement). Additionally, marked downregulation of granulysin in the FC analysis (FC -23.51) further supports immune dysregulation in decidua. [53]

Limitations

Specimens obtained from surplus CVS revealed genomic differences in first trimester placentas of pregnancies eventuating in preeclampsia. The indication for CVS, advanced maternal age, may limit the present findings for younger women. Our samples were from a homogeneous racial group, consistent with the population undergoing CVS at the clinical site, which may limit the findings for groups other than Caucasian women. It must be mentioned, however, that CVS is the only method to directly access first trimester placental genome in the context of known pregnancy outcomes. The procedure is not offered to women without risk factors because CVS is associated with risk, e.g., 0.33% pregnancy loss. [54] The specimens for this study provided a rare window into pregnancy disorders such as preeclampsia and to early normal placentation. Another precaution is that we presently lack microarray data on CVS tissues obtained from other obstetrical complications linked to abnormal placentation, e.g., normotensive IUGR and preterm labor. [46] The results may not be specific only to preeclampsia in all women.

Many genes regulated at the post-transcriptional level could be important in the pathogenesis of preeclampsia. Because post-transcriptional changes are not directly interrogated by mRNA gene expression microarray, important pathogenetic mechanisms could be overlooked using this approach.

Finally, this microarray study could be considered a pilot investigation due to the relatively low sample number; yet, appropriate techniques for power analysis in microarray studies remain controversial. [55] Previous microarray analyses used a range of 2 to 11 placentas from preeclamptic women. [14] The laborious and time-consuming nature of specimen collection and stringent inclusion criteria affected our sample size. This limitation may be at least partially offset by the analytic methods within caGEDA. [24]

Conclusion

This is the first known study of global gene expression in first trimester placental tissues of preeclamptic pregnancies, and it contributes to the systems biology of normal first trimester placentation. The 36 differentially expressed genes provide promising potential biomarkers of

preeclampsia and clues to etiology, including dysregulation of maternal-fetal immune interaction and altered decidualization in first trimester placental tissue of women destined to develop preeclampsia. More specific hypotheses will require testing maternal versus fetal origins of the genes of interest. Clearly, we consider the maternal genome evident in our samples as a crucial component providing important insights, rather than as “contamination.” Individualized prevention strategies and treatments could follow from the genes identified. Validation studies using data from larger cohorts are warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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B.

Figure 1.

Ingenuity Pathways Analysis Networks 1 and 2. IPA incorporated some of the genes identified in the microarray analysis into potential functional relationships based on human and animal literature. **A.** Network 1 includes 10 genes from this study related to Cancer, Respiratory Disease, and Cellular Movement. CCK is up-regulated and located in the extracellular space. FSTL3, IGFBP1 and SEMA3 are down-regulated and located in the extracellular space, as is the enzyme MMP12. The genes of interest are in direct (solid arrows) and indirect (dashed arrows) relationships with other molecules depicted in the Network. FN1 is depicted as a down-regulated enzyme in the plasma membrane interacting directly and indirectly with other molecules depicted. None of the genes of interest is located in the cytoplasm in Network 1. ASCL2, ELL2, EPAS1 are down-regulated transcription factors involved in direct and indirect relationships with other molecules. SART3 is a molecule acted upon indirectly by molecules outside the nucleus. **B.** Network 2 includes 7 genes from this study related to Inflammatory Disease, Cellular Movement, and Hematological System Development and Function. The enzyme MMP12 and molecule PAEP are both down-regulated and located in the extracellular space. The genes of interest are in direct (solid arrows) and indirect (dashed arrows) relationships with other molecules depicted in the Network. SLC16A6 is a down-regulated transporter in the plasma membrane, indirectly related to molecules in other compartments.

F11R is a plasma membrane molecule that is down-regulated and related directly and indirectly to other molecules depicted in Network 2. S100A8 is an up-regulated molecule, KRT14 is a down-regulated molecule, and LRAP is an enzyme down-regulated in the cytoplasm. All interact directly and indirectly with other molecules depicted in the Network. None of the genes of interest is located in the nucleus in Network 2.

Table 1
Clinical characteristics of microarray study sample (N = 12).

