Early pregnancy DNA methylation in stress–related genes and birth outcomes in mothers with mental health diagnoses

Elizabeth L. Minnick

Undergraduate BSN Honors Student

minnick.35@osu.edu

The Ohio State University College of Nursing

Shannon L. Gillespie

Assistant Professor

gillespie.175@osu.edu

The Ohio State University College of Nursing

Cindy M. Anderson

Professor

The Ohio State University College of Nursing

anderson.2765@osu.edu

Corresponding Author:

Cindy M. Anderson

The Ohio State University

College of Nursing

1585 Neil Avenue

Columbus, OH 43215

Phone: 614-292-4179

Fax: 614-292-4948

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Abstract

Background. Maternal mental health influences the intrauterine environment, triggering epigenetic changes at a critical time of embryonic and fetal development through DNA methylation (DNAm) in cytosine-guanine (CpG) sites that alter gene expression.

Purpose: The purpose of this study was to examine birth timing/birth weight and differences in DNAm in stress-related genes (*NR3C1, HSD11B2,* and *FKBP5*) in first trimester maternal blood from pregnant mothers with/without mental health diagnoses. **Methods.** A prospective convenience sample of nulliparous women (n=47) were recruited in the first trimester of pregnancy. Genome-wide DNA methylation was quantified in peripheral white blood cells. Maternal history of depression and/or anxiety and birth outcomes were determined by medical record abstraction. Linear regression models examined associations among mental health diagnoses, DNAm at individual *NR3C1*, *HSD11B2,* and *FKBP5*-associated CpG sites, birth timing, and birth weight, controlling for early pregnancy weight and infant sex. **Results.** A mental health diagnosis was associated with DNAm in CpGs associated with *NR3C1* (ten CpGs), *HSD11B2* (three CpGs), and *FKBP5* (eight CpGs) ($p<0.05$). After adjustment for multiple comparisons, the association between anxiety and DNAm at *NR3C1*-associated Cg12466613 remained significant ($p<0.001$). Mental health diagnoses were not associated with birth timing/birth weight. DNAm at four *NR3C1*-associated CpGs was significantly associated with birth weight ($p\leq0.038$). **Conclusions.** DNAm is altered in early pregnancy among women with mental health diagnoses, supporting epigenetic influences during development and association with birth outcomes. Promoting women's mental health prior to pregnancy has potential to foster the well-being and long-term health of the mother-infant dyad.

