Distribution Agreement

In presenting this thesis or dissertation as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis or dissertation in whole or in part in all forms of media, now or hereafter known, including display on the world wide web. I understand that I may select some access restrictions as part of the online submission of this thesis or dissertation. I retain all ownership rights to the copyright of the thesis or dissertation. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.

Signature:

_____ _____

Martina Albano Date

Approval Sheet

Exploring the Association of Maternal Socioeconomic Adversity, Epigenetics of Genes Regulating Fetal Glucocorticoid Intrauterine Exposure, and the Risk of Small for Gestational Age (SGA) in Term Infants

By

Martina Albano

MPH

Epidemiology

Carmen J. Marsit, PhD

Committee Chair

Exploring the Association of Maternal Socioeconomic Adversity, Epigenetics of Genes Regulating Fetal Glucocorticoid Intrauterine Exposure, and the Risk of Small for Gestational Age (SGA) in Term Infants

By

Martina Albano

MPH

B.M., Berklee College of Music, 2018

Thesis Committee Chair: Carmen J. Marsit, PhD

An abstract of

A thesis submitted to the Faculty of the

Rollins School of Public Health of Emory University

in partial fulfillment of the requirements for the degree of Master in Public Health in Epidemiology

2024

Abstract

Exploring the Association of Maternal Socioeconomic Adversity, Epigenetics of Genes Regulating Fetal Glucocorticoid Intrauterine Exposure, and the Risk of Small for Gestational Age (SGA) in Term Infants

By

Martina Albano

Socioeconomic status has been linked to adverse birth outcomes including reduced birth weight, and may have these effects because of impacts on maternal stress. This study examines the association between maternal socioeconomic adversity, epigenetic regulation of a stress response related pathway, and the risk of infants being born Small for Gestational Age (SGA). We hypothesize that socioeconomic adversity, quantified through a computed cumulative score that considered employment, tobacco use, marital status, household crowding, and education, may impact placental DNA methylation (DNAm) patterns of genes regulating fetal glucocorticoid exposure (HSD11B2, NR3C1, FKBP5), contributing to the occurrence of SGA. The findings revealed that, after adjusting for confounders, infants born to mothers with a higher cumulative socioeconomic adversity score have 2.53 (95% CI 1.30-4.93) times the odds of being classified as SGA compared to infants of mothers with a lower score. A significant association was also found between maternal socioeconomic adversity score and differential methylation at specific CpG sites within all three target genes. Significant DNAm patterns at CpG within the NR3C1 and FKBP5 genes were also associated with SGA risk. These findings hint at the potential for placental epigenetic regulation as a biological mechanism by which socioeconomic adversity influences birth outcomes.

Exploring the Association of Maternal Socioeconomic Adversity, Epigenetics of Genes Regulating Fetal Glucocorticoid Intrauterine Exposure, and the Risk of Small for Gestational Age (SGA) in Term Infants

By

Martina Albano

B.M., Berklee College of Music, 2018

Thesis Committee Chair: Carmen J. Marsit, PhD

A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of Master in Public Health in Epidemiology

2024

Introduction

At birth, infants are classified based on the level of appropriateness of their growth and development in utero as Adequate, Small, or Large for Gestational Age. Children born Small for Gestational Age (SGA), falling below the 10th percentile on a normative growth curve, face an increased risk for several health outcomes in adulthood, including coronary artery disease, hypertension, obesity, and insulin resistance [1]. Consequently, SGA emerges as a critical public health issue, given its important implications for the lifelong health of affected individuals.

In line with the Theory of Developmental Origin of Health and Diseases, which suggests that the intrauterine experiences influence individuals' risks for diseases in adulthood, SGA likely itself is not causal to that eventual disease risk. Instead, it represents the culmination of events of an adverse developmental environment in the womb which can be an important risk factor to address and serve as a target for prevention.

Socioeconomic status (SES), as measured by factors such as education, income, and occupation, is a critical predictor of various health issues across the life course [2, 3]. The impact of SES disparities is particularly evident in its association with adverse birth outcomes, with a well-documented association between maternal low SES and the risk for low birth weight [4-7]. Socioeconomic disadvantage often manifests as an accumulation of adversities, such as poverty, limited education, elevated stress levels, and inadequate nutrition, which collectively influence maternal physiological health [8]. These factors also contribute independently to an increased risk of Small for Gestational Age (SGA) [5, 8-10].

The role of stress stemming from socioeconomic adversity is significant in understanding the relationship between SES and health outcomes. In the context of this paper, stress is defined as an exposure that triggers the activation of the hypothalamic–pituitary–adrenal (HPA) axis, a

crucial component of the body's stress response system. Individuals from low SES backgrounds experiencing higher degrees of socioeconomic adversity and stress attributable to factors such as financial strains, insecure employment, and stressful life events that contribute to the impact that SES has on health [2]. Socioeconomic adversity can therefore be considered a chronic stressor that has the potential to increase the strain on biological regulatory mechanisms and lead to the dysregulation of the HPA axis, with cortisol, a key stress hormone, as a primary marker of this dysfunction [11]. Individuals from low SES backgrounds may experience dysregulations of the hypothalamic-pituitary-adrenal (HPA) axis and higher basal levels of stress hormones. The association between socioeconomic status and higher basal levels of stress hormones has been identified independently from race, age, gender, and BMI [12]. Furthermore, stress during the prenatal period is associated with a dysregulation of the fetal glucocorticoid response pathway, potentially resulting in increased glucocorticoid exposure for the developing fetus [13]. Studies have found that low income, used as a measure of SES, is linked to elevated amniotic fluid glucocorticoid concentrations, underlying the role of SES in this process [14].

To understand the mechanisms involved in the dysregulation of the fetal glucocorticoid response pathway, it is essential to acknowledge the role of the placenta in fetal development. The placenta serves as an interface between the mother and the fetus. Its primary functions are the transfer of essential nutrients and oxygen from the maternal bloodstream to the fetus, and the protection of the fetus from a range of potential adverse factors such as harmful substances, toxins, and pathogens. Acting as a protective barrier, the placenta regulates the fetal hormone levels, shielding the fetus from maternal hormonal fluctuations that could negatively impact fetal development.