	Control (N = 8)	Preeclampsia ^a (N = 4)
Maternal age (years)	38.1 ± 3.1 ^b	36.5 ± 0.6
Nulliparous (N)	7	4
BMI at CVS	24.5 ± 4.0	29.9 ± 4.2
Gestational weeks at CVS	11.3 ± 0.6	11.4 ± 0.7
Gestational weeks at delivery	39.8 ± 1.2	37.5 ± 2.9
Birth weight (grams)	3346.7 ± 437.4	2717.8 ± 643.1
Infant sex		
Female	4	2
Male	4	2
Smoking	1	0

PE case (Patient ID)	Maternal age at CVS	Gravidity	Parity	Race	BMI at CVS	Gestational age at CVS	Gestational age at delivery	Mode of delivery	Average systolic BP (prior to 20 wks)	Average diastolic BP (prior to 20 wks)
1 (19)	37	2	0	W	27.5	11.4	38	C- section	109	69
2 (21)	36	2	0	W	36.0	12.4	35.7	Vaginal	116	69
3 (58)	36	1	0	W	26.8	10.7	41.3	Vaginal	129	77
4 (147)	37	1	0	W	29.1	11.0	34.9	C- section	102	69

PE case (Patient ID)	Average systolic BP (at admission)	Average diastolic BPs (at admission)	Proteinuria (grams per 24 hour urine)	Uric acid (> 1SD for gestational age [+], mg/dL)	Birth weight (grams)	Birth weight percentile	Other
1 (19)	145	80	1.06	10.2 (+)	3241	>10	Max BP 155/90, Low platelets to 87K, creatinine 1.1 mg/dL
2 (21)	155 (145–164)	90 (80–88)	4.76	8.1 (+)	1965	<3	Max BP 164/88, IUGR
3 (58)	163 (149–177)	80 (69–95)	0.77	4.0 (–)	3265	>10	Max systolic BP 184
4 (147)	151 (134–176)	93 (84–115)	0.74	5.2 (+)	2400	>10	Max BP 176/115

^aPreeclampsia definition is based on the National High Blood Pressure Education Program Working Group. [21]

^bMean ± SD; level of significance, $p \leq 0.05$.

Table 2

Genes differentially expressed in first trimester PE.

Probe Set ID	J5 Score	Gene Title	Gene Symbol & MIM Number ^a	GO Biological Process Description	Localization and Function of Immune-related Genes (Cites with PE in Pubmed ^b)
205827_at	20.41	Cholecystokinin	CCK 118440	Neuron migration///signal transduction///axonogenesis///eating behavior	
215141_at	13.77	chromosome 4 open reading frame 10	C4orf10	---	
202917_s_at	13.19	S100 calcium binding protein A8	S100A8123885	inflammatory response	Chorionic villi, monocytes, macrophages, epithelial cells; [56] chronic inflammation; cystic fibrosis antigen [57] (1 related to HELLP) [58]
215733_x_at	8.52	cancer/testis antigen 2	CTAG2300396	biological_process	
234601_x_at	8.37	CDNA: FLJ22732 fis, clone HSI15880	---	---	
227238_at	-8.01	mucin 15, cell surface associated	MUC15 608566	---	
239010_at	-8.13	Hypothetical gene supported by AK096951; BC066547	LOC440157	---	
214702_at	-8.20	fibronectin 1	FN1 135600	acute-phase response///cell adhesion///transmembrane receptor protein tyrosine kinase signaling pathway///metabolism///response to wounding///cell migration///cell adhesion	plasma, epithelial cell surface, extracellular matrix (ECM); [59] migration stimulating factor, inflammation, binds collagen, binds complement, stimulates endocytosis and clearance of particulate material from the circulation [60] (114)
1553319_at	-8.25	oxoglutarate (alpha- ketoglutarate) receptor I	OXGR1 606922	signal transduction///G-protein coupled receptor protein signaling pathway///G-protein coupled receptor protein signaling pathway	
235592_at	-8.25	Elongation factor, RNA polymerase II, 2	ELL 2601874	transcription///regulation of transcription, DNA-dependent///RNA elongation from RNA polymerase II promoter	

