

# Genome-wide methylation profiling demonstrates hypermethylation in maternal leukocyte DNA in preeclamptic compared to normotensive pregnancies

Wendy M. White,<sup>1</sup> Brian Brost,<sup>1</sup> Zhifu Sun,<sup>2</sup> Carl Rose,<sup>1</sup> Iasmina Craici,<sup>3</sup> Steven J. Wagner,<sup>3</sup> Stephen T. Turner,<sup>3</sup> and Vesna D. Garovic<sup>3</sup>

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<sup>1</sup>Department of OB/GYN, Division of Maternal Fetal Medicine, Mayo Clinic College of Medicine, Rochester, MN, USA,

<sup>2</sup>Division of Biomedical Statistics and Informatics, Mayo Clinic College of Medicine, Rochester, MN, USA,

<sup>3</sup>Department of Internal Medicine, Division of Nephrology and Hypertension, Mayo Clinic College of Medicine, Rochester, MN, USA

*Objective:* To compare genome-wide methylation profiles in maternal leukocyte DNA between normotensive and preeclamptic pregnant women at delivery. *Methods:* Age, body mass index matched case-control comparison of methylation at 27,578 cytosine—guanine sites in 14,495 genes in maternal leukocyte DNA in women with preeclampsia (PE;  $n = 14$ ) and normotensive controls ( $n = 14$ ). *Results:* PE was associated with widespread differential methylation favoring hypermethylation. Pathway analysis identified the best matched process as a neuropeptide signaling pathway ( $p < 10^{-5}$ ); best matched disease as eclampsia ( $p < 9.97 \times 10^{-20}$ ). Significantly differentially methylated genes (*GRIN2b*, *GABRA1*, *PCDHB7*, and *BEX1*) are associated with seizures. *Conclusion:* Altered maternal leukocyte DNA methylation is associated with PE at delivery, and differential methylation of certain neuronal genes may explain the risk for eclampsia.

**Keywords** DNA methylation, Epigenetics, Leukocyte, Maternal, Pregnancy, Preeclampsia.

## INTRODUCTION

Preeclampsia, defined as new onset hypertension after 20 weeks gestation, accompanied by proteinuria  $>300$  mg in 24 h, occurs in 5–8% of pregnancies. Eclampsia, its convulsive form, complicates up to 3% of these pregnancies. Preeclampsia is an inflammatory state with significant immune system dysfunction. To date, alterations in multiple proteins which control immune

tolerance and inflammatory mechanisms have been noted to be associated with the clinical state of PE; however, it is not clear what mediates these changes and whether DNA methylation may play a role in controlling the maternal expression of these proteins.

Epigenetic phenomena, such as variable methylation, could explain certain differences in gene expression. An epigenetic trait is a stably inherited phenotype resulting from changes in a chromosome without alterations in its DNA sequence. The most studied epigenetic modification is DNA methylation, which is the addition of a methyl group to the DNA molecule at the sites of cytosine-guanine residue combinations called CpG sites. DNA methylation may result in an altered structure which blocks transcription factors, resulting in gene silencing or downregulation (1).

We hypothesize that altered DNA methylation patterns may contribute to the pathophysiology of PE by driving the differential expressions of maternal genes in preeclamptic compared to normal pregnancies. A review of the literature shows a paucity of studies in the area of DNA methylation changes and their possible roles in the manifestation of PE. What has been done, largely, has been directed at a few specific genes and/or in fetal-derived tissues (2–9). To our knowledge, there have been no previously published reports describing genome-wide methylation patterns from maternal leukocyte DNA in cases of PE.

In this study, we sought to characterize genome-wide differential methylation patterns in maternal blood leukocytes in 14 normotensive and 14 preeclamptic pregnancies at the time of delivery. We analyzed the DNA methylation status of 27,578 CpG dinucleotides in 14,495 genes using the Illumina Human Methylation-27 Assay (Illumina, San Diego, CA, USA). In addition, we performed gene-network analysis using GeneGo Metacore software (Thomson Reuters, Philadelphia, PA, USA) to determine candidate pathways, disease states, and gene sets.