One of the mechanisms involved in the protection of the fetus from elevated maternal stress hormones is the enzyme HSD11B2, which converts maternal cortisol into cortisone, rendering it biologically inactive for the fetus. Through this inactivation, the fetus is shielded from the potential adverse effect of high maternal cortisol levels. The expression of the HSD11B2 enzyme is dynamic and changes in response to different maternal hormone levels. Studies have found evidence of downregulation and lower activity of the HSD11B2 enzyme in mothers with prenatal anxiety and depression[15, 16]. A lower enzymatic activity could result in more cortisol passing through the placental barrier exposing the fetus to potentially harmful levels of cortisol. Additionally, if the glucocorticoid regulatory pathway is overwhelmed, for example during acute or chronic maternal prenatal stress, both the fetus and the placenta might be negatively impacted and their growth restricted. This harmful process might predispose the fetus to a greater risk of disease later in life [17]. Given the pivotal role of the placenta in fetal development, prenatal stress's impact on placental function is a significant factor in the risk for adverse birth outcomes.

The associations between prenatal maternal socioeconomic adversity and DNA methylation patterns in the placenta have also been documented. DNA methylation, a process governing gene expression, is susceptible to modification by environmental exposures. Placental DNA methylation patterns appear to be sensitive to the influences of socioeconomic adversity as found in both epigenome-wide studies [7] and target gene studies[18]. This association might be partially explained by the impact of SES on oxidative stress and inflammatory cellular processes [19]**.** Under acute stress conditions and excess glucocorticoids, genes involved in regulating fetal glucocorticoid exposure may undergo differential regulation to protect the developing fetus [20]. Differential DNA methylation patterns in genes involved in the regulation of fetal glucocorticoid

exposure may also be linked to birthweight [21]. In this study, three specific genes have been selected for investigation, each playing an important role in the regulation of fetal glucocorticoid exposure, a fundamental process for the developing fetus's well-being. HSD11B2, described above for its role in cortisol inactivation, has been found to be differentially methylated according to maternal prenatal socioeconomic adversity [18]. NR3C1, which encodes the glucocorticoid receptors to which cortisol binds to induce downstream gene expression regulation, and FKBP5, which regulates the glucocorticoid receptor's sensitivity, are additional genes of interest due to their direct involvement in the regulation of glucocorticoid levels. Studies have found evidence of differential methylation of the selected genes in relation to maternal stress [22-24]. While existing research has established associations between SES, prenatal stress, and adverse birth outcomes, the underlying biological mechanisms are still uncertain. The primary aim of this study is to propose a plausible mechanism through which socioeconomic adversity may be linked to an increased risk for SGA in full-term infants. This association will be explored through the examination of DNA methylation alterations occurring in genes central to the regulation of fetal glucocorticoid exposure.

This research aligns with the Theory of Developmental Origins of Health and Disease, emphasizing that the intrauterine environment and early-life experiences can have long-lasting effects on the risk for disease in adulthood. Preventing SGA births is of critical importance. Identifying factors and exposures associated with SGA presents an opportunity to develop targeted preventative interventions and treatments. While variables used to assess SES, such as maternal education and employment status, may remain relatively constant during pregnancies and may not be inherently modifiable, they represent easily accessible information that can aid in identifying mothers facing socioeconomic adversity. The identification of high-risk populations

is essential for the targeted development of interventions and the allocation of resources where they have the most substantial impact. The data from this investigation can have broad implications and serve as a valuable resource to inform and guide policy changes aimed at reducing socioeconomic disparities and their risk on lifelong health outcomes. This research not only aims to advance the understanding of the relationship between socioeconomic adversity, DNA methylation, and the risk for SGA, but also to serve as evidence to drive positive policy decisions and societal changes.

Methods

Ethics statement

Informed consent was obtained from all participants (n = 840). The protocol was approved by the registered Institutional Review Board of Women and Infants Hospital of Rhode Island and Emory University.

Study population

The study population comprises mother-infant dyads enrolled in the Rhode Island Child Health Study (RICHS) at the Woman and Infant's Hospital (Providence, RI, USA) between 2009 and 2014. The original study aimed to explore placental epigenetic modifications and their relationship with various prenatal factors and subsequent infants' health outcomes. The data allows researchers to explore how the prenatal environment, including factors about maternal stress, can influence specific epigenetic modifications in the placenta, and how different epigenetic patterns might be associated with low birth weight in the infants.

Inclusion criteria were singleton, full-term infants (≥37 weeks gestational age) born to mothers aged 18-40 years without serious pregnancy complications. Infants with congenital or chromosomal abnormalities were excluded. After birth, infants were categorized as Small for Gestational Age (SGA; <10th percentile), Large for Gestational Age (LGA; >90th percentile), or Adequate for Gestational Age (AGA; 10th-90th percentile), according to the 2013 Fenton Growth Curve. The study population was oversampled for SGA and LGA infants. Comprehensive data was collected from mothers post-delivery, encompassing demographic, socioeconomic, health behaviors, and environmental exposure information. None of the participants in the study were exposed to prenatal synthetic glucocorticoids. Placental methylation data was available on a subset of the sample (n=227). Analyses involving methylation were therefore only conducted on this subset.

Socioeconomic Adversity Measures

Based on Appleton, A.A., et al. (2013), several exposure measures were considered both independently and as a cumulative risk score to represent the experience of multiple co-occurring sources of socioeconomic adversity. The following dichotomous variables were included: maternal education (classified as low (less than high school) or high (high school graduate or above)), maternal employment status, household crowding (defined as the presence of 5 or more individuals in the home), maternal tobacco use, and marital status (categorized as partnered or unpartnered). To generate the cumulative score, for each factor in which mothers reported an experience of socioeconomic adversity, a point was assigned. Equal weight was given to each factor. A point was assigned if mothers reported having low education, unemployment, living in a household with more than 5 people, tobacco use, and being unpartnered, which were

considered factors contributing to socioeconomic adversity. A higher score is to be interpreted as a higher experience of socioeconomic adversity. For the analysis, the cumulative score was dichotomized as >=4 and <4 points to investigate the accumulation of adverse socioeconomic factors.

DNA methylation (DNAm)

DNAm is quantified based on bisulfite modification and Illumina MethylationEPIC Array analysis, and is represented as a beta values at specific cytosine-phosphate-guanine (CpG) sites within the specific target genes (HSD11B2, NR3C1, and FKBP5) involved in fetal glucocorticoid regulation. Beta values represent the proportion of methylation at the CpG sites, and are continuous, ranging from 0 (completely unmethylated) to 1(fully methylated). Placental samples and DNA methylation data from the original study were obtained with methodologies described in greater detail in Appleton, A.A., et al [18]. Genetic annotation is based on the GRCh37/hg19 reference genome.

