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DNA Methylation Patterns of Glucocorticoid Pathway Genes in Preterm Birth Among Black Women

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Abstract

Preterm birth (PTB; <37 weeks gestation) rates have increased for 5 of the last 6 consecutive years in the United States. These rates are particularly alarming for U.S. non-Hispanic Black women who give birth prematurely at 1.5 times the rate of non-Hispanic White women. Previous research suggests that psychological stress is associated with PTB in Black women. However, the biological pathways by which stress alters birth timing are not clear. We examined DNA methylation (DNAm) in peripheral blood leukocytes in 6 glucocorticoid, stress-related genes in 44 (22 PTB; 22 term birth) pregnant Black women. Four cytosine-phosphate-guanine (CpG) sites were identified as differentially methylated ($p < 0.05$) between women with PTB and women with term births. The ability to identify stress-related biological markers that are associated with PTB among Black women would provide a critical step toward decreasing the PTB disparity among these women. Future studies should include larger sample sizes and gene expression analyses of the stress-related biological pathways to PTB.

Keywords

preterm birth, epigenetics, maternal stress, hypothalamic-pituitary-adrenal axis, African American, Black

Preterm birth (PTB), birth at less than 37 completed weeks gestation, is a serious national health issue. Approximately 1 in 10 births or 360,000 babies are delivered prematurely in the U.S. each year (March of Dimes, 2021). Consequences of PTB include death, sepsis, necrotizing enterocolitis, respiratory disease, intellectual disabilities, blindness, and hearing loss (March of Dimes, 2021). Overall, PTB rates increased steadily in the U.S. for 6 years from 9.57% in 2014 to 10.23% in 2019 (Hamilton et al., 2021) before declining slightly in 2020 to 10.1% (Martin et al., 2021). However, this small decrease in 2020 was attributable to non-Hispanic White and Hispanic women while the PTB rate for non-Hispanic Black women remained stable (Martin et al., 2021). A considerable disparity exists in PTB rates between non-Hispanic White women and non-Hispanic Black women at 9.1% and 14.3%, respectively (Martin et al., 2020). Socioeconomic and sociodemographic factors (e.g., income, education, marital status) have been identified as contributing to the disparity (Braveman et al., 2015; Thoma et al., 2019) but do not fully account for the significant difference in risk for PTB faced by non-Hispanic Black (referred to hereafter as Black) women. Research suggests that psychological stress is related to the risk for PTB among Black women (Ding et al., 2021; Dunkel Schetter and Tanner, 2012; Giurgescu et al., 2022). However, research is limited on the biological mechanisms underlying this association.

Epigenetic modifications have been implicated as a mechanism by which environmental stressors may alter the protein expression of genes and affect health (Dirven et al.,

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2017). Epigenetics is defined as changes in the DNA structure that do not alter the underlying gene sequence. DNA methylation (DNAm) is an epigenetic mechanism that occurs when a methyl group covalently attaches to the 5′ carbon of a cytosine base of a cytosine-phosphate-guanine (CpG) dinucleotide. DNA methylation is heritable, reversible, and subject to psychological stress (McGowan et al., 2009; Weaver et al., 2004). Thus, DNAm offers a plausible and potentially modifiable biological pathway by which maternal psychological stress increases the risk for PTB. However, research is lacking for DNAm of stress-related genes associated with PTB among Black women. The purpose of this study was to examine differences in stress-related, glucocorticoid (GC) genes between women with PTB and women with term birth (≥37 weeks gestation) in a sample of Black women.

Stress-related, GC genes are involved in the neuroendocrine and hypothalamic-pituitary-adrenal (HPA) axis systems which work synergistically to regulate the stress response and maintain homeostasis. However, repeated stress exposure may lead to endocrine dysregulation that can alter the stress response system (Dirven et al., 2017). During stressful situations, the HPA axis is triggered when stress input is received by the hypothalamus from the limbic system (Lightman and Conway-Campbell, 2010) (See Figure 1). Corticotropin-releasing hormone (CRH) is secreted into the circulation from the periventricular nucleus (PVN) of the hypothalamus and transported via the hypophyseal portal system to the anterior pituitary gland (Timmermans et al., 2019). Arginine vasopressin (AVP) is also expressed in the PVN and subsequently potentiates the effects of CRH in stimulating the secretion of adrenocorticotropic hormone (ACTH) from the anterior pituitary gland (Smith, 2006). Circulating ACTH binds to receptors in the adrenal cortex which stimulates the cyclic adenosine monophosphate (cAMP) pathway leading to