## METHODS

### Ethical approval

This study was approved by the Mayo Clinic Institutional Review Board and informed consent was obtained prior to enrollment.

### Sources for case and control DNA

Preeclampsia was defined as the presence of new onset hypertension ( $>140/90$  mmHg), as documented by at least two readings 6 h apart, accompanied by proteinuria, as defined by a 24 h urine protein excretion  $>300$  mg, or the equivalent based on either the protein/creatinine ratio or urine dipstick. Preeclamptic participants ( $n = 14$ ) and selected normotensive controls ( $n = 14$ ) were matched for age (age,  $\pm 5$  years) and body mass index (BMI:  $\pm 5$  kg/m<sup>2</sup>). The medical records were abstracted for data, such as gravidity (primigravid being defined as no prior pregnancies), comorbidities (such as chronic hypertension or chronic kidney disease), BMI, ethnicity, intrapartum magnesium exposure, blood pressure readings, proteinuria, and gestational age at the time of delivery. All control and preeclamptic participants were in their first pregnancies, were non-smokers, and were of European descent.

### DNA extraction and processing

Blood was drawn within a 24 h window of delivery. Blood was collected in a 10-mL ethylenediaminetetraacetic acid (EDTA) tube, separated into a buffy coat, and stored at  $-80^{\circ}\text{C}$  until processed. Genomic DNA was extracted from the thawed buffy coat using the QIAGEN Flexigene Reagent kit (QIAGEN, Germantown, MD, USA), purified using the AutoGenFlex DNA purification kit (AutoGen, Holliston, MA, USA), quantified with NanoDrop spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA), normalized with standard PicoGreen methodology (Thermo Fisher Scientific, Wilmington, DE, USA), and plated in 1000 ng aliquots. Bisulfite modification was performed using the EZ DNA Methylation Kit (Zymo Research, Orange, CA, USA).

### Methylation assay

This article focuses on 28 samples plus technical replicates that were collected at the time of delivery. Plate maps were generated to determine the random location for each sample on the plate, as well as the samples that were run in duplicate. All samples were run in a single batch. We used the Illumina Human Methylation-27 Assay – a platform utilizing bead chip technology to evaluate the methylation status of over 27,578 CpG sites in 14,495 genes.

### Quality control

The raw data were processed using the BeadArray Reader from Illumina GenomeStudio (version 2010.2, Illumina, San Diego, CA, USA), with methylation module (version 1.7). Next, a series of quality control metrics were applied. Quality assessment of the array was conducted using the “Control Dashboard” in the software package, which includes a graphical inspection of the eight types of embedded control probes: staining, hybridization, target removal, extension, bisulfite conversion, guanine/thymine (G/T) mismatch, negative control, and non-polymorphic controls.

Overall sample performance was determined by the total number of detected CpGs, the average detection  $p$  value across all CpG sites, and the distribution of average  $f_i$  value for all CpGs. Call rates for each CpG site and sample were determined. Methylation sites and samples were excluded if the unreliable call rate (detection  $p$  value  $>0.05$ ) was greater than 5%. Technical replicate reproducibility was estimated by the Pearson correlation coefficient.

All samples were modified, plated, and run concurrently to avoid batch effect. However, BeadChips (Illumina, San Diego, CA, USA), even when processed at the same time, can have variations in assay integrity leading to chip effect. To assess for such effects, data were examined using principal components analysis and unsupervised hierarchical clustering (10–12).

### Data analysis

A mean  $\beta$  value between 0 and 1 was generated for each CpG site, representing the average ratio of methylated cytosine residues to the total number of cytosine residues at that site for each sample.

Data were analyzed as quantitative variables. The Student group  $t$ -test was used to compare the mean methylation level at each CpG site between the PE versus normotensive pregnant groups. All potential confounders were considered in developing the study design.