Birth Outcome Classification

Infants were classified as SGA, LGA, or AGA, based on the 2013 Fenton Growth Curve. In the analyses, these categories were dichotomized into SGA and not-SGA (combining AGA and LGA).

Covariates

The variables considered as confounders were maternal age, pre-pregnancy body mass index (BMI; weight (kg)/height(m)^2), both treated as continuous measures, and race, dichotomized as

"White" and "BIPOC" based on the sample distribution. In the methylation analyses, the dichotomous variable infant gender was also considered as a confounder. The models also controlled for cell-type proportions, derived from DNA methylation data, with syncytiotrophoblasts representing the most common cell type. These variables were identified *a priori* based on literature review and their confounding potential in the association between socioeconomic adversity, DNAm, and birth outcomes.

Analyses

The hypothesis proposes a mechanism that links differential DNAm patterns occurring at genes that regulate fetal glucocorticoid exposure to socioeconomic adversity and an increased risk for SGA. The nature of the cross-sectional study design precluded a formal mediation analysis. Three separate analyses were therefore conducted to examine the following relationships:

I- Maternal Socioeconomic Adversity and Birth Outcomes

II- Maternal Socioeconomic Adversity Score and DNAm at Candidate Genes

III- DNAm and Birth Outcomes

I. Maternal Socioeconomic Adversity and Birth Outcomes

Descriptive statistics were calculated on the whole sample (n=840) using means, SD, medians, or proportions. The association with birth outcomes was assessed for the computed cumulative socioeconomic adversity score as well as for the following variables: maternal education, maternal employment, household crowding, maternal tobacco use, and marital status. Bivariate analyses between exposure measures were conducted using chi-square tests. Bivariate analyses were also conducted between the covariates and the exposure measures, using independent t-tests

and simple linear regression, and between the covariates and the outcome, using independent t-tests and chi-square tests. While the choices of confounders were based on literature review, this step aimed to investigate the relation between confounding, exposure, and outcome variables within the sample. The associations between socioeconomic adversity measures and birth outcomes were analyzed with unadjusted logistic regression models and adjusted logistic regression models controlling for maternal age, maternal BMI, and maternal race. Statistical significance was set at $p<.05$. Confounding was assessed using the 10% change-in-estimate rule between the unadjusted and adjusted models. This analysis was also conducted on the subset for which placental methylation was available (n=227).

II. Maternal Socioeconomic Adversity Score and DNAm at Candidate Genes

This analysis was conducted on the subset (n=227). Descriptive statistics on the subset were generated using mean, SD, medians, and proportions. The association between the maternal socioeconomic adversity score and DNAm at the candidate genes (HSD11B2, NR3C1, FKBP5) was assessed using a robust linear regression model, adjusted for maternal age, maternal BMI, maternal race, infant sex, and cell type proportion. Robust linear regression was used to account for potential homoscedasticity issues. Statistical significance was set at $p<0.05$.

III. DNAm and Birth Outcomes

This analysis was conducted on the subset of the sample (n=227). The association between the DNAm at candidate genes (HSD11B2, NR3C1, FKBP5) and birth outcomes was assessed using a logistic regression model, adjusted for maternal age, maternal BMI, maternal race, infant sex, and cell type proportion. Statistical significance was set at $p<0.05$. In the logistic regression

analysis, for DNAm using beta values, a one-unit change would not meaningful as it corresponds to an unrealistic shift from 0% to 100% methylation. Therefore, the beta values were scaled based on the interquartile range (IQR). This method rescales the data by subtracting the median and subsequently dividing each value by the IQR. This process adjusts the distribution of the data, with the largest value representing the top of the IQR and the smallest value representing the bottom IQR. The subtraction of the median allows for centering around the median value to ensure that the distribution is less influenced by extreme values, making the scaling robust to outliers while maintaining the relative position of each data point within the dataset. This method allows for a more interpretable and biologically plausible assessment of methylation's impact on the odds of the outcome.

Results

Sample Characteristics

Characteristics of the study population (mothers and infants) are summarized in Table 1a for the whole sample (N=840) and Table 1b for the subset with methylation data (N=227), stratified by birth outcomes (SGA, Not-SGA). The mean maternal age was 29.7 years for the full sample and 30.9 years for the subset. Most participants identified as white (73.1% in the full sample, 77.1% in the subset) and had an average BMI of 26.6 in the full sample and 26.4 in the subset. Low education prevalence was 24.6% in the full sample and 15.4% in the subset. The majority of participants were unemployed (61.7% in the full sample, 67.0% in the subset) and partnered (64.8% in the full sample, 74.4% in the subset). Smoking was reported by 14.3% in the full sample and 8.8% in the subset, and 23.2% of the full sample and 26.4% of the subset resided in households with more than 5 people. Infants' sex distribution was nearly even, with an average birth weight of 3480g in the full sample and 3560g in the subset.

Table 1a. Maternal and Infant Characteristics in whole sample (N=840)

