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Preeclampsia/eclampsia Candidate Genes Show Altered Methylation in Maternal Leukocytes of Preeclamptic Women at the Time of Delivery

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Abstract

Objective—To analyze methylation profiles of known preeclampsia/eclampsia (PE) candidate genes in normal (NL) and preeclamptic (PE) women at delivery.

Methods—A matched case-control study comparing methylation in 79 CpG sites/33 genes from an independent gene set in maternal leukocyte DNA in PE and NL (n=14 each) on an Illumina beadchip platform. Replication performed on second cohort (PE=12; NL=32).

Results—PE demonstrates differential methylation in *POMC*, *AGT*, *CALCA*, and *DDAH1* compared with NL.

Conclusion—Differential methylation in 4 genes associated with PE may represent a potential biomarker or an epigenetic pathophysiologic mechanism altering gene transcription.

Keywords

AGT; *CALCA*; candidate gene; *DDAH1*; DNA methylation; leukocyte; *POMC*; preeclampsia; pregnancy

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INTRODUCTION

Preeclampsia can be diagnosed when new onset hypertension occurs in pregnancy after 20 weeks gestation in combination with either proteinuria >300 mg in 24 hours or other evidence of end organ dysfunction manifested by a strictly defined set of symptoms or other laboratory abnormalities. Of the 5%-8% of pregnancies meeting criteria for preeclampsia, a subset of these patients develop seizure activity leading to a diagnosis of eclampsia. Maternal and fetal morbidity and mortality related to preeclampsia/eclampsia (PE) is high.

Alterations in the levels of many plasma or serum proteins have been associated with PE; however, it is unclear what mediates these changes.[1-4] These proteins serve as agents of metabolic change, endothelial dysfunction, hypercoagulability, and inflammatory response, all of which are present to some degree in normal pregnancy, but are further accentuated in preeclampsia.[5] The regulation of the differential expression of these proteins and the potential role of epigenetic factors are not understood. [6] DNA methylation is an epigenetic mechanism that could explain changes in gene transcription that contribute to the PE phenotype.[7]

Epigenetic changes affect patterns of gene transcription without altering the primary DNA sequence. One example is DNA methylation, where the addition of a methyl group to the DNA molecule at the site of cytosine – guanine residue combinations called **CpG sites,** may result in an altered structure which blocks or enhances transcription. Methylation can result in gene silencing or downregulation if present in a promoter, and is associated with increased gene expression if present in a gene body.[8]

We have previously demonstrated that normal early pregnancy is associated with a transient state of altered methylation in maternal leukocyte DNA.[9] We have also shown that maternal leukocyte DNA in preeclampsia cases at the time of delivery has an altered genome-wide methylation profile and that performing pathway analysis on these differentially-methylated genes independently identifies the PE disease state with high statistical significance.[10] In the current study, we sought to characterize the methylation patterns of 33 candidate genes in maternal leukocyte DNA in normal and preeclamptic pregnancies at delivery.

MATERIALS AND METHODS

Ethical Approval

This study was approved by the Mayo Clinic Institutional Review Board (#2104-05), and informed consent was obtained prior to enrollment.

Sources for Original Cohort Case and Control DNA

This subanalysis was part of a larger experimental design, planned from the outset to study methylation changes in normal and preeclamptic pregnancies at various times in gestation from an agnostic (previously published for both normal and preeclamptics at delivery) and candidate gene set approach.

Preeclampsia was defined as the presence of new onset hypertension (>140/90 mm Hg), as documented by at least two readings 6 hours apart, accompanied by proteinuria, as defined by a 24-hour urine protein >300 mg or the equivalent protein/creatinine ratio. Preeclamptic patients (n=14) and normotensive controls (n=14) were matched by age (age±5 years) and body mass index (BMI [in kg/m^2±5]) by design. Electronic medical records were abstracted for data regarding gravidity (primigravid being defined as no prior pregnancies), absence of comorbidities (such as chronic hypertension or chronic kidney disease), BMI, ethnicity, intrapartum magnesium exposure, blood pressures, proteinuria, and gestational age at time of delivery. Selection criteria for control and preeclamptic patients included European ancestry, first pregnancy, and non-smoking status. The discovery cohort of 28 samples plus technical replicates were selected from a larger study (total samples n=96) of normotensive and preeclamptic women sampled at different time points during their gestations; we selected all the samples at the delivery time point for inclusion in this study.