**Table 1.** Demographics of preeclamptic cases and normotensive controls.

Demographics	Normotensive (n = 14) Mean ± SD	Preeclamptic (n = 14) Mean ± SD	p Value
Age (years)	24.9 ± 2.9	23.3 ± 4.3	NS
BMI (kg/m <sup>2</sup> )	26.9 ± 7	28.7 ± 7	NS
SBP at delivery (mmHg)	119 ± 14	151 ± 12	<0.0001
DBP at delivery (mmHg)	71 ± 11	96 ± 11	<0.0001
GA at delivery (weeks)	40.0 ± 1.0	36.6 (±2.6)	0.0001

All subjects were Gravida 1, non-smoking, and of European descent; BMI, body mass index;

SBP, systolic blood pressure; DBP, diastolic blood pressure; GA, gestational age; p value determined by *t*-test.

All sites with an absolute mean methylation difference of >2% and  $p < 0.05$  were used to characterize global methylation patterns, and the GeneGo Metacore software was used to determine candidate pathways, disease states, and gene sets (13).

## RESULTS

### Characteristics of cases and controls

By design, the mean values did not differ significantly between preeclamptic and normotensive pregnant control participants in age; BMI, tobacco use, comorbidities, or gravidity. The only exception was a significantly higher gestational age at delivery in the normotensive controls than in the preeclamptic women (mean = 40 versus 36.6 weeks,  $p < 0.0001$ ). Blood pressures were different reflecting case control status, that is, higher blood pressures, both systolic and diastolic, in women with preeclamptic compared to normotensive pregnancies. Magnesium prophylaxis was used in 9 of 14 PE cases who were considered at risk for eclamptic seizures; none of the controls were exposed to magnesium (Table 1).

### Quality control

Of the 28 samples, three were randomly chosen to be run in duplicate as technical replicates. The technical replicates were highly reproducible, as estimated by a Pearson correlation coefficient ( $r^2 > 0.994$ ). All samples were randomly allocated to one of the eight BeadChips, no two technical replicates randomized to the same BeadChip, and all chips were processed at the same time. Finally, whole genome amplification (WGA) DNA was used as a negative external control, was also run in duplicate, and performed as expected.

Review of the eight types of embedded control probes such as staining, hybridization, target removal, extension, bisulfite conversion, G/T mismatch, negative control, and non-polymorphic controls, demonstrated that the overall assay performance was as expected. Overall sample quality was determined by the total number of detected CpGs at a detection  $p$  value  $< 0.05$ , the average detection  $p$  value across all CpG sites, and the distribution of the average  $\beta$  for all CpGs. CpGs that were not reliably detected in 95% samples based on

detection  $p$  values ( $n = 475$ ), or samples that did not have >95% reliable calls for all CpG sites ( $n = 0$ ), were excluded. All 28 patient samples and 98%, or 27,103 CpG sites, met quality control standards and were included for analysis.

All samples were run concurrently, thus eliminating issues of batch effect. However, differences related to chip effect (first chip versus last chip) or placement on the chip, for example, left versus right can exist. All samples were randomly allocated to eight BeadChips. The initial principal components analysis of our entire cohort did not detect any significant chip effect. As normalization/batch correction made a negligible difference in the results, the original data were presented in this article (11).

### Differentially methylated CpG sites genome-wide

Our first analytic approach was from a genome-wide perspective. All  $\beta$ s with mean methylation differences >2% and  $p < 0.05$  were included, for a total number of CpG sites of 997. Of note, these differentially methylated genes are located on all 22 autosomes, and the X chromosome as well, demonstrating a true genome-wide distribution. (List as online addendum)

Seven hundred twenty-nine genes were hypermethylated in PE, as compared with normotensive controls. The set of CpG sites that were more methylated in PE had an absolute methylation difference of up to 12%.

Two hundred sixty-eight genes were hypomethylated in PE, as compared with normotensive controls. This set of CpG sites that were less methylated in PE had an absolute methylation percentile difference of up to 20%.