	Not Small for Gestational Age (Not-SGA) (N=700)	Small for Gestational Age (SGA) (N=140)	Total (N=840)
Age (Yrs)			
Mean (SD)	30.0 (5.24)	28.5 (6.26)	29.7 (5.45)
Median [Min, Max]	30.0 [18.0, 40.0]	29.0 [18.0, 40.0]	30.0 [18.0, 40.0]
BMI (kg/m2)			
Mean (SD)	26.9 (6.99)	25.3 (6.85)	26.6 (6.98)
Median [Min, Max]	24.9 [16.0, 58.4]	23.2 [15.9, 46.1]	24.7 [15.9, 58.4]
Missing	52 (7.4%)	0 (0%)	52 (6.2%)
Race			
Asian	19 (2.7%)	9 (6.4%)	28 (3.3%)
Black of African American	39 (5.6%)	22 (15.7%)	61 (7.3%)
Indian	5 (0.7%)	1 (0.7%)	6 (0.7%)
Multiracial	21 (3.0%)	6 (4.3%)	27 (3.2%)
Unknown/Not Reported	79 (11.3%)	25 (17.9%)	104 (12.4%)
White	537 (76.7%)	77 (55.0%)	614 (73.1%)
Education			
High School or Less	166 (23.7%)	41 (29.3%)	207 (24.6%)
More than High School	533 (76.1%)	99 (70.7%)	632 (75.2%)
Missing	1 (0.1%)	0 (0%)	1 (0.1%)
Employment Status			
No	429 (61.3%)	89 (63.6%)	518 (61.7%)
Yes	201 (28.7%)	41 (29.3%)	242 (28.8%)
Missing	70 (10.0%)	10 (7.1%)	80 (9.5%)
Tobacco Smoking			
No	602 (86.0%)	110 (78.6%)	712 (84.8%)
Yes	91 (13.0%)	29 (20.7%)	120 (14.3%)
Missing	7 (1.0%)	1 (0.7%)	8 (1.0%)
Number of People in Household			
Less than 5 people	475 (67.9%)	95 (67.9%)	570 (67.9%)
5 people or more	168 (24.0%)	27 (19.3%)	195 (23.2%)
Missing	57 (8.1%)	18 (12.9%)	75 (8.9%)
Marital Status			
Partnered	479 (68.4%)	65 (46.4%)	544 (64.8%)
Unpartnered	220 (31.4%)	75 (53.6%)	295 (35.1%)
Missing	1 (0.1%)	0 (0%)	1 (0.1%)
Adversity Score			
0	102 (14.6%)	17 (12.1%)	119 (14.2%)
1	209 (29.9%)	32 (22.9%)	241 (28.7%)
2	136 (19.4%)	31 (22.1%)	167 (19.9%)
3	97 (13.9%)	20 (14.3%)	117 (13.9%)
4	32 (4.6%)	14 (10.0%)	46 (5.5%)
5	5 (0.7%)	2 (1.4%)	7 (0.8%)
Missing	119 (17.0%)	24 (17.1%)	143 (17.0%)
Infant Birth Weight (g)			
Mean (SD)	3660 (542)	2570 (299)	3480 (652)
Median [Min, Max]	3650 [2410, 5470]	2620 [1710, 4110]	3480 [1710, 5470]
Infant Gender			
Female	329 (47.0%)	95 (67.9%)	424 (50.5%)
Male	371 (53.0%)	45 (32.1%)	416 (49.5%)

Table 1b. Maternal and Infant Characteristics in subset (N=221)

	Not Small for Gestational Age (Not-SGA) (N=194)	Small for Gestational Age (SGA) (N=33)	Total (N=227)
Age (Yrs)			
Mean (SD)	30.8 (4.79)	31.4 (5.57)	30.9 (4.90)
Median [Min, Max]	31.0 [18.0, 40.0]	31.0 [18.0, 40.0]	31.0 [18.0, 40.0]
BMI (kg/m2)			
Mean (SD)	26.4 (6.22)	26.2 (7.35)	26.4 (6.38)
Median [Min, Max]	24.8 [16.0, 46.7]	23.9 [18.1, 44.1]	24.7 [16.0, 46.7]
Race			
Asian	5 (2.6%)	5 (15.2%)	10 (4.4%)
Black	5 (2.6%)	7 (21.2%)	12 (5.3%)
Indian	2 (1.0%)	0 (0%)	2 (0.9%)
More than one	4 (2.1%)	0 (0%)	4 (1.8%)
Unknown/Not Reported	21 (10.8%)	3 (9.1%)	24 (10.6%)
White	157 (80.9%)	18 (54.5%)	175 (77.1%)
Education			
High School or Less	30 (15.5%)	5 (15.2%)	35 (15.4%)
More than High School	164 (84.5%)	28 (84.8%)	192 (84.6%)
Employment Status			
No	131 (67.5%)	21 (63.6%)	152 (67.0%)
Yes	60 (30.9%)	11 (33.3%)	71 (31.3%)
Missing	3 (1.5%)	1 (3.0%)	4 (1.8%)
Tobacco Smoking			
No	176 (90.7%)	30 (90.9%)	206 (90.7%)
Yes	17 (8.8%)	3 (9.1%)	20 (8.8%)
Missing	1 (0.5%)	0 (0%)	1 (0.4%)
Number of People in Household			
Less than 5 people	139 (71.6%)	26 (78.8%)	165 (72.7%)
5 people or more	53 (27.3%)	7 (21.2%)	60 (26.4%)
Missing	2 (1.0%)	0 (0%)	2 (0.9%)
Marital Status			
Partnered	146 (75.3%)	23 (69.7%)	169 (74.4%)
Unpartnered	48 (24.7%)	10 (30.3%)	58 (25.6%)
Adversity Score			
0	39 (20.1%)	6 (18.2%)	45 (19.8%)
1	76 (39.2%)	13 (39.4%)	89 (39.2%)
2	40 (20.6%)	7 (21.2%)	47 (20.7%)
3	27 (13.9%)	5 (15.2%)	32 (14.1%)
4	4 (2.1%)	1 (3.0%)	5 (2.2%)
5	3 (1.5%)	0 (0%)	3 (1.3%)
Missing	5 (2.6%)	1 (3.0%)	6 (2.6%)
Infant Birth Weight (g)			
Mean (SD)	3720 (547)	2570 (358)	3560 (662)
Median [Min, Max]	3700 [2520, 5470]	2570 [2030, 4110]	3540 [2030, 5470]
Infant Gender			
Female	86 (44.3%)	25 (75.8%)	111 (48.9%)
Male	108 (55.7%)	8 (24.2%)	116 (51.1%)

Bivariate Analyses

Table 2 displays the relationship among socioeconomic adversity measures. Most factors were correlated at or slightly above the alpha significance level, with the exception of tobacco and household crowding (p=.34). The presence of correlation among the different socioeconomic adversity factors justifies the use of a cumulative score method to capture the exposure comprehensively. Using these factors as independent exposures in the model would have yielded collinearity issues and would not have been appropriate.

Table 2. Association between socioeconomic adversity factors[a]

	Education		Employment		Tobacco		Household		Marital Status	
	χ2	p value	χ2	p value	χ2	p value	χ2	p value	χ2	p value
Education	-	-	-	-	-	-	-	-	-	-
Employment	11.3	<.001	-	-	-	-	-	-	-	-
Tobacco	32.4	<.001	3.5	.061	-	-	-	-	-	-
Household	6.1	.013	15.2	<.001	0.9	-	-	-	-	-
Marital Status	136.7	<.001	3.6	.057	48.3	<.001	0	1	-	-

[a]Chi-squared ($\chi 2$) test statistics for categorical variables.