DNA Extraction and Processing

Blood was drawn within a 24-hour window of delivery and was collected in a 10 mL EDTA tube, separated into a buffy coat, and stored at −80 degrees C until processed. Genomic DNA was extracted from the thawed buffy coat using the Qiagen FlexiGene DNA kit, purified using an AutoGen Flex DNA purification kit, quantified using a NanoDrop spectrophotometer (Thermo Fisher Scientific), normalized with standard PicoGreen methodology, and plated in 1,000 ng aliquots. Bisulfite modification was performed using the EZ DNA Methylation Kit (Zymo Research).

Methylation Assay

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 27,000 CpG sites in 14,495 genes.

Quality Control

The raw data were processed using the BeadArray Reader from Illumina GenomeStudio (version 2010.2), with methylation module (version 1.7) Quality assessment of the array was conducted using the “Control Dashboard” in the software package, which includes a graphical inspection of the 8 types of embedded control probes: staining, hybridization, target removal, extension, bisulfite conversion, 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 beta values 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) was greater than 5%. Technical replicate reproducibility was estimated by the Pearson correlation coefficient.

All samples were bisulfite modified, plated, and run concurrently to avoid batch effect. However, different BeadChips, even when processed at the same time, can have variations in

assay integrity leading to the “chip” effect. Data were examined using principal components analysis and unsupervised hierarchical clustering.[11]

Statistical Analysis

The average beta value between 0 and 1 was generated for each CpG site, representing the ratio of methylated cytosine residues (methylated signal intensity) to the total number of cytosine residues (the sum of methylated and unmethylated signal intensity) at that site for each sample. Using GeneGo MetaCore version July 2010 (Thomson Reuters), a candidate gene set for preeclampsia/eclampsia was selected. For all CpG sites in each of the candidate genes on our platform, a Student’s group *t*-test was used to compare the mean methylation levels between the preeclampsia and normotensive pregnant groups. A significant difference in methylation between the two groups was defined as an absolute difference between beta values ≥ 0.02 and a *P* value <.05. A correction for multiple comparisons was not performed, as the comparison was confined to a predetermined gene set.

Replication Dataset

The replication cohort included 12 independent preeclampsia subjects and 32 normotensive controls; DNA was extracted and processed in the same way as the discovery cohort. The subjects’ case or control status was confirmed by chart review, but they were not matched based on any other characteristics such as parity or smoking. These samples were run on an Illumina HumanMethylation450 BeadChip Kit. The raw data were processed using Illumina GenomeStudio (version 2011.1), with methylation module (version 1.9) to get the average beta value. The probe II bias was adjusted to be more similar to probe I using the BMIQ algorithm.[12] Potential batch effects were evaluated using principal components analysis. Some minor batch effect was observed between two plates of samples and subsequently corrected using empirical Bayes method.[11, 13] After the data processing, all CpG sites (n=140) in the six genes previously identified (from the discovery dataset) as differentially methylated were extracted from the whole dataset for analysis, i.e., the beta values were compared between the cases and controls with a *t*-test, and a *P* value <.05 was considered significant. Differential methylation as a potential epigenetic regulatory mechanism was confirmed if differential methylation occurred at any CpG site in any of these six genes in the replication cohort.

RESULTS

Clinical Characteristics of Discovery and Replication Cohort

Demographic and clinical parameters were similar between both preeclamptic and normal pregnancy groups of the discovery cohort by design (age, BMI, tobacco use, comorbidities, parity), with the exception of gestational age at delivery (normal mean=40 weeks; preeclampsia mean=37 weeks) (Table 1). Magnesium prophylaxis was used in 9 of 14 preeclamptic cases who were considered at risk for eclamptic seizures; none of the normal pregnancy controls were exposed to magnesium.

Age, BMI and gestational age at delivery were also similar between both preeclamptic (n = 12) and normal pregnancy (n = 32) groups of the Replication Cohort (Table 2).