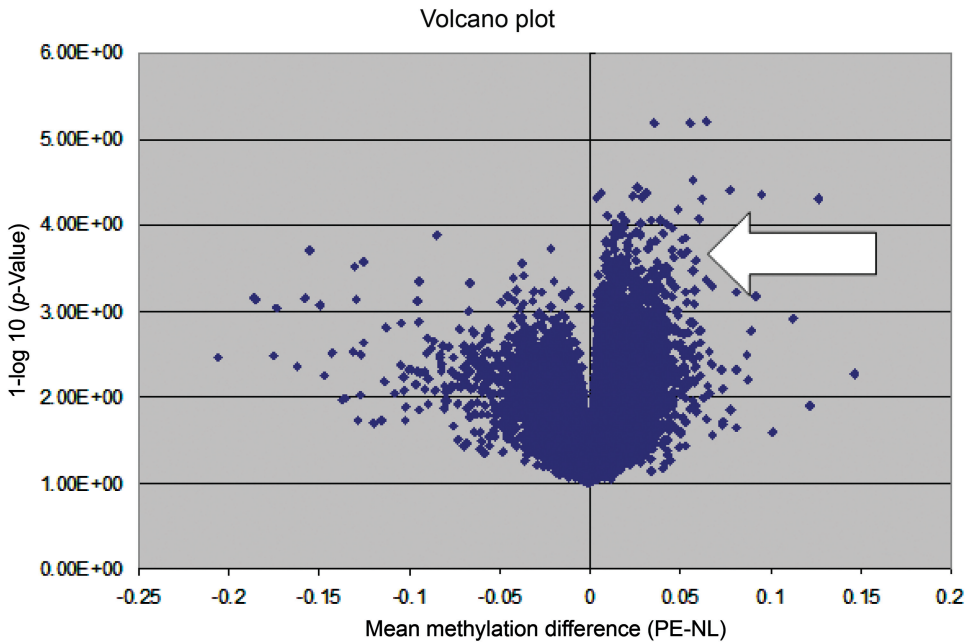
Overall, PE appears to be a hypermethylated state when compared with normotensive pregnancy. This is represented graphically in the Volcano plot in Figure 1.

### Pathway analysis

In putting all genes with statistically significant differences in methylation between preeclamptic and normotensive pregnancies into the GeneGo Metacore program identified the cellular and molecular process most likely to be involved as signal transduction involving neuropeptide signaling ( $p < 10^{-5}$ ). Other pathways identified were as follows: macrophage differentiation and vascular tone ( $p < 10^{-3}$ ). Using the same gene set analysis, the best-matched disease state was identified as eclampsia ( $p < 9.97 \times 10^{-20}$ ) (Figure 2).

### Differential methylation identifies potential candidate genes

There are 19 CpG sites in 18 genes that have statistically significant ( $p < 0.001$ ) differences in methylation in maternal leukocyte DNA, when measured at the time of delivery in normotensive and preeclamptic pregnancies. Of these 18 genes, four genes are highly expressed in the brain and associated with certain neurologic diseases, including seizure disorders. *GRIN2b* is 6% more methylated in PE ( $p = 0.00006$ ). *PCDHB7* is 6% more methylated in PE ( $p = 0.00007$ ). *BEX1* is 3% more methylated in PE ( $p = 0.0003$ ). *GABRA1* is 5% more methylated in PE ( $p = 0.00066$ ; see Tables 2 and 3). Differential methylation measured at the time of delivery did not appear to be associated with peripartum exposure to magnesium (data not shown).