Probe Set ID	J5 Score	Gene Title	Gene Symbol & MIM Number ^a	GO Biological Process Description	Localization and Function of Immune-related Genes (Cites with PE in Pubmed ^b)
229839_at	-8.30	Scavenger receptor class A, member 5 (putative)	SCARA5 611306	phosphate transport	
230748_at	-8.37	solute carrier family 16, member 6 (monocarboxylic acid transporter 7)	SLC16A6 603880	transport//monocarboxylic acid transport	
203789_s_at	-8.42	sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3C	SEMA3C 602645	immune response//transmembrane receptor protein tyrosine kinase signaling pathway//development//response to drug	axons; [61] secreted Ig; integrin inhibition [62] (0)
226482_s_at	-8.44	F11 receptor	F11R 605721	cell motility//inflammatory response//cell adhesion//epithelial cell differentiation	tight junctions of epithelial and endothelial cells, hematopoietic cells; [63, 64] tight junction assembly and stabilization, leukocyte transmigration, and platelet activation (0)
215388_s_at	-8.45	complement factor H//complement factor H- related 1	CFH//CFHR1 134370//134371//235400//609814//610698	complement activation, alternative pathway//complement activation//immune response//innate immune response	liver; monocytes, human umbilical vein endothelial cells (HUVEC), fibroblasts, [65] placenta; [66] alternative complement pathway in innate immune system (1 postpartum case study) [67]
1562053_at	-8.58	CDNA clone IMAGE:5267797	---	---	
219911_s_at	-8.59	solute carrier organic anion transporter family, member 4A1	SLCO4A1	ion transport//transport	
209351_at	-8.70	keratin 14 (epidermolysis bullosa simplex, Dowling- Meara, Koebner)	KRT14 125595//131800//148066//161000//601001	cell morphogenesis//biological_process//epidermis development//epidermis development	
1552858_at	-8.92	melanoma antigen family B, 6 300467	MAGEB6	biological_process	
215108_x_at	-8.95	trinucleotide repeat containing 9 611416	TNRC9	regulation of transcription, DNA- dependent	
226403_at	-8.97	transmembrane channel- like 4	TMC4	---	
207607_at	-9.23	achaete-scute complex homolog 2 (Drosophila)	ASCL2601886	regulation of transcription, DNA- dependent//central nervous system development//peripheral nervous system development//cell differentiation//regulation of transcription	

Probe Set ID	J5 Score	Gene Title	Gene Symbol & MIM Number ^a	GO Biological Process Description	Localization and Function of Immune-related Genes (Cites with PE in Pubmed ^b)
228293_at	-9.41	DEP domain containing 7	DEPDC7	intracellular signaling cascade//biological_process	
210251_s_at	-9.55	RUN and FYVE domain containing 3	RUFY3 611194	---	
1561318_at	-9.69	CDNA clone IMAGE:5287025	---	---	
241036_at	-9.76	Hermansky-Pudlak syndrome 3	HPS3 606118	organelle organization and biogenesis	kidney, liver placental cells; [68] lysosomal vesicles, platelet storage deficiency [69] (0)
219759_at	-9.92	leukocyte-derived arginine aminopeptidase	LRAP 609497	proteolysis//blood pressure regulation//antigen processing and presentation of endogenous peptide antigen via MHC class I	endoplasmic reticulum; maintenance of homeostasis; maintenance of normal pregnancy, memory retention, blood pressure regulation, antigen presentation [70] (0)
203592_s_at	-10.05	folliculin-like 3 (secreted glycoprotein)	FSTL3 605343	negative regulation of BMP signaling pathway	
203502_at	-10.35	insulin-like growth factor binding protein 1	IGFBP1 146730	regulation of cell growth//signal transduction	liver; secretory endometrium, and decidua; decidualization and trophoblast invasion [42] (34)
1568554_x_at	-10.67	Chromosome 6 open reading frame 142	C6orf142	---	
1554276_at	-13.80	squamous cell carcinoma antigen recognized by T cells 3	SART3 611684	RNA processing	RNA-binding nuclear protein; tumor-rejection antigen; induces HLA-A24-restricted and tumor-specific cytotoxic T lymphocytes in cancer [71] (0)
242842_at	-14.51	Transcribed locus	---	---	
242868_at	-15.34	Endothelial PAS domain protein 1	EPAS1 603349	angiogenesis//response to hypoxia//regulation of transcription, DNA-dependent//transcription from RNA polymerase II promoter//signal transduction//cell differentiation//transcription//signal transduction//development//regulation of transcription transport//development	