The mean systolic and diastolic blood pressures at admission for delivery for cases and controls were different in both the Discovery and Replication cohorts as expected.

Discovery Dataset from the Illumina 27K Platform

Of a total of 27,578 sites, 27,104 CpGs and all of the 28 samples passed our quality assurance steps. Technical replicates were highly reproducible (r2 >0.994).

The candidate gene set implicated in the preeclampsia/eclampsia disease state was predefined in GeneGo MetaCore (http://thomsonreuters.com/metacore as of July 2010), which consisted of 39 genes. Of these, 33 were present on the Illumina 27K platform with a total of 73 CpG sites (Table 3). Six of these genes were differentially methylated in our discovery comparison. Table 4 summarizes these genes, mean beta methylation levels, and the absolute methylation difference in the discovery experiment.

Validation of the Six Genes in an Independent Cohort with the Illumina 450k Platform

Of these six genes, *POMC* (proopiomelanocortin), *AGT* (angiotensinogen), *CALCA* (calcitonin-related polypeptide alpha), and *DDHA1* (dimethylarginine dimethylaminohydrolase 1) had two or more CpG sites within or proximate to the gene region with differential methylation in our replication experiment. Details about the absolute level of change, the location of these CpG sites within the gene, association with known single nucleotide polymorphisms (SNPs), and the Illumina 27k results are presented in Table 5.

DISCUSSION

In our previous work, we demonstrated that normal early pregnancy is associated with a relative shift to hypomethylation in many genes across the genome and that this shift reverts back to a baseline non-pregnant state, similar to the nulligravid population, by 6 weeks postpartum.[9] We also demonstrated that, compared to normotensive controls, preeclamptic pregnancies are associated with an incomplete shift in normal hypomethylation resulting in a relative hypermethylation of much of the genome when measured at the time of delivery.[10] Pathway analysis of these differentially-methylated genes independently implicated the preeclampsia/eclampsia disease process with a $P<9.97 \times 10^{-20}$.[10]

In the current study, we characterized the methylation profiles of 33 candidate genes from an independently-defined gene set known to be involved in PE. These candidate genes have been characterized by others at the transcriptional or protein levels, and differences have been associated with the PE clinical state. We found evidence of differential methylation in 6 genes, *AGT, CALCA , DDAH1, MTHFR, POMC*, and *PTGS2*, in our initial cohort and replicated this finding in 4 (*AGT*, *CALCA*, *DDAH1*, *POMC*) in a second independent cohort.

DNA methylation changes in preeclampsia have not been widely studied. When such epigenetic changes were studied, most researchers have focused on candidate genes or genome-wide approaches in DNA originating from **fetal** derived tissues, mainly placental[14151617] or free fetal DNA in maternal plasma[18], but not from maternal DNA.

In addition to our work, only a few other authors have focused on maternal tissues – omental arteries and leukocytes. Moussa et al. have studied maternal omental vessels, looking at methylation patterns in specific genes like the thromboxane synthase[19] and collagen metabolism genes,[20] as well as using a more genome-wide approach.[21] Anderson et al.[22] looked at methylation patterns in maternal leukocytes obtained during the first trimester in 6 PE and 6 NL cases genome wide using a 450K Illumina array and identified 207 CpG sites that had >0.20 difference in beta value between cases and controls. Their list of top differentially-methylated sites was generated without a correction for multiple comparisons despite >450,000 independent *t*-tests. Consequently, the resultant CpG sites demonstrated extreme heterogeneity in beta methylation values; no replication was performed to assess if these sites could predict preeclampsia cases from controls.

We have taken a novel approach, focusing on candidate genes expressed by maternal leukocytes, which are known to both alter immunomodulation (a potential cause of preeclampsia) and impact the inflammatory response (a potential effect from preeclampsia). We looked for altered methylation in maternal leukocytes at the time of acute preeclampsia in late pregnancy.