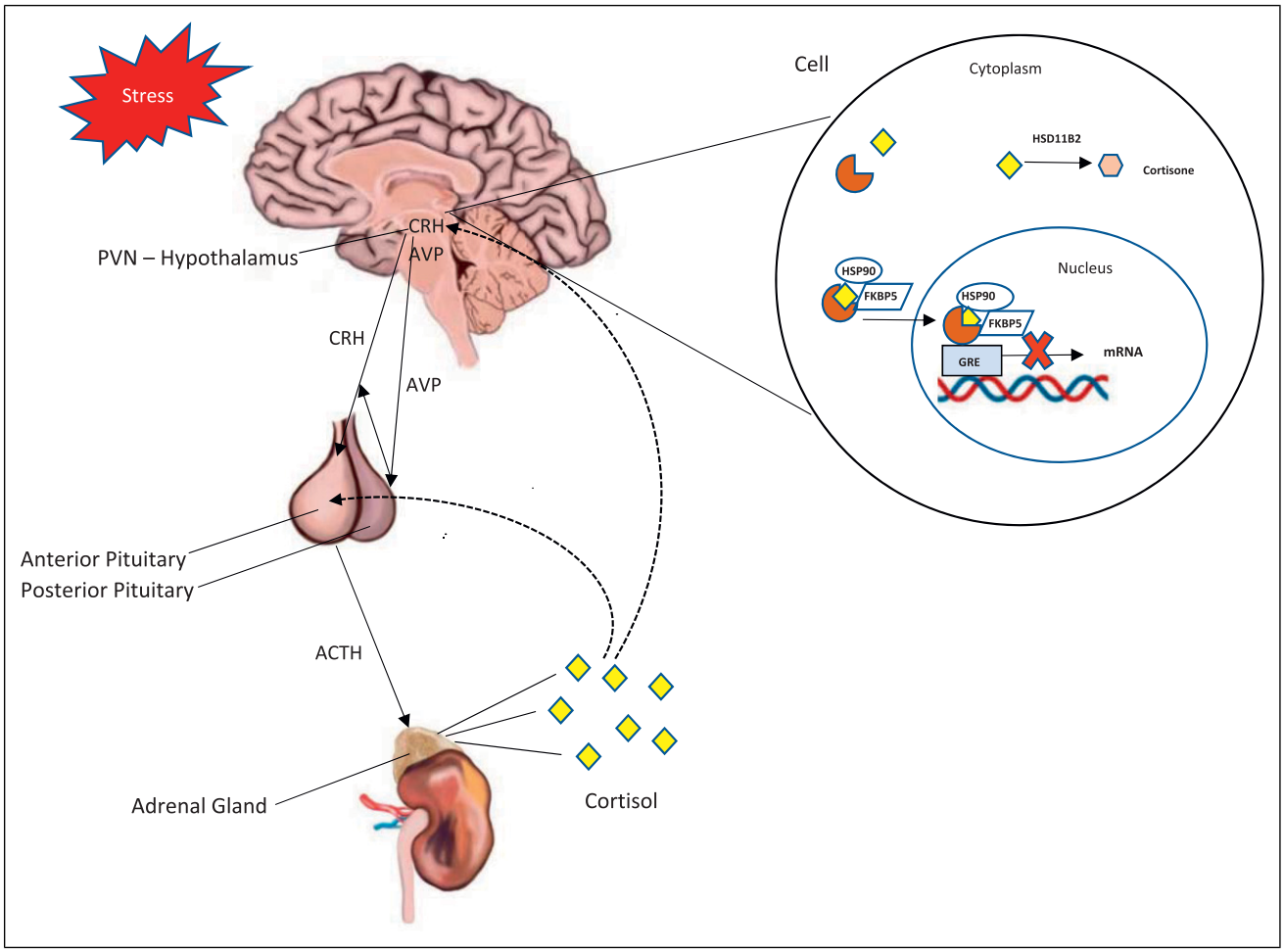

Figure 1. The HPA axis stress response mechanism. During active stress, the HPA axis is triggered when stress input is received by the hypothalamus from the amygdala and the limbic system. Corticotropin-releasing hormone (CRH) is secreted into the circulation from the periventricular nucleus (PVN) of the hypothalamus. Arginine vasopressin (AVP) is also expressed in the PVN and subsequently potentiates the effects of CRH in stimulating the secretion of adrenocorticotropic hormone (ACTH) from the anterior pituitary gland. Circulating ACTH binds to receptors in the adrenal cortex leading to the secretion of GCs (cortisol in humans) into the circulation. GC binds with the glucocorticoid receptor (GR) in the anterior pituitary signaling the start of the negative feedback mechanism.

the secretion of GCs (cortisol in humans) into the systemic circulation where it is able to bind with the glucocorticoid receptor (GR) in the anterior pituitary, signaling the start of the negative feedback mechanism.