Bivariate analyses were conducted between the confounders (age, BMI, and race) and the exposures (socioeconomic adversity factors and cumulative score). Maternal age was significantly associated with all of the exposure measures ($p<.05$). Associations between exposures and BMI were statistically significant for education, household crowding, and the cumulative score, but not for employment status, tobacco use, and marital status. Race showed statistical association with education, marital status, and cumulative score but not with employment status, tobacco use, and household crowding. A bivariate analysis was also conducted for the confounders and the birth outcome variable. All confounders were significantly associated with the birth outcome.

Association Between Maternal Socioeconomic Adversity and Birth Outcomes

The results from the logistic regression analyses conducted in the whole sample (N=840) on the association between individual socioeconomic adversity factors, the cumulative score, and the birth outcomes are presented in Table 3. The cumulative socioeconomic adversity score showed a significant positive association with the risk of SGA (OR 2.53, 95% CI 1.30-4.93, p<.05). After adjusting for maternal BMI, race, and age, infants born to mothers with a cumulative socioeconomic adversity score of 4 and above have 2.53 times the odds of being classified as SGA compared to infants of mothers with a score of less than 4. The odds ratios (ORs) of the individual socioeconomic adversity measures suggest varying degrees of association. Tobacco use and marital status were significantly associated with birth outcomes in both the unadjusted and adjusted models. Education, Employment, and Household Crowding did not show a significant association.

Table 3. Results of association analysis between socioeconomic adversity factors, cumulative score, and birth outcomes (N=840).

Socioeconomic Adversity Measures	Reference	OR (95% CI) Unadjusted	OR (95% CI) Adjusted**
Education	<=HS	0.75 (0.50,1.12)	1.08 (0.68, 1.73)
Employment	Unemployed	0.98 (0.66, 1.48)	1.04 (0.69, 1.58)
Tobacco	No	**1.74 (1.10,2.78)***	**2.07 (1.25,3.40)***
Household	<5 in Household	0.80 (0.51, 1.28)	0.80 (0.49, 1.30)
Marital Status	Partnered	**2.51 (1.74, 3.63)***	**2.20 (1.40, 3.47)***
Cumulative Score (reference <4)	Score <4	**2.35 (1.26, 4.39)***	**2.53 (1.30, 4.93)***

*p < .05
**The variables treated as confounders in the model were maternal age, maternal BMI, and maternal race.

The analysis of the association between the cumulative socioeconomic adversity score and the birth outcomes was also conducted on the subset for which placental epigenetic data was available (N=227). The results from this analysis presented odds ratios that were in the same direction as the odds ratios of this analysis in the whole sample but were not statistically

significant (OR 1.15, 95% CI(0.12, 10.89)). This is likely due to the subset sample being unstable due to the small sample size and small number of individuals with high adversity scores.

Association Between Maternal Socioeconomic Adversity Score and DNAm

Results from the robust linear regression between maternal socioeconomic adversity score as the exposure and DNAm at the candidate genes as the outcome are displayed in Table 4. The β values represent the ratio of methylated portion in the CpG to the overall combined methylation and unmethylated portion of the CpG per unit difference in socioeconomic adversity score. A β value of 0 means that all of the CpG were completely unmethylated, while a value of 1 (or -1) means that all of the CpG were completely methylated. A positive β value signifies an increase in methylation, while a negative β value indicates a decrease in methylation. This implies that as the socioeconomic adversity score increases, CpG sites with a positive β value have a higher degree of methylation, whereas those with a negative β value have a lower degree of methylation. Because the socioeconomic adversity score was dichotomized to a score of <4 and a score of >=4, the estimate represents the difference in methylation between low and high score values. As shown in Table 4, nine CpG sites within the genes NR3C1, FKBP5, and HSD11B2 were significantly associated with the exposure ($p<.05$). This may reflect a differential epigenetic regulation in response to socioeconomic adversity. Specifically, within the NR3C1 gene, sites cg03906910, cg12969488, and cg16219186 showed an increase in methylated as the socioeconomic adversity score increased, whereas sites cg04111177, cg15910486, and cg27345592 displayed a decrease in methylation. In the HSD11B2 gene, the cg02734600 and cg08711598 sites present an increased methylation. In the FKBP5 gene, the cg07696519 site has a decreased methylation.

Table 4. Results of association analysis between socioeconomic adversity cumulative score and DNAm

CpG	β Value	β SE	P Value	Gene	Chromosome	Position
cg00862770	0.00258	0.00149	.084	FKBP5	chr6	35655764
cg02734600	**0.01194**	**0.00606**	**.049***	**HSD11B2**	**chr16**	67467751
cg03906910	**0.03592**	**0.01308**	**.0060***	**NR3C1**	**chr5**	**142814388**
cg04111177	**-0.00353**	**0.00149**	**.018***	**NR3C1**	**chr5**	**142783607**
cg07485685	-0.00721	0.00376	.055	FKBP5	chr6	35696061
cg07696519	**-0.04165**	**0.02079**	**.045***	**FKBP5**	**chr6**	**35619165**
cg08711598	**0.02400**	**0.01077**	**.026***	**HSD11B2**	**chr16**	**67464483**
cg10913456	0.00095	0.00055	.086	FKBP5	chr6	35656590
cg12670061	-0.00098	0.00052	.061	HSD11B2	chr16	67465042
cg12969488	**0.03675**	**0.01733**	**.034***	**NR3C1**	**chr5**	**142780984**
cg15910486	**-0.00958**	**0.00204**	**2.7E-06***	**NR3C1**	**chr5**	**142783621**
cg16219186	**0.04197**	**0.00864**	**1.2E-06***	**NR3C1**	**chr5**	**142780531**
cg17342132	0.02763	0.01615	.087	NR3C1	chr5	142780254
cg17349736	-0.01158	0.00613	.059	NR3C1	chr5	142802329
cg19176661	-0.03015	0.01814	.097	NR3C1	chr5	142718549
cg21979215	-0.02131	0.01202	.076	NR3C1	chr5	142815807
cg22233604	0.01304	0.00721	.070	NR3C1	chr5	142729377
cg23776787	0.03786	0.02265	.095	NR3C1	chr5	142814315
cg26720913	0.00876	0.00485	.071	NR3C1	chr5	142814934
cg27345592	**-0.00412**	**0.00188**	**.029***	**NR3C1**	**chr5**	**142786405**

*p < .05

Association Between DNAm and Birth Outcomes

Table 5 presents the significant (p<.05) results from the logistic regression model assessing the relationship between DNAm at various CpG sites in the candidate genes and birth outcomes. Several CpG sites in the NR3C1 and FKBP5 genes were significantly associated with birth outcomes after controlling for confounders.