AGT produces angiotensinogen, which goes on to produce angiotensin I and angiotensin II (Ang II) after cleavage by renin and angiotensin converting enzyme, respectively. *AGT* is expressed in leukocytes,[23] as well as in liver and placenta. A specific form of AGT, high molecular weight angiotensinogen, is formed from complexes between angiotensinogen, complement C3dg, and the pro-form of eosinophil major basic protein, and is elevated in the serum in pregnancy.[24] Total AGT levels are raised 4-fold in normal pregnancy. Preeclampsia is associated with profound differences in the function of the renin-angiotensin system, with increased high molecular weight Ang levels in serum, and decreased Ang II and Ang (1-7) levels, despite overall unchanged, unbound levels of angiotensingen. In contrast, increased AGT transcription has been seen in PE placenta.[25, 26]

CALC, calcitonin- related polypeptide alpha, is a widely-expressed gene, including in leukocytes, placenta, uterus, and blood vessels, that, through alternative mRNA splicing, makes (pro)calcitonin and calcitonin gene-related peptide (CGRP), which are important in Ca++ regulation, pain and neurotransmission, inflammatory response, angiogenesis and vasodilatory adaptation in pregnancy. In normal pregnancy, maternal serum CGRP levels increase as gestation advances and then fall after delivery[27]; this same rise is not observed in untreated preeclamptic pregnancies, although levels do seem to increase acutely with magnesium exposure.[4, 28]

DDAH1, dimethylarginine dimethylamniohydrolase 1, is involved in nitric oxide generation via asymmetric dimethylarginine (ADMA) metabolism, the levels of which are known to be altered in preeclampsia[29] and which have been implicated in hypertension and endothelial dysfunction. There are no studies of changes in *DDAH1* expression associated with preeclampsia, although known SNPs in these genes have been associated with susceptibility to preeclampsia.[3031] Alterations in *DDAH1* methylation have been shown to change mRNA expression in other diseases, such as idiopathic pulmonary fibrosis[32]; it is possible that changes in *DDAH1* methylation regulate gene expression in preeclampsia as well.

POMC, proopiomelanocortin, produces a hormone precursor that can, after tissue-specific processing, lead to 10 different proteins, including beta-endorphin, alpha-melanocyte-stimulating hormone (MSH), and adrenocorticotropic hormone (ACTH). Serum levels of beta-endorphin and ACTH are known to be higher in the pregnant versus nonpregnant state. *POMC* is expressed in several white blood cell lineages, as well as in the pituitary and placenta.[33] Leukocyte-derived proteins, especially alpha-MSH and ACTH, have been implicated in immune function and inflammation. Interestingly, intact POMC is not usually measurable in blood; however, it can be found in pregnancy early in the first trimester, and it disappears within days after delivery.[34] There have not been specific studies of gene expression or protein levels in maternal leukocytes in the pregnant or preeclamptic state.

Our rationale for using leukocytes and our attempts to correct for known confounders of DNA methylation, such as age, BMI, and tobacco, have been detailed in previous papers. [9, 10] Potential uncontrolled confounders in this work include gestational age and exposure to magnesium. We are currently addressing the change in methylation across different time points in normal and pathological pregnancy to evaluate whether a gestational age difference of 3-4 weeks at the time of assessment is important. It is unknown if magnesium infusion impacts methylation.

Another limitation of this type of study is the possibility of SNP confounding effects. A common polymorphism, M235T, is associated with higher AGT levels, and a recent meta-analysis confirmed the relationship between this polymorphism and increased risk for PE.[35] One known SNP in the *AGT* gene located approximately 35 base pairs from the CpG site was found to be differentially methylated in the original cohort; however, it is not clear if differences in gene frequencies between cases and controls impacted our methylation analysis. Other genes did not have known SNPs associated with them. Our replication cohort had 3 SNPs in the 10 CpGs, with differential methylation as noted in Table 4. Thus, the confounding SNP effects may explain some of the differential methylation that was observed.