The GR (coded by nuclear receptor subfamily 3 group C member 1—*NR3C1*) plays a key regulatory role in the negative feedback response to stress-induced increases in cortisol. The ligand-bound GR forms a complex with FKBP5 (FK506 Prolyl Isomerase 5) and other co-chaperone molecules (e.g., heat shock proteins) initiating a conformational change in the GR, leading to hyperphosphorylation, and translocation into the nucleus (Wochnik et al., 2005). Once in the nucleus, GR binds with glucocorticoid response elements (GREs) and transcription factors (TF) to repress RNA transcription of CRH (Ratman et al., 2013). When functioning properly, the HPA axis neuroregulatory systems counterbalance the stress reaction. However, under conditions of repeated or prolonged stressors, the GR develops resistance to cortisol, decreasing the number of GR-GC ligand binding opportunities, and further dysregulating the HPA axis negative feedback mechanism (Rogac and Peterlin, 2018). CRH binding protein (CRHBP) plays a role in CRH regulation through its binding affinity and removal of active CRH from circulation (Wadhwa et al., 2012). FKBP5, when binding with unliganded GR, decreases its affinity for cortisol, inhibiting the efficiency of GR and making the negative feedback mechanism less effective (Binder, 2009). Moreover, the GR/FKBP5 binding initiates *FKBP5* expression creating a short negative feedback loop and continuing to build GR resistance to cortisol (Vermeer et al., 2003). Limited availability of GR reduces the number of mature GR heterocomplexes for binding, nuclear translocation, and transcription in target genes (e.g., *AVP* and *CRH*) (Binder, 2009). This activity diminishes the response to incoming stressors thereby blunting the HPA axis negative feedback mechanism (Binder, 2009). Another GC regulator, the enzyme hydroxysteroid 11-beta dehydrogenase 2 (HSD11β2) resides in the cellular cytoplasm where it degrades cortisol to its inactive form, cortisone (Smith, 2006). Stress induced DNAm and subsequent GR-induced changes in gene expression dampen the effectiveness of the genomic HPA axis negative feedback mechanism.

The HPA axis has been recognized as a pathway by which psychological stress influences birth timing and gestational length (Ding et al., 2021; Gilles et al., 2018). During pregnancy, maternal serum CRH and ACTH levels increase exponentially with length of gestation and decrease to nonpregnant levels after the birth (Gangestad et al., 2012). The rise in CRH levels is due in part to a rise in estrogen levels and in part to increasing amounts of placental CRH secretion into the maternal bloodstream (Gangestad et al., 2012). Later in pregnancy, the sustained high levels of CRH downregulate the reactivity of the HPA axis resulting in an attenuated stress response with women becoming less physiologically and psychologically sensitive to stress (De Weerth and Buitelaar, 2005; Yu et al., 2018). At the end of pregnancy, changes in receptor affinity and sensitivity increase placental CRH binding with receptors in the myometrium and promote uterine contractility (Sandman and Glynn, 2009). Pregnancy-related HPA axis changes in CRH occur outside of typical HPA axis regulation, increasing the biological complexity of the pathways that mediate maternal psychological stress and PTB.

DNAm at the cytosine bases of CpG dinucleotides may interfere with gene transcription and expression of protein products (Szyf and Bick, 2013). In general, the addition of methyl groups is known to reduce gene expression, while the removal of methyl groups increases gene expression. CpG site location in relation to gene promoters suggests a greater likelihood of gene expression changes. CpG islands (CGI) are at least 200 base pairs (bp) long, rich in CpG dinucleotides, and conservatively unmethylated. CGIs are most often found within proximal promoter regions at the 5′ end of transcription start sites (TSS). Cytosine methylation at TSSs is associated with transcription repression by blocking TF access or by recruiting repressor proteins to block TFs and prevent the assembly of the protein product (Edgar et al., 2014). DNAm also affects gene expression at CpG "shores" and "shelves" which are located up to 2000 bp and up to 4000 bp from CpG islands respectively, both upstream and downstream of the CGIs (Moore et al., 2013; Ohm, 2019). Thus, DNAm and subsequent gene expression at these regions may result in changes in the rate or amount of RNA transcription and the resulting protein synthesis.