In the NR3C1 gene, most of the significant CpGs found in promoter regions had ORs below 1, suggesting an inverse association with birth outcomes: cg13648501 (OR=0.39), cg14438279 (OR=0.71), cg14558428 (OR=0.28), cg21702128 (OR=0.45), cg23430507 (OR=0.48), cg24026230 (OR=0.34). After scaling DNAm by the IQR and centering around the median, a one IQR-unit increase in DNAm at most of the sites in the promoter region were

associated with a lower likelihood of being classified as SGA. One significant CpG site in the promoter region, cg01751279, had an OR=2.34 suggesting a positive association with birth outcome. In the NR3C1 gene body, four significant CpG sites were identified: cg08845721 (OR=2.40), cg22233604 (OR=2.28), cg03857453 (OR=0.51), cg16586394 (OR=0.44).

In the FKBP5 gene, CpG sites cg00130530 (OR=0.54), and cg24295963 (OR=0.55), in the promoter region, present ORs in suggesting an inverse association with birth outcomes: after scaling DNAm by the IQR and centering around the median, a one IQR-unit increase in DNAm at the promoter sites were associated with a lower likelihood of being classified as SGA. Within the FKBP5 gene body, site cg04791658 presented a OR of 1.63, suggesting that an increase in methylation is associated with a higher likelihood of being classified as SGA, while sites cg09268536 presented an OR of 0.61, suggesting an inverse association with birth outcomes: an increase in DNAm was associated with a lower likelihood of being classified as SGA.

Table 5. Results of association analysis between DNAm and birth outcomes.

CpGs	Estimate	SE	Z Value	P Value	ORs	Gene	Chromosome	Position
cg00130530	-0.613	0.303	-2.03	.043	0.54	FKBP5	chr6	35657202
cg01751279	0.851	0.387	2.20	.028	2.34	NR3C1	chr5	142793924
cg03857453	-0.668	0.286	-2.33	.020	0.51	NR3C1	chr5	142729913
cg04791658	0.491	0.224	2.19	.028	1.63	FKBP5	chr6	35611554
cg08845721	0.877	0.368	2.38	.017	2.40	NR3C1	chr5	142780693
cg09268536	-0.491	0.242	-2.03	.042	0.61	FKBP5	chr6	35611576
cg13648501	-0.940	0.417	-2.25	.024	0.39	NR3C1	chr5	142785258
cg14438279	-0.345	0.157	-2.20	.028	0.71	NR3C1	chr5	142806343
cg14558428	-1.265	0.473	-2.67	.0075	0.28	NR3C1	chr5	142784982
cg16586394	-0.816	0.406	-2.01	.044	0.44	NR3C1	chr5	142757011
cg21702128	-0.793	0.393	-2.02	.044	0.45	NR3C1	chr5	142784721
cg22233604	0.826	0.335	2.47	.014	2.28	NR3C1	chr5	142729377
cg23430507	-0.726	0.367	-1.98	.048	0.48	NR3C1	chr5	142798375
cg24026230	-1.077	0.450	-2.39	.017	0.34	NR3C1	chr5	142785172
cg24295963	-0.590	0.301	-1.96	.050	0.55	FKBP5	chr6	35681420

Discussion

This study aimed to explore a mechanism linking maternal socioeconomic adversity to differential methylation of target genes in the placenta and to an increased risk for SGA in full-term infants. The results of the analyses showed an association between maternal socioeconomic adversity cumulative score and an increased risk for SGA, consistent with previous findings [4,6,7,9,10]. Further, a significant association was identified between socioeconomic adversity and differential DNAm at several CpG sites within the target genes. This is consistent with previous studies suggesting that placental DNAm are sensitive to socioeconomic adversity [7,18]. In the association between DNAm and birth outcomes, several CpG sites within the NR3C1 and FKBP5 gene were associated with SGA.

When examining the relationship between maternal socioeconomic adversity and DNAm, and increased methylation was observed within the HSD11B2 gene. This gene is responsible for inactivating cortisol to protect the fetus from potential harmful effects of high maternal cortisol levels. These findings are in agreement with previous studies showing evidence of downregulation of the HSD11B2 enzyme in conjunction with maternal indicators like prenatal anxiety, depression, and perceived stress [15,16, 22]. A lower enzymatic activity might contribute to excessive placental cortisol levels, resulting in a potentially unfavorable fetal environment and increasing the risk of adverse birth outcomes [17]. In contrast, other studies found lower methylation of HSD11B2 in response to higher socioeconomic adversity in mothers [18], hinting at an adaptive response to higher maternal cortisol levels. It is important to note that this prior study [18] used a different technique to assess DNA methylation and was looking at a different genomic region, which could also contribute to the difference in findings.

The FKBP5 gene, involved in the regulation of the sensitivity of glucocorticoid receptors, exhibited decreased methylation associated with socioeconomic adversity. These results are in contrast with previous studies that identified higher DNAm at the FKBP5 gene associated with higher perceived maternal stress [22, 24], with the difference related potentially to the differences between socioeconomic adversity considered here, with more formal measures of perceived stress. Within the NR3C1 gene, which encodes receptors involved in gene downregulation, CpG sites were identified where methylation was both increased and decreased relative to socioeconomic adversity. This methylation pattern might reflect the complexity of the regulatory responses to stress and the complex nature of NR3C1 genomic regulation. Previous studies had identified decreased methylation of the NR3C1 gene in association with various maternal stress measures [24], which might lead to less response to circulating cortisol. Overall, these findings suggest that prenatal exposure to socioeconomic adversity could impact placental DNAm. Differential methylation suggests the adaptability of the epigenome in response to increased exposure to stress linked to socioeconomic adversity. As described in previous literature, a differential regulation might occur as a protective mechanism against excessive glucocorticoids [20], or as a response to increased oxidative stress and inflammation, often found in lower socioeconomic contexts [19].

The small-magnitude effect size is not uncommon in epigenetic analyses and even expected since variations in methylation occur across cell populations [25]. The specific method for the categorization of methylation at individual CpG sites should be considered. In somatic cell carrying two copies of each chromosome, there are two copies of each CpG site. For any given CpG site there are two potential methylation states: methylated or unmethylated. If both copies of a CpG site in somatic cell are methylated, then the CpG site is 100% methylated, if

none of the CpG copy is methylated, then the CpG site is 0% methylated (unmethylated), if only one CpG copy is methylated, then the site is 50% methylated. The methylation status of each CpG site contributes to the overall methylation profile observed across the cell population within an individual. Therefore, a shift in beta value indicates a variation in methylation amongst a proportion of the cells. A small effect size implies that a small proportion of cells displays the specific variation in DNAm. However, it does not mean that the impact of such difference is biologically unimportant [25].