Methylation as a mechanism of gene transcription control is incompletely understood. Unlike a single point mutation in a primary DNA sequence, methylation at a specific unique CpG does not have a direct and predictable correlation with changes in gene transcription.[8] The methylation levels at specific CpG loci, outside of a CpG island (CGI), can be highly variable in terms of mean and standard deviation.[36] It is difficult to predict the relationship between the direction of methylation change and transcriptional activity, as increased methylation in the areas of promotors is associated with decreased transcription, whereas methylation in a gene body and other untranslated regions is associated with increased transcription.[8] Although this is suggestive of epigenetic regulation of gene transcription, in all studies where differential methylation is associated with a disease state, it is important to correlate these methylation changes with actual changes in RNA or protein, as well as explore the relationship with other epigenetic mechanisms such as histone modification.

CONCLUSION

We have shown in two independent cohorts that leukocyte DNA from women with preeclampsia at the time of delivery demonstrates differential methylation in CpG sites associated with 4 candidate genes: *POMC*, *AGT*, *CALCA*, and *DDAH1*. We postulate that the differential methylation we found in these four candidate gene may contribute to the alterations in gene expression and protein levels previously demonstrated by others in preeclampsia. The presence of any altered methylation may implicate epigenetic control as an explanation for why these genes act differently, and identifying which environmental factors influence methylation may allow us to better understand the pathophysiology of the disease. In future work, we plan to correlate gene transcription with altered methylation within the same individuals.

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REFERENCES

1. Molvarec A, Szarka A, Walentin S, et al. Serum leptin levels in relation to circulating cytokines, chemokines, adhesion molecules and angiogenic factors in normal pregnancy and preeclampsia. Reprod Biol Endocrinol. 2011; 9:124. [PubMed: 21906313]
2. LaMarca BD, Ryan MJ, Gilbert JS, Murphy SR, Granger JP. Inflammatory cytokines in the pathophysiology of hypertension during preeclampsia. Curr Hypertens Rep. 2007; 9:480–5. [PubMed: 18367011]
3. Romero R, Nien JK, Espinoza J, et al. A longitudinal study of angiogenic (placental growth factor) and anti-angiogenic (soluble endoglin and soluble vascular endothelial growth factor receptor-1) factors in normal pregnancy and patients destined to develop preeclampsia and deliver a small for gestational age neonate. J Matern Fetal Neona. 2008; 21:9–23.
4. Halhali A, Wimalawansa SJ, Berentsen V, Avila E, Thota CS, Larrea F. Calcitonin gene- and parathyroid hormone-related peptides in preeclampsia: effects of magnesium sulfate. Obstet Gynecol. 2001; 97:893–7. [PubMed: 11384692]
5. Ahn H, Park J, Gilman-Sachs A, Kwak-Kim J. Immunologic characteristics of preeclampsia, a comprehensive review. Am J Reprod Immunol. 2011; 65:377–94. [PubMed: 20825381]
6. Chappell S, Morgan L. Searching for genetic clues to the causes of pre-eclampsia. Clin Sci (Lond). 2006; 110:443–58. [PubMed: 16526948]
7. Oudejans CB, van Dijk M, Oosterkamp M, Lachmeijer A, Blankenstein MA. Genetics of preeclampsia: paradigm shifts. Hum Genet. 2007; 120:607–12. [PubMed: 17024365]
8. Jones PA. Functions of DNA methylation: islands, start sites, gene bodies and beyond. Nat Rev Genet. 2012; 13:484–92. [PubMed: 22641018]
9. White WM, Brost BC, Sun Z, et al. Normal early pregnancy: a transient state of epigenetic change favoring hypomethylation. Epigenetics. 2012; 7:729–34. [PubMed: 22647708]