In light of the influence of DNAm on gene expression combined with the influence of psychological stress on birth timing, we examined DNAm differences in 6 GC genes between women with PTB and women with term birth. The 6 candidate genes (*AVP, CRH, CRHBP, FKBP5, HSD11β2, NR3C1*) were selected based on their physiologic function in HPA axis activation, downstream effects of cortisol release (see Table 1), or prior research associated with DNAm, gene expression, and birth outcomes (Barcelona de Mendoza et al., 2017; Hong et al., 2018; Togher et al., 2018). We hypothesized that these CG candidate genes would be differentially expressed between women with PTB and women with term birth.

Methods

Design

This nested, case-control study is a subset of the Biosocial Impact on Black Births (BIBB) study. The BIBB study uses a longitudinal design to examine psychosocial factors and systemic inflammation markers (including C-Reactive Protein and pro- and anti-inflammatory cytokines) related to PTB among Black women.

Table 1. Glucocorticoid Pathway Genes and Functions.

HGNC Gene Names	Gene description	Gene function summary
AVP	Arginine vasopressin	Member of the vasopressin/oxytocin family. Posterior pituitary hormone that is synthesized in the PVN of the hypothalamus. Can contract smooth muscle during parturition.
CRH	Corticotropin releasing hormone	Member of the corticotropin-releasing factor family secreted by the PVN of the hypothalamus. Binds to corticotropin releasing hormone receptors and stimulates release of ADHC. Determines length of gestation and timing of parturition.
CRHBP	Corticotropin releasing hormone binding protein	Binds to and inactivates CRH. May prevent inappropriate pituitary-adrenal stimulation in pregnancy.
FKBP5	FK506 binding protein 5	Member of immunophilin protein family. Involved in trafficking of steroid hormone receptors maintaining the receptor in the cytoplasm.
HSD11β2	Hydroxysteroid 11-beta dehydrogenase 2	Responsible for the conversion of cortisol to its inactive form, cortisone.
NR3C1	Nuclear receptor subfamily 3 group C member 1	Glucocorticoid receptor; promotes glucocorticoid responsive genes to activate transcription. Also functions as a transcription factor.

Note. Source: The Human Protein Atlas. Retrieved from https://www.proteinatlas.org

Sample

Women were included in the BIBB study if they self-described as African American or Black, were 18–45 years of age, had a singleton pregnancy, were 8–29 weeks gestation, and spoke and read English language. Women were excluded from the BIBB study if they had multiple pregnancies (e.g., twins), autoimmune disorders, fever at the time of data collection, or were receiving steroid or anti-inflammatory medications. Women were recruited into the BIBB study from prenatal clinics in the metropolitan Detroit, Michigan, and Columbus, Ohio areas. A sample of 618 women were enrolled into the BIBB study prior to the COVID-19 pandemic. Of the 618 women, 44 pregnant Black women [22 women with PTB (due to preterm labor or preterm premature rupture of membranes) and 22 women with term birth] were selected after the births for the current study. Of the 44 women, 32 were recruited from the Columbus, Ohio clinical site and 12 were recruited from the Detroit, Michigan site. Women of any parity were included in the current study if they completed the questionnaires and had blood drawn between 8-18 weeks gestation. This gestational period was selected in order to examine a homogenous sample of DNAm which is likely to fluctuate over the course of pregnancy (Das and Maitra, 2021) and to detect differences in DNAm as early predictors of PTB. Women with PTB were matched with women with term birth on maternal age, smoking status, and history of prior PTB.

Variables and Instruments

Maternal Characteristics. Maternal level of education, marital status, employment, and annual household income were collected by self-report questionnaires as part of the BIBB study. Age, smoking status, gestational age (GA) at birth, GA at the time of data collection, and history of prior PTB were abstracted from the maternal medical records by research assistants.