Probe Set ID	J5 Score	Gene Title	Gene Symbol & MIM Number ^a	GO Biological Process Description	Localization and Function of Immune-related Genes (Cites with PE in Pubmed ^b)
206859_s_at	-15.64	progesteragen-associated endometrial protein (placental protein 14, pregnancy-associated endometrial alpha-2- globulin, alpha uterine protein)	PAEP 173310		genital tract, secretory endometrium and decidua; [43] inhibits E-selectin-mediated cell adhesion [43]; neovascularization [72] (3)
204580_at	-17.18	matrix metalloproteinase 12 (macrophage elastase)	MMP12 601046	peptidoglycan metabolism//proteolysis//cell motility//proteolysis	macrophages, ECM; [73] Degrades elastin, tissue remodeling and repair during development and inflammation [74] (1 in vitro) [75]
207509_s_at	-18.87	leukocyte-associated immunoglobulin-like receptor 2	LAIR2 602993	---	homologous with LAIR1 on NK cells, T cells, B cells, macrophages, and dendritic cells; [76] secreted Ig-like receptor (0)

Table downloaded from Affymetrix. [25]

^aMIM Numbers accessed February 15, 2008; MIM absent if none available for gene transcript. [77]

^bPubmed search February 15, 2008; References in Supplement

^cUnmapped or function unknown

Table 3

Top Functions and Diseases in IPA associated with genes of interest.

Function and Disease Category	-log(p-value)	Genes ^a
Cellular Movement	1.31E-06–2.89E-02	F11R, FN1, S100A8, PAEP, SEMA3C, IGFBP1, CCK, MMP12
Hematological System Development and Function	1.31E-06–2.82E-02	F11R, FN1, S100A8, IGFBP1, PAEP, MMP12
Cancer	2.5E-06–2.89E-02	KRT14, LRAP, FN1, EPAS1, FSTL3, CCK, PAEP, HPS3, F11R, S100A8, IGFBP1, SEMA3C, MMP12, CFHR1
Immune Response	4.11E-06–2.82E-02	LRAP, F11R, FN1, S100A8, IGFBP1, PAEP, SEMA3C, MMP12, CFHR1
Reproductive System Disease	4.81E-06–2.75E-02	HPS3, EPAS1, FN1, FSTL3, S100A8, SEMA3C, PAEP, IGFBP1, ELL2
Hematological Disease	3.83E-05–2.91E-02	F11R, FN1, EPAS1, IGFBP1
Gastrointestinal Disease	6.36E-04–3.05E-02	KRT14, LRAP, FN1, FSTL3, CCK, IGFBP1, CFHR1
Nutritional Disease	7.52E-04–2.89E-02	FN1, IGFBP1, CCK
Reproductive System Development and Function	8.14E-04–2.26E-02	EPAS1, FN1, IGFBP1
Embryonic Development	1.56E-03–2.26E-02	EPAS1, FN1, ASCL2, SEMA3C

Table created in IPA for genes of interest, December 23, 2007. [26]

^a All genes are down-regulated except CCK and S100A8.

Table 4

Functional groups other than immune incorporating IPA-omitted genes of interest in PE.

Functional group	Genes of Interest
Neural/Axonal	CCK [78–80] ^a SEMA3C [62, 81] RUFY3 [82] ASCL2 [83] DEPDC7 [84]
Imprinting	CTAG2 [28, 85] ASCL [86, 87]
Angiogenesis	SEMA3C [88] EPAS1 [89] PAEP [72] CCK [90] FN1 [91]
VEGF related	PAEP [72] KRT14 [92] FR11 [93] CCK [90]
Epithelium	S100A8 [94] PAEP [43] F11R (Table 2) MUC15 [41] SCARA5 [95] SCLO4A1 [96] KRT14 [97] LAIR2 [98]
Melanoma	CTAG2 [99, 100] MAGEB [100] LAIR2 [98]
Endothelium	CCK HUVEC [109] PAEP HUVEC [72] LAIR2 [98] CFH [102] S100A8 [56] SEMA3C [62] EPAS1 [103]
Transcription factor	ASCL2 EPAS1 TNRC9 ELL2 (all in Table 2)
Metabolic homeostasis Insulin/glucose metabolism	CCK [80] FSTL3 [104] IGFBP1 (Table 2) EPAS1 [105] SLCO4A1 [106] SLC16A6 (Table 2)
Tyrosine kinase	SEMA3C (Table 2) RUFY3 [107] FN1 (Table 2)
G protein-coupled receptor	OXGR1 (Table 2) DEPDC7 [84]

^aReferences in Supplement