10. White WM, Brost B, Sun Z, et al. Genome-wide methylation profiling demonstrates hypermethylation in maternal leukocyte DNA in preeclamptic compared to normotensive pregnancies. Hypertens Pregnancy. 2013; 32:257–69. [PubMed: 23782156]
11. Sun Z, Chai HS, Wu Y, et al. Batch effect correction for genome-wide methylation data with Illumina Infinium platform. BMC Med Genomics. 2011; 4:84. [PubMed: 22171553]
12. Teschendorff AE, Marabita F, Lechner M, et al. A beta-mixture quantile normalization method for correcting probe design bias in Illumina Infinium 450 k DNA methylation data. Bioinformatics. 2013; 29:189–96. [PubMed: 23175756]
13. Johnson WE, Li C, Rabinovic A. Adjusting batch effects in microarray expression data using empirical Bayes methods. Biostatistics. 2007; 8:118–27. [PubMed: 16632515]
14. Avila L, Yuen RK, Diego-Alvarez D, Penaherrera MS, Jiang R, Robinson WP. Evaluating DNA methylation and gene expression variability in the human term placenta. Placenta. 2010; 31:1070–7. [PubMed: 20947161]
15. Katari S, Turan N, Bibikova M, et al. DNA methylation and gene expression differences in children conceived in vitro or in vivo. Hum Mol Genet. 2009; 18:3769–78. [PubMed: 19605411]
16. Yuen RK, Penaherrera MS, von Dadelszen P, McFadden DE, Robinson WP. DNA methylation profiling of human placentas reveals promoter hypomethylation of multiple genes in early-onset preeclampsia. Eur J Hum Genet. 2010; 18:1006–12. [PubMed: 20442742]
17. Zhao A, Cheng Y, Li X, et al. Promoter hypomethylation of COMT in human placenta is not associated with the development of pre-eclampsia. Mol Hum Reprod. 2011; 17:199–206. [PubMed: 21047975]
18. Bellido ML, Radpour R, Lapaire O, et al. MALDI-TOF mass array analysis of RASSF1A and SERPINB5 methylation patterns in human placenta and plasma. Biol Reprod. 2010; 82:745–50. [PubMed: 20075396]
19. Mousa AA, Strauss JF 3rd, Walsh SW. Reduced methylation of the thromboxane synthase gene is correlated with its increased vascular expression in preeclampsia. Hypertension. 2012; 59:1249–55. [PubMed: 22493072]
20. Mousa AA, Cappello RE, Estrada-Gutierrez G, et al. Preeclampsia is associated with alterations in DNA methylation of genes involved in collagen metabolism. Am J Pathol. 2012; 181:1455–63. [PubMed: 22863954]
21. Mousa AA, Archer KJ, Cappello R, et al. DNA methylation is altered in maternal blood vessels of women with preeclampsia. Reprod Sci. 2012; 19:1332–42. [PubMed: 22902744]
22. Anderson CM, Ralph JL, Wright ML, Linggi B, Ohm JE. DNA methylation as a biomarker for preeclampsia. Biol Res Nurs. 2013
23. Gomez RA, Norling LL, Wilfong N, et al. Leukocytes synthesize angiotensinogen. Hypertension. 1993; 21:470–5. [PubMed: 7681424]
24. Tewksbury DA. Quantitation of five forms of high molecular weight angiotensinogen from human placenta. Am J Hypertens. 1996; 9:1029–34. [PubMed: 8896657]
25. Anton L, Brosnihan KB. Systemic and uteroplacental renin--angiotensin system in normal and pre-eclamptic pregnancies. Ther Adv Cardiovasc Dis. 2008; 2:349–62. [PubMed: 19124433]
26. Merrill DC, Karoly M, Chen K, Ferrario CM, Brosnihan KB. Angiotensin-(1-7) in normal and preeclamptic pregnancy. Endocrine. 2002; 18:239–45. [PubMed: 12450315]
27. Yallampalli C, Chauhan M, Thota CS, Kondapaka S, Wimalawansa SJ. Calcitonin gene-related peptide in pregnancy and its emerging receptor heterogeneity. Trends Endocrinol Metab. 2002; 13:263–9. [PubMed: 12128288]
28. Ariza AC, Bobadilla N, Diaz L, Avila E, Larrea F, Halhali A. Placental gene expression of calcitonin gene-related peptide and nitric oxide synthases in preeclampsia: effects of magnesium sulfate. Magnes Res. 2009; 22:44–9. [PubMed: 19441274]
29. Savvidou MD, Hingorani AD, Tsikas D, Frolich JC, Vallance P, Nicolaides KH. Endothelial dysfunction and raised plasma concentrations of asymmetric dimethylarginine in pregnant women who subsequently develop pre-eclampsia. Lancet. 2003; 361:1511–7. [PubMed: 12737861]
30. Akbar F, Heinonen S, Pirskanen M, Uimari P, Tuomainen TP, Salonen JT. Haplotypic association of DDAH1 with susceptibility to pre-eclampsia. Mol Hum Reprod. 2005; 11:73–7. [PubMed: 15501905]