DNA methylation. DNAm processing was performed using the Illumina Infinium® Methylation EPIC 850K BeadChip array (Illumina, Inc., San Diego, CA) and conducted at Roswell Park Comprehensive Cancer Center Shared Genomics Core (Buffalo, NY). The PAXgene® Blood DNA tubes were thawed and incubated for 2 hours at room temperature to ensure complete lysis of blood cells. The purification then began with adding blood into a processing tube with lysis buffer. The samples were centrifuged to pellet the nuclei and mitochondria, which are washed in the digestion buffer with protease. The resuspended pellet was incubated at 65°C to digest and remove protein contamination. Isopropanol was added and the sample was mixed before being centrifuged to pellet DNA. The remaining contaminants from the pellet were removed with 70% ethanol wash and centrifuged again. The supernatant was removed, and the DNA pellet was dried for 5 mins before being resuspended. DNA was then incubated at 65°C for 1 hour followed by room temperature incubation overnight. Quantitative assessment of the purified genomic DNA was accomplished by using a Qubit Broad Range DNA kit (ThermoFisher Scientific, Waltham, MA). The samples underwent amplification and fragmentation prior to hybridization. EPIC Methylation chip scanning was performed using the Illumina iScan System (Illuminia). Total methylation was determined using CpG site-specific probes and GenomeStudio software (Illumina) calculated the ratio of fluorescent signals from methylated and unmethylated sites. Data were first normalized using background subtraction to minimize the amount of background signal variation between arrays and the average beta scores were then calculated for each CpG site. The values assigned for methylation at CpG

sites range from 0 (no methylation) to 1 (completely methylated) for each cytosine nucleotide. Beta scores served as the basis for comparison between cases and controls. The results identified CpG locations within genes (e.g., TSS1500, CGI).

Procedures

The Institutional Review Boards of the participating universities and clinical sites approved both the BIBB study and the current study. Potential participants at the clinical sites were screened for eligibility. Eligible women were approached by trained research assistants and invited to participate in the study. Women completed an informed consent process prior to participating in the BIBB study. Over the course of their prenatal visits, participants completed self-report questionnaires on computer tablets using QualtricsXM data collection software (Qualtrics International Inc., Provo, UT) and had venous blood drawn for measures of systemic inflammation (e.g., C-Reactive Protein). Additional venous blood samples were collected in PAXgene® DNA in vitro diagnostic (IVD) 2.5 mL tubes (PreAnalytiX Qiagen/BD, Hombrechtikon, Switzerland) for the current study. After blood collection, the PAXgene® DNA tubes were inverted 8–10 times, stored at −20°C for 24–72 hours and then at −80°C until DNA extraction and purification. Trained research assistants collected birth data from maternal medical records. Women received a $30 store gift card as part of the BIBB study.

Statistical Analysis

Data were analyzed using SPSS 27 (IBM, Inc., Armonk, NY). Descriptive statistics were used to describe maternal characteristics. Differences in maternal characteristics between women with PTB and women with term birth were examined using independent samples *t* tests for continuous variables (maternal age, gestational age at data collection, gestational age at birth) and Chi-squared tests for categorical variables (level of education, marital status, employment, annual household income, history of prior PTB, cigarette smoking).

Differential methylation analyses of the 218 CpG sites in the 6 candidate genes between women with PTB and women with term births were performed using a robust linear regression model using the lmFit() function in the limma Bioconductor package (Ritchie et al., 2015). We controlled for potential confounding effects due to maternal age, gestational age at data collection, and smoking history in the models. After the model fittings, the robust Empirical Bayes (EBayes) statistical tests were performed to identify differentially expressed CpG sites. The robust EBayes method has shown to be very effective, especially when the sample size is small (Phipson et al., 2016). We calculated Benjamini-Hochberg adjusted *q*-values for differential methylation between women with PTB and women with term births at each CpG site in order to account for multiple comparisons (Benjamini and Hochberg, 1995). The test significance level was set to a *q*-value threshold of 0.05 level. Women with PTB and women with term births did not differ significantly in cell type proportions. Therefore, cell type heterogeneity was not adjusted in our models. Our data did not show significant batch effects.

Results

Maternal Characteristics

Socio-demographic characteristics and clinical factors for the 44 women are shown in Table 2. The mean maternal age was 27.6 ± 5.4 years. The mean gestational age at birth was 33.6 ± 4.5 weeks for women with PTB and 39 ± 0.9 weeks for women with term births. There were no significant differences in maternal socio-demographic characteristics or clinical factors between the 2 groups.