Key Words: Epigenetics, Pregnancy, Depression, Anxiety

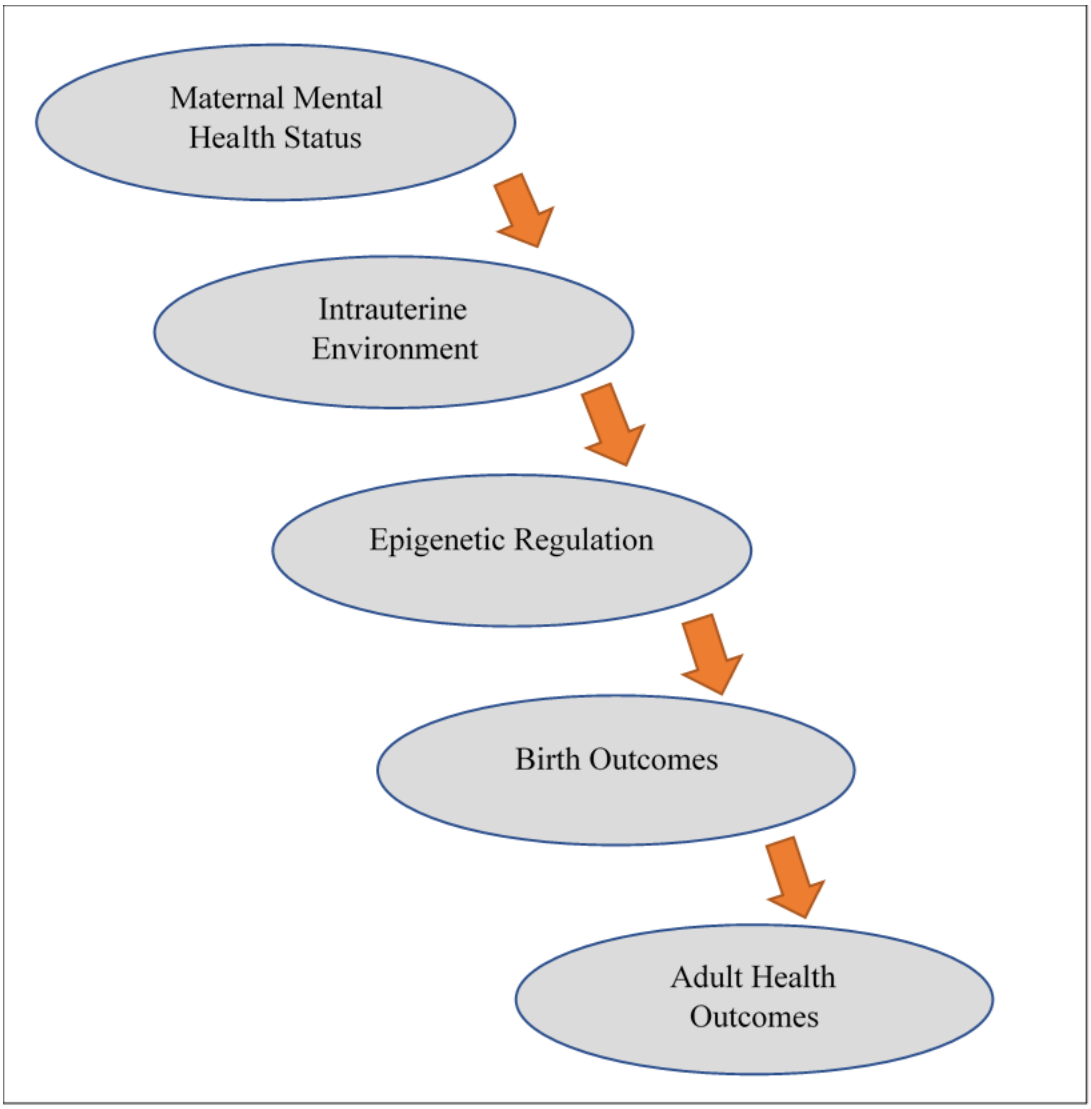
Introduction

Mental illness is a widespread health crisis within the United States. In the 2019 National Survey on Drug Use and Health, 20.6% of American adults reported having experienced a mental illness within the past year (SAMHSA, 2020). Pregnant women are a particularly vulnerable population to the effects of mental illness, with potential consequences for both mother and child. Prenatal mental illness is linked to adverse birth outcomes, including low birth weight and preterm birth (Ciesielski et al., 2015; Uguz et al., 2019). In a recent study, the offspring of mothers with mental health diagnoses were more likely to have a low birth weight (Ciesielski et al., 2015), with poor growth reported among 62% of the infants born to mothers with prenatal mental illness compared to 29% of the infants born to mothers without prenatal mental illness (Ciesielski et al., 2015). Preterm birth was also more common among infants born to mothers with mental illness (Uguz et al., 2019). Moreover, mothers with concurrent major depression and anxiety were most likely to have a preterm birth in comparison to mothers with only depression, only anxiety, or no mental illness (Uguz et al., 2019).

While it is known that maternal mental illness and adverse birth outcomes are associated, the biological underpinnings for this association are uncertain (Braithwaite et al., 2015). The Developmental Origins of Health and Disease (DOHaD) hypothesis provides a framework for epigenetics as a potential impetus for this relationship (Monk et al., 2016; Mulligan et al., 2012; Sosnowski et al., 2018). According to the DOHaD hypothesis, the conditions which affect the intrauterine environment impact fetal development and lead to implications for the health and development of offspring throughout their lifetime (Barker, 1995). As shown in Figure 1, the DOHaD hypothesis provides a potential explanation by which prenatal maternal mental illness is associated with poor birth outcomes in the infant.

Figure 1

Developmental Origins of Health and Disease Pathway: Maternal Mental Health and Offspring Development



Note. Following the DOHaD hypothesis, maternal mental health status may create conditions that impact the intrauterine environment, potentially mediating differential fetal DNAm. These early DNAm patterns could lead to differential birth outcomes that have implications for long term offspring health.