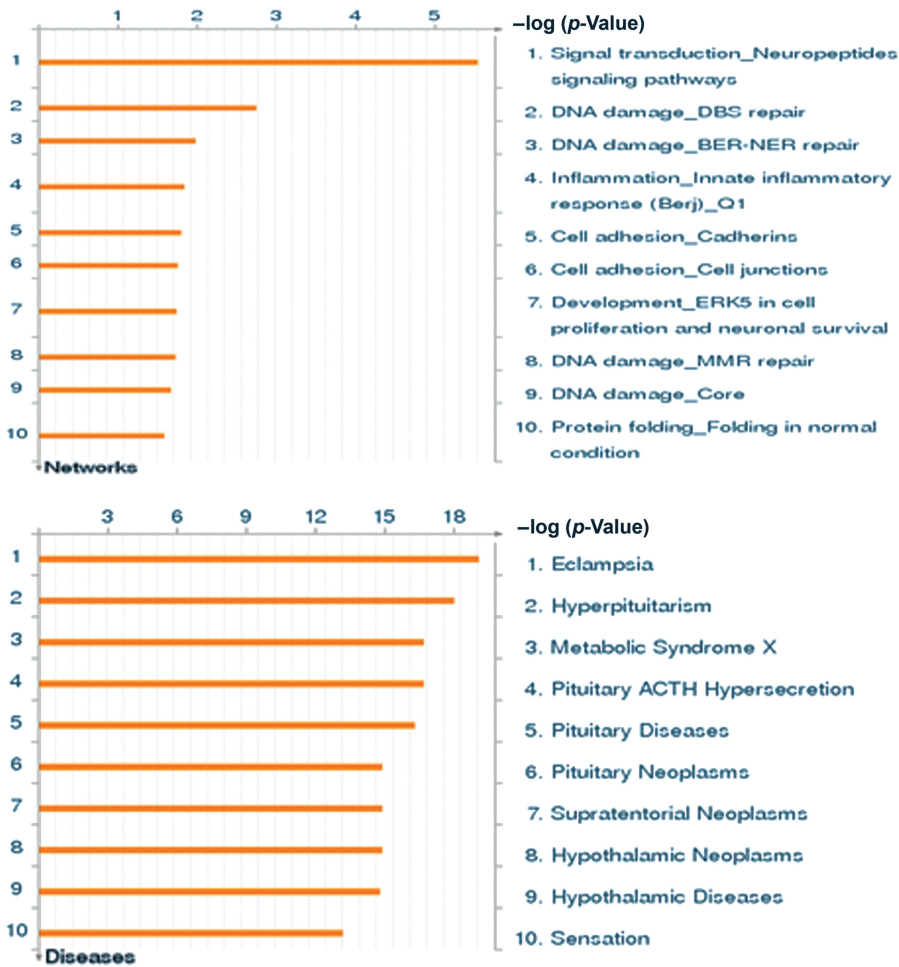
**Figure 1.** A volcano plot illustrating the global methylation differences between preeclamptic and normotensive samples. Each dot represents a comparison of mean methylation at an individual CpG site. The x-axis is the methylation mean difference (preeclampsia-normotensive). The y-axis is the negative log<sub>10</sub> of the p value. Note the large number of significant differentially hypermethylated sites in preeclamptic pregnancy (arrow).

## DISCUSSION

Our study indicates that genome-wide methylation profiles in maternal leukocyte DNA at the time of delivery are more methylated in PE cases than in matched normotensive controls. The presence of a methyl group at key CpG sites can lead to structural changes in the DNA molecule, which block gene transcription. Increased methylation at promoter sites in maternal DNA in PE is consistent with the idea that many genes may be either turned off or down-regulated compared to normal pregnancy. Our previous work has identified normal pregnancy as a hypomethylated state compared with non-pregnancy. It is possible that the relative hypermethylation, we see in this study, is actually a state of failed hypomethylation that accompanies normal maternal adaptation to pregnancy (14).

Many factors can influence global DNA methylation – both in the establishment of methylation, and also in the maintenance of methylation over time. Putative factors investigated to date have included age, ethnicity, nutrition, chemical exposures, and tobacco (1). As our sample size was too small to stratify by these factors, we controlled for potential confounding factors by matching the two groups of women for age (within 5 years), tobacco status (all non-smokers), BMI (within 5 kg/m<sup>2</sup>), and ethnicity. All women were free of major medical comorbidities.