Differential DNAm

Two hundred eighteen CpG dinucleotide sites were examined for DNAm across the 6 candidate genes. Four CpG sites in 2 of the 6 candidate genes (*CRH and NR3C1)* were differentially methylated ($p < 0.05$) between women with PTB and women with term birth (see Table 3). Two CpG sites (cg16664570, cg19035496) of *CRH* are located in the south shore region, TSS1500 and TSS200 respectively, of an identified CGI. These CpG sites showed increased methylation for women with PTB compared with women with term births. The 2 remaining differentially methylated CpG sites *NR3C1* (GR), cg12741214 and cg25579735, were located within the gene body and 5′UTR respectively and showed a decrease in methylation in women with PTB compared with women with term births. The magnitude of the change in DNAm between women with PTB and women with term births (Δβ) ranged from .004 to 037.

Discussion

This study is novel as, to our knowledge, there are no reports of DNAm analysis of GC candidate genes in maternal blood collected early in pregnancy in a cohort of Black women as a biological indicator of birth timing. We compared DNAm in maternal leukocytes between women who had PTB with women who had term births. We identified 4 differentially methylated CpG dinucleotides in 2 GC pathway genes (*CRH, NR3C1)*. Identified CpG sites included 2 sites with locations at a CGI flanking shore region. DNAm at or near TF binding sites within shore and shelf regions are associated with gene expression changes (Maurano et al., 2015). Two of the differently methylated CpG sites were at gene body and

Table 2. Maternal Characteristics (*N*=44).

Variable	Preterm birth (N = 22)	Term birth (*N* = 22)	*p*-value*
	Mean (SD)	Mean (SD)	
Maternal age (years)	27.95 (5.32)	27.23 (5.48)	.868
GA at data Collection (weeks)	13.5 (3.8)	12.5 (2.9)	.336
GA at birth (weeks)	33.6 (4.5)	39 (0.9)	< .001
	n (%)	*n* (%)	
Level of education			
< High School	14 (63.6)	13 (59.1)	.910
≥ High School	8 (36.4)	7 (31.8)	
Missing		2 (09.1)	
Marital status			
Married or living with partner	13 (46.4)	9 (56.2)	.531
Single	15 (53.6)	7 (43.8)	
Employment			
Employed	11 (50.0)	14 (63.6)	.346
Unemployed	10 (45.5)	7 (31.8)	
Missing	1 (04.5)	1 (04.5)	
Annual household income			
< $10,000	9 (41.0)	7 (31.8)	.808
$10,000 – $29,999	10 (45.5)	10 (45.5)	
≥ $30,000	2 (09.1)	3 (13.6)	
Missing	1(04.5)	2 (09.1)	
History of prior PTB			
Yes	10 (45.5)	10 (45.5)	1.00
No	12 (54.5)	12 (54.5)	
Cigarette smoking			
Never			
Prior to pregnancy	10 (45.5)	10 (45.5)	.687
Prior to and during	9 (40.9)	7 (31.8)	
Pregnancy	3 (13.6)	5 (22.7)	

Note. *Age, GA (gestational age) at data collection, GA at birth - Independent Samples *t*-test, 2-tailed significance. Level of education, marital status, employment, annual household income, history of prior PTB, cigarette smoking - Pearson X^2, 2-sided significance.

Table 3. Differentially Methylated CpG Sites (*N* = 44).

Gene	CpG[a]	CpG location	Chromosomal position	Relation to CpG Island	PTB mean (SD)	Term birth mean (SD)	DNAm[b] Δβ	*t*	*p*-value[c]
CRH	cg16664570	TSS1500	Ch.8:67091580	S. Shore	.821 (.031)	.784 (.034)	.039	5.98	<.001
CRH	cg19035496	TSS200	Ch.8:67090792	S. Shore	.657 (.043)	.635 (.026)	.026	3.77	.033
NR3C1	cg12741214	Body	Ch.5:142695619	Ukn	.882 (.011)	.893 (.013)	-.013	−3.79	.033
NR3C1	cg25579735	5′UTR	Ch.5:142807171	Ukn	.873 (.013)	.885 (.015)	-.013	−3.73	.033

Note. Linear regression/Empirical Bayes.
[a]continuous variable.
[b]Estimated percentage difference in DNAm (Δβ).
[c]Benjamini-Hochberg adjusted *q*-values.

5′UTR locations. Although some researchers have reported that gene body methylation is correlated with increased gene expression and may play a role in regulating gene function (Jones, 2012), more research is needed to gain a clearer understanding of the methylation influence on gene bodies and UTRs.