Epigenetic alterations, specifically DNA methylation (DNAm), provide a putative explanation underlying the biological link between maternal mental health and birth outcomes. Epigenetic changes include heritable DNA modifications influenced by the environment that do not alter the genetic code itself, leading to differential gene transcription and function (Moore et al., 2013; Zhang et al., 2020). DNAm is one of the most studied examples of epigenetic modification. DNAm involves the addition of a methyl group (CH_3) to a cytosine base, typically preceding a guanine base (CpG site) (Moore et al., 2013). The amount and location of DNAm along the genetic sequence determines the impact on gene transcription. For instance, increased DNAm within the promoter region of a gene can lead to silencing of that gene so that its transcription is essentially "turned off" (Zhang et al., 2020).

DNAm is active in development (conception, embryonic and fetal) and disease, influenced by endogenous and exogenous factors (Zhang et al., 2020). The effects that DNAm can have on gene transcription may lead to significant ramifications for phenotypic characteristics (Zhang et al., 2020). The first trimester of gestation, which includes the process of organogenesis during fetal development in which lasting physiologic changes are made, is especially sensitive to the impacts of DNAm on tissue differentiation. Consistent with the DOHaD hypothesis, the intrauterine environment mediates differential DNAm patterns within the fetal genome (Barker, 1995). Maternal health status, including mental health diagnoses, may impact the intrauterine environment, potentially altering DNAm patterns within the fetal genome. In addition to the profound impact that DNAm can have on fetal development, DNAm represents an important mechanism in disease formation that can impact the future health of the offspring. Differential DNAm patterns associated with diseases may serve as biomarkers, utilized to inform disease prevention, identification, and treatment (Zhang et al., 2020). There is

increasing evidence supporting associations between differential DNAm patterns and mental health diagnoses, representing a potential biomarker for mental health diagnoses (Moore et al., 2013). As the association between mental health diagnoses and DNAm is better understood, researchers are looking to elucidate how differential DNAm patterns of women with mental health diagnoses may be linked to altered fetal development and birth outcomes. However, the question remains as to which genes and genetic pathways are subject to DNAm changes in the presence of maternal mental illness.

Identifying potential gene targets that are susceptible to differential DNAm when exposed to maternal mental illness is important in determining the associations among DNAm, maternal mental health, and adverse birth outcomes. While gene specific maternal DNAm alterations associated with maternal mental illness and adverse birth outcomes are not known, stress pathway genes have been implicated. In pregnancies complicated by mental health diagnoses, findings of increased DNAm of *NR3C1*, a glucocorticoid receptor gene, in newborn and placental DNA, respectively, have been reported (Braithwaite et al., 2015; Conradt et al., 2013). Prenatal maternal anxiety and stress, respectively, were associated with increased DNAm of *HSD11B2*, which helps modulate active cortisol levels within the body (Conradt et al., 2013; Monk et al., 2016). Increased DNAm of *FKBP5*, which assists in steroid transportation, has been found in mothers with prenatal stress (Monk et al., 2016). Therefore, these three stress-related genes—*NR3C1*, *HSD11B2*, and *FKBP5*—were selected for examination of maternal DNAm in early pregnancy in this current study among women with and without mental health diagnosis (see Table 1). The purpose of this study was to examine birth timing/birth weight and differences in DNAm in stress-related genes (*NR3C1*, *HSD11B2* and *FKBP5*) in first trimester maternal

peripheral white blood cells from pregnant mothers with or without depression and/or anxiety mental health diagnoses.

Table 1

Target Gene Function

	Target Genes		
	NR3C1	*HSD11B2*	*FKBP5*
Chromosome Number	5	16	6
Gene Function	Encodes glucocorticoid receptor	Controls active cortisol levels	Helps transport steroids

Materials and methods

Research design

This study is a secondary analysis of the parent study designed to identify distinct DNAm patterns in women during early pregnancy that may predict preeclampsia (Anderson et al., 2014). The study was approved by the Institutional Review Boards of the University of North Dakota and the linked hospital network. During the study, safety and protection of participants was ensured.

Sample and setting

In the parent study, a convenience sample of nulliparous pregnant women was recruited prospectively in the first trimester and followed longitudinally through birth. Women were invited to participate in the study through community recruitment using televised advertisements and distributed publications. Eligibility criteria included: no prior births; age 18 or over; English speaking; singleton pregnancy of less than 14 weeks gestation; and plan to deliver at the local community hospital. Exclusion criteria included: prior completed pregnancy; less than 18 years

of age; and pregnancy with more than one fetus. Upon meeting inclusion criteria, subjects were invited to participate in the study. Women whose pregnancies terminated prior to 20 weeks gestation were dismissed.