In the analysis of the association between DNAm and birth outcomes, several CpGs within the NR3C1 and FKBP5 gene showed associations with SGA. Previous findings also linked differential methylation of genes involved in fetal glucocorticoid regulation with birthweight [21]. In the NR3C1 gene, most significant CpG sites in promoter regions displayed ORs<1. Methylation in the promoter region of NR3C1 may lead to decrease expression of the glucocorticoid receptors, affecting placental glucocorticoid signaling and potentially impacting fetal development and risk for SGA. Increasing methylation in the gene body was found to be both associated with higher and lower risk of SGA depending on the specific CpG sites. Based on these associations, epigenetic regulation of the NR3C1 and FKBP5 gene might be important in fetal development and could potentially affect birth outcomes. Further research into the functional implications of the DNAm patterns at specific CpG sites may provide insights into prenatal development and potential preventive strategies for adverse birth outcomes.

The cg22233604 site, located in the gene body of NR3C1, exhibited an overlapping association between both birth outcome and DNA methylation ($p=0.014$) and cumulative socioeconomic diversity score and DNAm ($p=.07$). In both analyses, the association was in the same direction as indicated by a positive β value ($\beta=0.013$), which suggests a greater

socioeconomic adversity score is associated with increased methylation in the specific CpG site in NR3C1, and an OR >1 (OR=2.28), implying that higher DNAm at this site may be associated with an increased risk for SGA. This particular CpG site could represent a target to understand the interplay between socioeconomic factors, DNAm patterns, and birth outcomes. Future studies, ideally involving a more extensive cohort with a broader range of socioeconomic backgrounds, should further explore the potential mediating effect of DNAm in the relationship between socioeconomic adversity and risk for SGA.

Considering the study population, the overall SES was high, indicated by the distribution of the cumulative socioeconomic adversity score. The majority of women did not experience high levels of socioeconomic adversity and had a cumulative score of 0 and 1. While this distribution was expected, it impacted the framework of the analysis and the results. In contexts of higher SES, we might hypothesize that it would take substantially more compounding adversity to generate enough stress to impact both methylation and birth outcomes, compared to lower SES contexts. Access to better resources and support systems, something to be expected in high SES contexts, could buffer the effect of socioeconomic challenges that would otherwise be detrimental in lower SES contexts. Therefore, the significance of the same adversity score may vary across different SES landscapes. In the analysis, the dichotomization of the cumulative socioeconomic adversity score separated people with a score of 4 or above from people with a score of less than 4. The cut-point was intentionally set at an extreme to emphasize the potential impact of severe accumulation of socioeconomic adversity.

The selection of socioeconomic indicators considered their relevance within existing research and accessibility of data. While interconnected, the selected indicators represent different facets of socioeconomic adversity and might not be interchangeable. Each has the

potential to uniquely influence maternal stress levels and birth outcomes, as seen in Table 3. While the different factors can independently affect birth outcomes, when they are combined, the effect might be more pronounced. The computation of a score allowed us to consider the accumulation and the compounding effect of socioeconomic adversity as a multifaceted experience. It should be noted that while the indicators selected are reflective of various aspects of adversity, they may not comprehensively capture the entire spectrum of factors contributing to maternal stress. Further, the chosen indicators were framed within an etic perspective applying conventional metrics to measure variables such as employment status, educational attainment, and household size for the quantification of socioeconomic adversity. For instance, unemployment, low educational attainment, and living in a large household were predetermined as indicators of socioeconomic adversities. It should be recognized that such standards are not absolute but relative to the culture. What might be indicative of adversity in one cultural context may not necessarily hold the same meaning in another. For example, household crowding might have different connotations across cultural settings where extended family support is normative and not indicative of socioeconomic strain. The findings from this research hint at a protective association between household crowding and the risk of SGA, as evidenced by the odds ratio displayed in Table 3. While the results did not reach statistically significant, they suggest a direction opposite to what was initially expected. This relationship could reflect positive aspects of communal living during pregnancy that could potentially enhance maternal support rather than contributing to the experience of adversity.

This study has many strengths. The overall sample size was large and allowed for the investigation of several indicators of socioeconomic adversity measures. This allows for a broad consideration of the potential impact of socioeconomic adversity on the risk for SGA. While the

subset for which placental methylation data was available was smaller, it was powered enough to detect small effects in the association between socioeconomic adversity and DNAm. Further, there are not many studies that integrate social epidemiology factors with epigenetics. This study aims to contribute to the understanding of the underlying mechanisms linking socioeconomic challenges with adverse health outcomes.

The study has also some limitations. The high SES of the sample may not reflect the experiences of populations with lower SES, limiting generalizability. Moreover, socioeconomic adversity is a latent construct that is not directly measurable. In this study, we attempted to capture the experience of socioeconomic adversity through the selected measures as proxies, but they might not comprehensively report the complex and intricate nature of socioeconomic challenges. Furthermore, this study assumed that stress mediated the relationship between the experience of socioeconomic adversity and birth outcomes. Yet, prenatal maternal stress was not directly measured and therefore maternal experience of stress cannot be explicitly assessed. Similarly, while differential regulation of the target genes is assumed to be reflected in the expression of the genes, placental RNA was not considered in this analysis. Lastly, exposure to stress, both prenatal and over the life course, might affect several systems beyond the function of the HPA axis. The proposed mechanisms by which exposure to stress impacts placental methylation and birth outcomes might only be a portion of the complete mechanism linking maternal exposure to adversity to the risk of harmful birth outcomes.

Positionality statement

My perspective as a researcher is intrinsically shaped by a consideration of the multifaceted nature of health outcomes, which are not the solely result of individual choices but also the

product of complex interplay between social determinants and systemic inequalities. While the research is rooted in the Theory of the Developmental Origins of Health and Diseases, the study does not wish to attribute health outcomes to individual behaviors. Instead, it aims to investigate the broader socioeconomic circumstances that exert impact on specific populations and their life-long health.