It is unclear what level of differential methylation is clinically meaningful when defining “significantly different,” as this area of study in epigenetics is in



**Figure 2.** Pathway analysis of most highly differentially methylated genes. The GeneGo program compared the genes that were differentially methylated (defined as >2% different and  $p < 0.05$ ) in our data with >500 diseases and >100 cellular and molecular processes to generate a list of best matched pathways without a prior knowledge of the phenotype. The best matched process was the neuropeptide signaling pathway ( $p < 10^{-5}$ ). The best matched disease was eclampsia ( $p < 9.97 \times 10^{-20}$ ). See <http://www.genego.com>

its infancy. We chose an absolute change of 2% as the lower limit for this initial exploratory analysis, as our goal was not to exclude any statistically significant genes, accepting that some of these changes might not be clinically significant. This absolute change may represent a *relative* change in methylation at any given CpG, ranging from 2% to >100%. For example, a specific CpG site in the *PCDHB7* gene is methylated 43% of the time in preeclamptic subjects, but only 38% of the time in normotensive pregnancy, representing a 5% absolute difference, but a 15% relative change in methylation.

The GeneGo Metacore program examined our differential methylation gene data and compared these biomarkers with over 500 diseases and 110 cellular and molecular processes to come up with likely matches. The program has no information about the patients from whom our data was derived. Even without *a priori* knowledge of our patient population, the best matched disease state

**Table 2.** Differentially methylated genes in maternal leukocyte DNA from preeclampsia cases compared with normotensive controls at delivery.

	Mean PE	Mean NL	Mean (PE-NL)	Fold change (PE/NL)	p Value	Chromosome	CpG island	Marker
GRIN2B	0.691	0.626	0.064	1.103	6.41 E-05	12	FALSE	cg04016326
SNX19	0.113	0.078	0.036	1.460	6.49 E-05	11	TRUE	cg12179176
PCDHB7	0.431	0.376	0.056	1.148	6.56E-05	5	TRUE	cg03780733
CXX1	0.509	0.452	0.057	1.127	0.0003	X	TRUE	cg27198071
BEX1	0.539	0.512	0.027	1.052	0.000371	X	TRUE	cg21509846
MLLT6	0.166	0.140	0.026	1.187	0.000385	17	TRUE	cg01485645
MTCP1	0.550	0.472	0.078	1.165	0.000405	X	TRUE	cg24341236
C9orf23	0.1577	0.126	0.032	1.252	0.000423	9	TRUE	cg07557424
CCL26	0.342	0.247	0.095	1.386	0.000443	7	FALSE	cg05556717
C11orf1	0.092	0.069	0.024	1.345	0.000466	11	TRUE	cg10219037
FGF20	0.147	0.118	0.029	1.244	0.000492	8	TRUE	cg24030449
TMEM100	0.432	0.369	0.063	1.169	0.000498	17	FALSE	cg08762247
CCL26	0.542	0.415	0.127	1.306	0.000503	7	FALSE	cg12943082
GABRA1	0.278	0.229	0.049	1.213	0.000658	5	FALSE	cg24523000
RBBP7	0.475	0.415	0.060	1.145	0.00085	X	TRUE	cg14719055
GM2A	0.110	0.072	0.039	1.544	0.000871	5	TRUE	cg22775896
C5orf15	0.170	0.135	0.034	1.254	0.00089	5	TRUE	cg22065439
FTSJ3	0.126	0.098	0.028	1.285	0.000952	17	TRUE	cg11296363
ZBP1	0.340	0.297	0.043	1.146	0.000999	20	TRUE	cg22772698

There are 19 CpG sites in 18 genes that have significantly different levels of methylation in preeclampsia (PE) versus normotensive (NL) groups ( $p$  values  $<0.001$ ). None remain significant after the FDR correction for multiple comparisons ( $q \sim 0.5$ ). Genes presented in bold are highly expressed in brain and have been previously described as related to cortical excitability and/or being regulated by magnesium (see Table 3).

identified was eclampsia ( $p < 9.97 \times 10^{-20}$ ). The best matched cellular and molecular process was signal transduction in neuropeptide signaling pathways ( $p < 10^{-5}$ ).