Data collection

Participants met with research staff at a site in the upper Midwest during the first trimester (10-14 weeks gestation). After informed consent, venipuncture was completed and whole blood was collected in EDTA coated collection tubes. After birth, medical records were reviewed in detail, and obstetric and birth outcome data were abstracted. Variables reviewed included maternal mental health diagnoses, maternal age (years), maternal first trimester weight (pounds), gestational age at birth (days), birth weight (grams), and offspring sex.

Measurement

DNA methylation

White blood cells were isolated from buffy coat in samples used for DNAm analysis as previously described (Anderson et al., 2014). DNA purification was performed using phenol-chloroform-isoamyl alcohol, yielding 3 μg in each analytic sample. Isolated DNA samples were analyzed for genome-wide DNAm analysis using the Illumina Infinium DNAm 450K bead-based array at the University of Minnesota. The GenomeStudio DNAm program was applied to analyze DNAm at individual CpG sites across the genome. DNAm was quantified and reported as percentage of methylation at each of the >450,000 individual CpG sites. In this secondary analysis, DNAm in CpGs from the candidate genes are reported including *NR3C1* (41 CpGs), *HSD11B2* (13 CpGs) and *FKBP5* (32 CpGs).

Mental health diagnoses

Prenatal maternal mental health status was obtained from participants utilizing medical record abstraction. Participants with available mental health data were sorted into two groups – those with and without mental health diagnoses. The participants with a mental health diagnosis were then further sorted into three subcategories according to diagnosis: anxiety, depression, and psychiatric. The psychiatric subcategory was understood as designating a diagnosis of anxiety and/or depression when the participants had "depression/anxiety," "anxiety/depression," or "anxiety depression" listed in their medical record. Participants with past diagnoses were not included in the group with mental health diagnoses. Only current mental health diagnoses were included in the group with mental health diagnoses. Other mental health diagnoses, like Oppositional Defiant Disorder, were not included in the group with mental health diagnoses.

Birth outcomes

Medical record abstraction of data related to pregnancy outcome and neonatal status were collected, serving as the source for data related to birth outcomes, including birth weight and gestational age. Birth weight of the offspring was determined after the mother gave birth and is recorded in grams (g). Low birth weight was understood to be less than 2,500 g. Gestational age at birth was calculated as the number of days from the day of conception until the day of birth. A preterm birth was understood to occur when the gestation age at birth was less than 259 days (37 weeks).

Statistical Analysis

Descriptive statistics were used to describe sample characteristics. Multivariable linear regression models were built to examine associations among DNAm at each individual CpG sites in each of the candidate genes, maternal mental health diagnoses, birth weight, and gestational age at birth. Each model controlled for maternal weight at the first trimester visit and fetal sex.

The Liu method was used to adjust for multiple comparisons (Liu, 2013; Unal, 2017). Significance was determined at $p<0.05$.

Results

Participant characteristics

For this current study, we conducted analyses among participants with availability of primary variables, including DNAm (n=50), documentation of mental health diagnosis (n=51), and birth weight and gestational age at birth (n=50). All women in the study reported race and ethnicity as white, non-Hispanic. Women with a mental health diagnosis (n = 16) included women with anxiety and/or depression if not specified (n = 3), anxiety specifically (n = 3), and depression specifically (n = 10). Women without a mental health diagnosis (n = 35) did not report an active diagnosis. Participant characteristics are described in Table 2.

Table 2

Participants Characteristics

	Mental Health Diagnosis	
	Yes (n = 16)	No (n = 35)
Maternal Age (years)	24.23 ± 4.44	25.86 ± 5.73
Maternal Prenatal Weight (pounds)	184.88 ± 44.29	154.30 ± 49.30
Offspring sex (male)	12 (75%)	13 (39%)
Gestational age at birth (days gestation)	274.75 ± 8.61	274.40 ± 11.21

Birth weight (grams)	3331.19 ± 434.61	3264.97 ± 610.11)

Note. Numerical data given are means ± standard deviation or count (frequency), as appropriate. Mental health diagnosis status refers to documentation of prenatal diagnosis of mental illness in the medical record.

Maternal mental health and DNA methylation

As shown in Table 3, diagnoses of depression and/or anxiety, depression specifically, and anxiety specifically were associated with percent DNAm at ten *NR3C1*-associated, three *HSD11B2*-associated, and eight *FKBP5*-associated CpGs, controlling for first trimester maternal weight and fetal sex. After adjustment for multiple comparisons, the association between anxiety and DNAm at *NR3C1*-associated Cg12466613 remained significant (p<0.001).