31. Best LG, Nadeau M, Bercier S, et al. Genetic variants, endothelial function, and risk of preeclampsia among American Indians. Hypertens Pregnancy. 2012; 31:1–10. [PubMed: 21174581]

32. Sanders YY, Ambalavanan N, Halloran B, et al. Altered DNA methylation profile in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2012; 186:525–35. [PubMed: 22700861]

33. Stephanou A, Fitzharris P, Knight RA, Lightman SL. Characteristics and kinetics of proopiomelanocortin mRNA expression by human leucocytes. Brain Behav Immun. 1991; 5:319–27. [PubMed: 1685686]

34. Raffin-Sanson ML, Ferre F, Coste J, Oliver C, Cabrol D, Bertagna X. Pro-opiomelanocortin in human pregnancy: evolution of maternal plasma levels, concentrations in cord blood, amniotic fluid and at the feto-maternal interface. Eur J Endocrinol. 2000; 142:53–9. [PubMed: 10633222]

35. Ni S, Zhang Y, Deng Y, et al. AGT M235T polymorphism contributes to risk of preeclampsia: evidence from a meta-analysis. J Renin Angiotensin Aldosterone Syst. 2012; 13:379–86. [PubMed: 22513276]

36. Wagner JR, Busche S, Ge B, Kwan T, Pastinen T, Blanchette M. The relationship between DNA methylation, genetic and expression inter-individual variation in untransformed human fibroblasts. Genome Biol. 2014; 15:R37. [PubMed: 24555846]

Table 1

Subject Characteristics in Discovery Cohort

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/m2	26.9±7	28.7±7	NS
SBP at delivery, mm Hg	119±14	151±12	<.0001
DBP at delivery, mm Hg	71±11	96±11	<.0001
GA at delivery, weeks	40.0±1.0	36.6±2.6	.0001

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; GA, gestational age; SBP, systolic blood pressure; SD, standard deviation

Table 2

Subject Characteristics in Replication Cohort

Demographics	**Normotensive (n = 32) Mean±SD**	**Preeclamptic (n= 12) Mean±SD**	***P* value**
Age, years	30.6±4.3	29.6±6.8	NS
BMI, kg/m2	26.0±6	28.2±8	NS
SBP at delivery, mm Hg	121±15	148±9	<.0001
DBP at delivery, mm Hg	75±13	97±9	<.0001
GA at delivery, weeks	39.3±1.5	38.4±2.0	NS

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; GA, gestational age; SBP, systolic blood pressure; SD, standard deviation

Table 3

Discovery Cohort Candidate Gene Set

GENE SYMBOL	GENE NAME
ADM	adrenomedullin
AGT	angiotensinogen
AGTR1	angiotensin II receptor, type 1
BOK	BCL2-related ovarian killer
CALCA	calcitonin-related polypeptide alpha
CYP11B2	aldosterone synthase
CYR61	cysteine-rich, angiogenic inducer, 61
DDAH1	dimethylarginine dimethylaminohydrolase-1
ENG	endoglin
EPHX1	epoxide hydroxylase 1
ERVWE1	syncytin
F5	factor V Leiden
FAS	Fas cell surface death receptor
GCM1	glial cells missing homolog 1
HLA-G	major histocompatibility complex; class I; G
IFNG	interferon gamma
IFNGR2	interferon gamma receptor 2
IL10	interleukin 10
KIR3DL3	killer cell Ig-like receptor, 3 domains, long intracytoplasmic tail, 3
LEPR	leptin receptor
MTHFR	methylenetetrahydrofolate reductase
NOS3	nitric oxide synthase 3 (endothelial)
NOV	nephroblastoma overexpressed
POMC	proopiomelanocortin
PTGS2	prostaglandin-endoperoxide synthase 2 and cyclooxygenase
S100B	S100 calcium-binding protein B