Given the above results, we closely examined our top differentially methylated sites. When ranked by  $p$  value, an unexpected finding was that 4 of the top 18 differentially methylated genes are highly expressed in brain, and are associated with cortical excitability and neuronal connections. These genes, the related proteins, and a description of their basic functions are summarized in Table 3. The relationships between these genes and seizure disorders are particularly intriguing given the development of eclamptic seizures in a subset of preeclamptic patients, suggesting the possibility that altered methylation may be involved. Three of these genes, *GABRA1*, *BEX1*, and *GRIN2B*, have also been shown to be expressed in blood at low levels. Though their role in normal leukocyte physiology is not established, *BEX1* and *GRIN2B* have putative roles in hematopoietic cancers (15,16).

There has been limited work by others addressing the methylation changes found in PE. Most have focused on specific genes (2,5–8) rather than a genome-wide approach (3,4). The changes in methylation were described in fetal-derived tissues, such as placenta, (3–5,8,17–19) or free fetal DNA in maternal plasma, (5,7) but not in maternal leukocyte DNA.



**Table 3.** Four genes differentially methylated in preeclampsia cases compared with normotensive controls at the time of delivery.

Gene	Protein function	Tissue expression	Disease associations	Differential methylation in preeclampsia
GRIN2b glutamate receptor, ionotropic, N-methyl-D-aspartate 2B	Excitatory neurotransmitter receptor in the brain; voltage-dependent sensitivity to magnesium	Nervous system, cardiovascular system, and leukocytes	Cancer Neurologic diseases	6% more methylated in preeclampsia ( $p < 0.0001$ )
PCDH7 protocadherin $\beta$ 7	Calcium-dependent cell-adhesion protein with putative importance in establishment and maintenance of specific	Widely expressed including in nervous system	Not well studied Cancer Mental disorders Neurologic disorders	6% more methylated in preeclampsia ( $p < 0.0001$ )
BEX1 brain expressed X-linked protein	Cell cycle progression and neuronal differentiation; implicated as a tumor suppressor as well	Highly expressed in all areas of brain; low expression: leukocytes, liver, and testes	Cancer Cardiovascular disease Neurologic diseases Spontaneous abortion	3% more methylated in preeclampsia ( $p = 0.0003$ )
GABRA1 gamma-aminobutyric acid receptor subunit $\alpha$ -1	Component of receptor for major inhibitory neurotransmitter in the mammalian brain	Highly expressed in all areas of brain; low expression in skeletal muscle; leukocytes	Cancers Neurological disease –especially seizure disorders Multiple other diseases involving many body systems	5% more methylated in preeclampsia ( $p = 0.0007$ )

Data regarding protein function, tissue expression, and disease association were gathered from expression databases (see <http://www.genecards.org> and <http://www.nextbio.com>).

Maternal leukocyte DNA from blood can be easily isolated from the buffy coat containing white blood cells. Neutrophils and lymphocytes produce circulating cytokines which promote immunotolerance of the antigenic challenge associated with the developing fetus, thus helping to establish and maintain pregnancy. Alterations in maternal immune system function have been associated with PE. In fact, the inflammatory actions mediated by leukocytes have been associated with the endothelial dysfunction seen in PE. If differential methylation proves predictive of or diagnostic for pathologic states such as PE, knowledge of these patterns in a readily accessible tissue, such as blood, will be essential.

We recognize that although some of these genes have been shown to be expressed in leukocytes, most are primarily expressed in neurons (20). Blood cells interact with every tissue in the body, including leukocyte transmigration across the blood–brain barrier. Peripheral changes in the leukocyte epigenome could be part of the pathophysiology of disease or, alternatively, may mirror epigenetic changes of distant tissues, including the brain (21,22). A growing body of literature suggests that the presence of alterations in the peripheral blood epigenome or transcriptome may serve as a biomarker for non-hematologic diseases (22). Given the inaccessibility of brain tissue for analysis, this concept of developing a marker from peripheral blood for central neurologic diseases or treatments has been widely explored. For example, microarray analysis has demonstrated unique patterns of differentially expressed genes in peripheral blood mononuclear cells associated with amyotrophic lateral sclerosis, migraines, and schizophrenia (23,24). Altered methylation of neuropsychiatric genes in DNA obtained from blood in patients with borderline personality disorder has been demonstrated (25). Altered gamma-aminobutyric acid receptor expression in peripheral blood mononuclear cells in patients with schizophrenia after treatment with selective serotonin re-uptake inhibitors has also been demonstrated (26).