Table 3

DNAm and Mental Health Diagnosis

Gene	Mental Health Diagnosis		
	Psychiatric[a]	Depression	Anxiety
NR3C1 (n=41 cg)	cg12466613 (0.008)	cg13648501 (0.037)	cg06521673 (0.040)
Chromosome 5	cg13648501 (0.025)		cg07589972 (0.024)
			cg12466613 (0.000)[b]
			cg18484679 (0.027)
			cg25535999 (0.030)
			cg26464411 (0.002)
			cg27122725 (0.013)

HSD11B (n=13 cg)	cg20981893 (0.045)		cg04750517 (0.008)
Chromosome 16			cg10686375 (0.017)
FKBP5 (n= 32 cg)	cg00140191 (0.024)	cg08586216 (0.053)	cg00862770 (0.010)
Chromosome 6	cg07061368 (0.013)		cg2665568 (0.021)
			cg07061368 (0.022)
			cg17085721 (0.023)
			cg19014730 (0.023)

* Numbers in parentheses correspond to the p value ($p \leq 0.05$) rounded to the nearest thousandth.

[a] Psychiatric includes diagnosis of depression and/or anxiety; [b] One CpG site with a significant *p* value following multiple comparison analysis.

Maternal mental health and birth outcomes

Maternal mental health diagnoses were found to not be significantly associated with gestational age at birth and birth weight ($p<0.05$). The statistical relationships between maternal mental health diagnoses and birth outcomes are displayed in Table 4.

Table 4

Mental Health Diagnosis and Birth Outcomes

Birth Outcome	Mental Health Diagnosis		
	Psychiatric[a]	Depression	Anxiety
Birth Weight	0.699	0.550	0.837
Gestational Age	0.904	0.913	0.987

* Numerical values represent *p* values rounded to the nearest thousandth ($p<0.05$).

[a] Psychiatric represents anxiety and/or depression.

DNA methylation and birth outcomes

The 21 CpGs associated with candidate genes that were determined as potentially responsive to perturbations in mental health were analyzed further for the prediction of birth outcomes. Of the eight individual CpGs associated with maternal mental health diagnosis, DNAm at four *NR3C1*-associated CpGs (cg06521673, cg18484679, cg2553599, cg2646441) was significantly associated with birth weight ($p \leq 0.038$) controlling for gestational age at birth, fetal sex, and first trimester maternal weight (Table 5).

Table 5

Associations between Responsive NR3C1 CpGs and Birth Weight

NR3C1-Associated CpGs	*P* Values[*]
cg06521673	0.020[a]
cg07589972	0.105
cg12466613	0.532
cg13648501	0.165
cg18484679	0.038[a]
cg25535999	0.006[a]
cg26464411	0.018[a]
cg27122725	0.112

[*] *P* values rounded to the nearest thousandth ($p<0.05$)

[a] Significant *p* values

Discussion

Maternal mental health diagnoses have long been understood to be linked to adverse birth outcomes. However, the guiding biological process behind this relationship has remained elusive. Researchers have begun examining the role of epigenetics in pregnancies complicated by maternal mental illness. In this study, DNAm of three stress-related target genes was examined among mothers with and without a mental health diagnosis. Maternal leukocyte DNA was collected during the first trimester and analyzed for DNAm quantification using the Illumina Infinium 450K bead-based array. Statistical analysis was focused on DNAm levels of the identified CpG sites of the target genes: *NR3C1*, *HSD11B2*, and *FKBP5*. Associations were examined among DNAm at target gene-associated sites, birth weight, gestational age at birth, and maternal mental health status using multivariable linear regression and multiple comparison analysis.