GENE SYMBOL	GENE NAME
SCNN1B	sodium Channel, Non-Voltage-Gated 1, Beta Subunit
SERPIND1	serpin Peptidase Inhibitor, Clade D (Heparin Cofactor), Member 1
TAP1	transporter 1, ATP-Binding Cassette, Sub-Family B (MDR/TAP)
TAP2	transporter 2, ATP-Binding Cassette, Sub-Family B (MDR/TAP)
TNF	tumor necrosis factor
UTS2	urotensin 2
VEGF	vascular endothelial growth factor

The preeclampsia/eclampsia candidate gene set was predefined in MetaCore

(http://thomsonreuters.com/metacore/) as of July 2010. Of 39 genes in the gene set, 33 were present on the Illumina 27K platform, with a total of 73 CpG sites analyzed in the discovery cohort.

Table 4

Differentially-Methylated Candidate Genes in Discovery Cohort

Gene Name	Symbol	Absolute Mean methylation difference in PE PE – NL (%)	*P* value
Angiotensinogen	***AGT***	**0.757 – 0.787 (− 3%)**	**.027**
Dimethylarginine dimethylamino-hydrolase 1	***DDAH1***	**0.047 – 0.102 (− 6%)**	**.031**
Calcitonin-related polypeptide alpha	***CALCA***	**0.215 – 0.175 (+ 4%)**	**.001**
Methylenetetra hydrofolate reductase	*MTHFR*	**0.323 – 0.293 (+ 3%)**	.030
Proopiomelanocortin	***POMC***	**0.412 – 0.372 (+ 4%)**	**.014**
Prostaglandin-endoperoxide synthase 2	*PTGS2*	**0.156 – 0.125 (+ 3%)**	.030

Six genes had evidence of differential methylation in at least one CpG site in the initial discovery cohort run the Illumina 27k platform. The absolute difference in beta value was determined by subtracting the average beta value in the preeclampsia subjects at the time of delivery (PE) minus the average beta value in the normotensive subjects at the time of delivery (NL) and expressed as a percentage. By definition, the absolute difference had to exceed 2% to be considered differentially methylated. A *t*-test was performed and a *P* value that was <.05 was considered significant. Of the six genes, four, in bold, were also found to have differential methylation in the replication cohort.

Table 5

List of Differentially-Methylated CpG Sites in Four Genes in the Replication Cohort on the Illumina 450K Platform

Gene Name (Symbol)	Total # CpG tested	CpG site differentially methylated	Absolute Mean methylation difference in PE PE–NL (% in PE)	*P* value	Notes
Angiotensinogen *(AGT)*	8	cg0750241 cg0108371	0.34-0.30 (+4%) 0.20-0.18 (+2%)	.04 .02	+ SNP; enhancer No SNP; 5'UTR
Dimethylarginine dimethylamino-hydrolase 1 (*DDAH1)*	45	cg24515368 cg24662614	0.87-0.85 (+2%) 0.76-0.78 (−2%)	.04 .01	No SNP; Body No SNP; N Shelf of CPI
Calcitonin-related polypeptide alpha (*CALCA)*	39	cg01971122 cg07529966	0.18-0.17 (+1%) 0.07-0.08 (−1%)	.03 .03	No SNP; CGI/ enhancer No SNP; CGI/Enhancer
Proopiomelanocortin (*POMC)*	23	cg09527270 cg03560973 cg02079741	0.75-0.79 (−4%) 0.42-0.44 (−2%) 0.57-0.60 (−3%)	.01 .05 .03	+ SNP; N Shore of CPI + SNP; N Shore of CPI No SNP; N Shore of CPI

The absolute difference in beta value was determined by subtracting the average beta value in the preeclampsia subjects at the time of delivery (PE) minus the average beta value in the normotensive subjects at the time of delivery (NL) and expressed as a percentage. A *t*-test was performed and a *P* value that was <.05 was considered significant. + SNP indicates a single nucleotide polymorphism is present in this CpG site; however, the genotype of the individual is not known. Gene locations include the gene body, CGI = CpG island; 5UTR (5 prime end untranslated region), N shores (North shore is up to 2 kilobases upstream from a CpG island) and regions that function as known enhancers. None of these CpGs differentially-methylated in the replication cohort were present on the 27k array in the discovery cohort.