We identified 2 differentially methylated CpG sites, cg16664570 and cg19035496, at the S. shore region of

CRH. Stress-induced epigenetic changes may modulate gene expression and subsequent levels of both GC gene products (e.g., CRH) and cortisol. The "placental clock" theory posits that the stress-induced, HPA axis cascade downregulates the normally-expected rise in maternal cortisol but upregulates placental CRH potentially initiating the start of labor (Sandman et al., 2006). Indeed, Ruiz et al. (2016) measured CRH levels in maternal blood and found that increased levels of CRH predicted PTB, particularly for Black American women. High levels of circulating CRH may lead to increased adrenal cortisol secretion. Studies have reported that increased levels of maternal cortisol mediate the association between different types of stressors and PTB (Gillespie et al., 2017; Hoffman et al., 2016). We report that CpG site cg16664570 (*CRH*) is significantly differentially methylated ($p < 0.001$) between women with PTB and term birth. The same CpG site has previously been reported to be related to maternal stress (Kertes et al., 2016). The authors of that study examined 24 mother-newborn pairs in the Democratic Republic of Congo and found that DNAm of cg16664570 predicted maternal stress from war trauma. DNAm of *CRH* at this CGI promoter region warrants further examination and may hold promise as a biological indicator of the relationship between maternal stress and birth timing.

We also identified 2 differentially methylated CPG sites in the gene body and 5′UTR of the *NR3C1* gene (GR) showing reduced methylation in women with PTB compared to women with term birth. Altered DNAm levels at the promoter region of *NR3C1* have been found in mothers exposed to various levels and types of stress during pregnancy in a number of studies (Palma-Gudiel et al., 2015). For example, Hompes et al. (2013) reported that maternal anxiety was related to DNAm status of *NR3C1* in 83 cord blood samples. The authors suggest that DNAm at the location of CpG sites within the exon 1D (*NR3C1*) region may be particularly susceptible to environmental influence (Hompes et al., 2013). These findings suggest that the *NR3C1* gene may be promising as a component gene of the biological pathway between maternal psychological stress and birth outcomes. Taken together, the results of this study add to the evidence that stress-induced epigenetic modifications of GC genes dysregulate the HPA axis and potentially alter birth timing.

Strengths and Limitations

This study is the first to analyze GC candidate genes in maternal blood in a cohort of Black women as an indicator of birth timing. Strengths of this study include its prospective design that allowed for the collection of biological samples prior to the births and may provide evidence key to revealing potential biomarkers of PTB. Identifying women who meet the criteria for increased risk of early parturition would allow for the immediate initiation of treatment for those at risk for PTB. The study is limited by its small sample size and should be interpreted with caution. In addition, the study used a candidate gene approach to identify methylation markers associated with PTB. Interested readers can refer to Mansell et al., 2019 for general statistical considerations in sample size, design, and analyses of epigenome-wide association studies. While *p*-values were statistically significant, the magnitude of change between women with PTB and women with term births was relatively small. However, these results are encouraging in that they are consistent with findings of other studies examining DNAm and PTB or maternal prenatal stress. Our study recruited Black women participants based on self-report race information. Studies show that the Black population have a complex genetic structure. Due to lack of GWAS SNP data and underdeveloped methods for inferring genetic ancestry from DNA methylation data, we did not control for genetic ancestry estimation in the analyses. Methylation of CpG dinucleotides at transcription start sites is associated with gene expression changes. While DNAm changes occurred in regions that may alter gene expression, the degree to which the locations or change in methylation influence expression is not known. Thus, gene expression analysis of DNAm findings is needed to determine the biological significance of the methylation differences at these CpG sites. This study lends itself to future work that includes gene expression analysis, replication, and application to larger sample sizes.

Conclusion

Glucocorticoid pathway regulatory genes are associated with birth timing. Developing an understanding of how psychosocial and biological stressors affect DNAm of glucocorticoid production and regulation is a crucial link to determining the pathways by which maternal psychological stress relates to PTB. Confirmation of the underlying physiology and subsequent development of a biomarker panel would allow for early identification of women at risk for PTB. Potential interventions include nutritional modification of nutrients that influence methylation status including folate, vitamin B12, Vitamin B6, and choline as well as social and psychological therapeutic modalities to decrease stress and reduce the incidence of PTB and neonatal morbidity and mortality among Black Americans.

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