Multivariable linear regression models were created to explore if there were significant associations between DNAm of *NR3C1*, *HSD11B2*, and *FKBP5* and adverse birth outcomes. Associations were examined among the 21 CpG sites potentially sensitive to maternal mental health status and gestational age at birth and birth weight. These models did not find significant associations between DNAm levels of the target genes' associated CpG sites and gestational age at birth. However, DNAm at four *NR3C1*-associated CpG sites (cg06521673, cg18484679, cg25535999, cg26464411) was found to be significantly associated with birth weight ($p<0.05$). This finding is notable because it supports that DNAm changes within *NR3C1* could impact birth weight. Similarly, in one study, DNAm of newborn *NR3C1* was strongly correlated with birth weight and maternal stress exposure (Mulligan et al., 2012). Multivariable linear regression models found that DNAm at ten *NR3C1*-associated CpG sites was significantly associated to maternal mental health status (see Table 3). Thus, if a maternal mental health diagnosis could

affect DNAm levels at *NR3C1*-associated CpG sites, then it could also potentially impact birth weight in offspring of mothers with a mental health history.

Statistical analysis using multivariable linear regressions found that DNAm of 21 CpG sites associated with *NR3C1*, *HSD11B2*, and *FKBP5* was significantly associated to maternal mental health diagnoses (see Table 3). However, only one of these associations remained significant to multiple comparison testing. This was the *NR3C1*-associated CpG site cg12466613 ($p<0.001$). This finding demonstrates a strong association between maternal mental health and DNAm of *NR3C1* at this CpG site. While it is unlikely that differential DNAm at one CpG site would alter gene transcription, this significant association supports that maternal mental health may lead to differential DNAm of stress-related genes in maternal leukocyte DNA.

Differential DNAm of *NR3C1*, *HSD11B2*, and *FKBP5* of maternal leukocyte DNA could lead to alterations in the stress-response pathway for the mother. All three of these target genes have integral roles in the regulation of glucocorticoids, which are hormones that influence the body's physiologic reaction to stress. Thus, differential DNAm of these target genes potentially mediated by exposure to maternal mental health diagnosis could lead to glucocorticoid dysregulation. This impaired stress-response pathway could be a vital contributor to the link between maternal mental health status and adverse birth outcomes.

This current study has unique implications because it considers how maternal mental health status may impact maternal leukocyte DNAm status in the first trimester. Most other researchers investigating the impact of maternal mental health status on epigenetics and birth outcomes have focused their inquiry on DNAm status during late pregnancy. Prior research has examined third trimester maternal DNAm levels of *NR3C1* in relation to maternal mood status, finding no correlation between DNAm and mood (Oberlander et al., 2008). These same

researchers, however, found that infant DNAm of *NR3C1* was potentially responsive to maternal mood status (Oberlander et al., 2008). Similarly, in a study by Mulligan et al. (2012), post-natal maternal DNAm of *NR3C1* was not significantly associated to maternal stress levels while newborn DNAm of *NR3C1* was significantly associated.

It is important to discuss the strengths and limitations of this current study to validate the conclusions and inform future research. This current study utilized multivariable linear regression to determine if there were statistically significant associations among maternal mental health diagnoses, birth outcomes, and DNAm. To examine these associations more stringently, multiple comparison analysis was performed. All statistical analyses controlled for maternal weight at the first trimester visit and fetal sex. While this thorough statistical approach provides strength and confidence to this study, there still are several limitations to be noted. The sample group is small and lacks diversity, potentially hindering generalizability of the results. Further, this study relied on medical record abstraction to classify maternal mental health diagnoses. Future studies should utilize validated scales to assess anxiety and depression in mothers during the first trimester to determine their mental health status more accurately and thoroughly. Lastly, this study was not able to attain sufficient placental samples from the subjects to assess for differential placental DNAm. This is an area that future research can expand upon to research how placental DNAm is associated with maternal mental health status, maternal leukocyte DNAm, birth weight, and gestation age at birth.

Adverse birth outcomes, like low birth weight and preterm birth, can have profound impacts on the immediate and long-term health of offspring. Pregnancies complicated by maternal mental illness are known to have higher incidence of low birth weight and preterm birth. Unfortunately, the prevalence of mental illness within the United States has been steadily

increasing over the past decade (SAMHSA, 2020). Therefore, to prevent adverse birth outcomes occurring due to the impact of maternal mental health status, the healthcare system must consider the potential role that DNAm may have in mediating this relationship. There needs to be further research regarding differential DNAm levels of stress-related genes in pregnancies complicated by mental illness, particularly relating to DNAm during the first trimester. The first trimester of pregnancy is a pivotal period for fetal development. Therefore, potential epigenetic alterations occurring during this time could lead to lasting implications for fetal and long-term development and health outcomes. Clinicians have a responsibility to better understand this relationship between maternal mental health and birth outcomes so that healthy pregnancies can be achieved for all.

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