Differential methylation analysis can be confounded by many factors including age, BMI, and race; we matched on the factors we could control (age and BMI), or excluded them as variables (homogeneous ethnicity in our study). The possibility still exists though that there is confounding by factors for which we were unable to completely control. For example, we attempted to match for gestational age but still there was almost a 4-week difference (cases 36.6 gestational weeks versus 40.0 in controls), which reflects widely accepted notion that delivery, which is the gold standard for treatment of PE, frequently results in prematurity. However, our longitudinal data for normal pregnancy suggest that methylation changes occur over months, not weeks, making this an unlikely explanation for the differential methylation (unpublished data).

Also the presence of cell-free DNA from the fetus could vary between cases and controls, however, this DNA is typically derived from the plasma fraction, not the buffy coat, and comprises only up to 6% of the total DNA measured (27). As we utilized the cellular buffy coat, although some fetal DNA contamination was possible, it likely represented only a small fraction of the total DNA measured and, therefore, is unlikely to significantly alter our results.

The major limitation of our study is the relatively small number of women in each group. There are hundreds of genes differentially methylated at  $p < 0.05$ . The genes discussed above, with  $p$  values ranging from 0.0003 to 0.00006, did

not remain significant after correction for multiple comparisons using the false discovery rate method. The false discovery rate, commonly used in genomics, produces a  $q$  value, which is the expected proportion of false discoveries among all results deemed significant. Type II error is inherent in pilot experiments involving high throughput platforms whose expense on a per sample basis limit the number of samples that can be assessed.

Given the Type 2 error associated with high throughput platforms, candidate genes identified by genome-wide association studies involving single nucleotide polymorphisms (SNPs) often require replication utilizing either an independent sample set or a different methodology. Although replication in epigenome-wide studies is not a requirement for data to be published, and (28) to establish an association between these candidate genes and the preeclamptic phenotype, additional confirmatory studies are needed. Although the best mechanism for confirming an association is not clear, one possible approach is to repeat genome-wide analysis on a larger independent sample, utilization of pyrosequencing to confirm altered methylation in individual genes, or exploration of downstream transcriptional and translational implications via gene expression arrays such as quantitative real time reverse transcriptase polymerase chain reaction and protein product quantification (29). However, the fact that the most pronounced methylation changes were observed in genes which were, in a blind fashion, matched subsequently to eclampsia, may represent indirect evidence of the validity of our findings. In addition, these genes are involved in biologically plausible neurologic pathways that are likely affected in both PE and eclampsia.

Another limitation includes the possible confounding effect by SNPs in the CpG sites, which may falsely implicate or obscure differential methylation patterns in individual genes. In future studies, we plan to use the latest version of the Illumina chip, the Infinium HumanMethylation450 BeadChip (Illumina, San Diego, CA, USA), which takes SNPs into account when analyzing output data.

Despite the small sample size and lack of replication, there is a high likelihood that some of our identified genes are in fact differentially methylated in PE. We hope to replicate the present findings in a larger, independent sample, and identify differentially methylated genes that are crucial to human reproduction –both normal and pathologic.

## CONCLUSION

In summary, we have demonstrated a widespread trend toward hypermethylation in maternal leukocyte DNA in preeclamptic compared to normotensive pregnancies at the time of delivery. Pathway analysis of these differentially methylated genes implicates the involvement of neuropeptide signaling and the eclampsia disease state. Several of the genes with the most pronounced methylation changes between preeclamptic and normotensive pregnancies produce proteins that have previously been implicated in seizure disorders, suggesting possible novel pathways in the pathogenesis of eclampsia. The study of genome-wide differential methylation patterns, in both normal pregnancy and in pregnancy disease states, may be a new and powerful technique to identify novel genes involved in these processes.

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Methylation data can be found at <http://www.ncbi.nlm.nih.gov/geo/> using GEO submission ID: GSE37722.

## DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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