While exploring biological pathways that may link socioeconomic adversity to the risk for SGA, my research does not place the burden of responsibility on the individual, specifically the mother, but rather on the system that perpetuates adversities and inequalities. This perspective recognizes the nuanced and often difficult to measure constraints that shape the 'choices' within a disadvantaged community. The chosen indicators of socioeconomic adversity are not interpreted as outcomes of individual choices but as manifestations of complex socioeconomic challenges. My goal is not solely to propose a plausible biological mechanism but to highlight how socioeconomic adversity can have implications on the health of both mothers and infants, reinforcing the need for policy interventions and resource allocation aimed at mitigating these impacts.

In summary, my positionality as a researcher is guided by a recognition of the systemic and social factors that shape health outcomes. This perspective has informed every aspect of my research, from literature review to hypothesis formulation and data interpretation.

Public Health Implications

Based on the Theory of Developmental Origins of Health and Disease, which suggests that the intrauterine environment and early life experiences can impact the risk for disease in adulthood, SGA can affect development and increase the susceptibility to chronic disease in adulthood.

Prevention of adverse exposures that have the potential to impact birth outcomes and long-term health is therefore of critical importance. The findings of this study serve as evidence that socioeconomic adversity is not only a social issue but might also be a factor involved in the mechanisms that predispose infants to be born Small for Gestational Age (SGA). Recognizing the impact of maternal exposure to socioeconomic adversity on the risk of SGA emphasizes the need for comprehensive policy approaches that address socioeconomic disparities. Identifying exposures associated with SGA can propose targets for preventative interventions. Effective social support programs are important, specifically those that enhance and promote access to smoking cessation and comprehensive financial, educational, and employment assistance for women of childbearing age, especially in socioeconomically disadvantaged communities. Such initiatives could alleviate stress associated with low socioeconomic status and ultimately positively impact birth outcomes. Integrating social and economic support frameworks in prenatal care alongside traditional healthcare could not only aim to reduce incidence of SGA but also contribute to the prevention of long-term health outcomes in infants, echoing the necessity for early interventions that address the multifaceted nature of health determinants.

References

1. Calkins, K. and S.U. Devaskar, *Fetal origins of adult disease.* Curr Probl Pediatr Adolesc Health Care, 2011. **41**(6): p. 158-76.
2. Szanton, S.L., J.M. Gill, and J.K. Allen, *Allostatic load: a mechanism of socioeconomic health disparities?* Biol Res Nurs, 2005. **7**(1): p. 7-15.
3. Adler, N.E. and K. Newman, *Socioeconomic disparities in health: pathways and policies.* Health Aff (Millwood), 2002. **21**(2): p. 60-76.
4. Blumenshine, P., et al., *Socioeconomic disparities in adverse birth outcomes: a systematic review.* Am J Prev Med, 2010. **39**(3): p. 263-72.
5. Ruiz, M., et al., *Mother's education and the risk of preterm and small for gestational age birth: a DRIVERS meta-analysis of 12 European cohorts.* J Epidemiol Community Health, 2015. **69**(9): p. 826-33.
6. Vos, A.A., et al., *Deprived neighborhoods and adverse perinatal outcome: a systematic review and meta-analysis.* Acta Obstet Gynecol Scand, 2014. **93**(8): p. 727-40.
7. Santos, H.P., Jr., et al., *Epigenome-wide DNA methylation in placentas from preterm infants: association with maternal socioeconomic status.* Epigenetics, 2019. **14**(8): p. 751-765.
8. van den Berg, G., et al., *Smoking overrules many other risk factors for small for gestational age birth in less educated mothers.* Early Hum Dev, 2013. **89**(7): p. 497-501.
9. Wilding, S., et al., *Are socioeconomic inequalities in the incidence of small-for-gestational-age birth narrowing? Findings from a population-based cohort in the South of England.* BMJ Open, 2019. **9**(7): p. e026998.
10. Love, C., et al., *Exploring weathering: effects of lifelong economic environment and maternal age on low birth weight, small for gestational age, and preterm birth in African-American and white women.* Am J Epidemiol, 2010. **172**(2): p. 127-34.
11. Aguilera, G., *HPA axis responsiveness to stress: implications for healthy aging.* Exp Gerontol, 2011. **46**(2-3): p. 90-5.
12. Cohen, S., W.J. Doyle, and A. Baum, *Socioeconomic Status Is Associated With Stress Hormones.* Psychosomatic Medicine, 2006. **68**(3): p. 414-420.
13. Kapoor, A., et al., *Fetal programming of hypothalamo-pituitary-adrenal function: prenatal stress and glucocorticoids.* J Physiol, 2006. **572**(Pt 1): p. 31-44.
14. Deuschle, M., et al., *Cortisol, cortisone, and BDNF in amniotic fluid in the second trimester of pregnancy: Effect of early life and current maternal stress and socioeconomic status.* Dev Psychopathol, 2018. **30**(3): p. 971-980.
15. O'Donnell, K.J., et al., *Maternal prenatal anxiety and downregulation of placental 11beta-HSD2.* Psychoneuroendocrinology, 2012. **37**(6): p. 818-26.
16. Seth, S., et al., *Maternal Prenatal Mental Health and Placental 11beta-HSD2 Gene Expression: Initial Findings from the Mercy Pregnancy and Emotional Wellbeing Study.* Int J Mol Sci, 2015. **16**(11): p. 27482-96.
17. Busada, J.T. and J.A. Cidlowski, *Mechanisms of Glucocorticoid Action During Development.* Curr Top Dev Biol, 2017. **125**: p. 147-170.
18. Appleton, A.A., et al., *Patterning in placental 11-B hydroxysteroid dehydrogenase methylation according to prenatal socioeconomic adversity.* PLoS One, 2013. **8**(9): p. e74691.

19. Menezo, Y.J., et al., *Oxidative stress and alterations in DNA methylation: two sides of the same coin in reproduction.* Reprod Biomed Online, 2016. **33**(6): p. 668-683.
20. Ghaemmaghami, P., et al., *The association between the acute psychobiological stress response in second trimester pregnant women, amniotic fluid glucocorticoids, and neonatal birth outcome.* Dev Psychobiol, 2014. **56**(4): p. 734-47.
21. Filiberto, A.C., et al., *Birthweight is associated with DNA promoter methylation of the glucocorticoid receptor in human placenta.* Epigenetics, 2011. **6**(5): p. 566-72.
22. Monk, C., et al., *Distress During Pregnancy: Epigenetic Regulation of Placenta Glucocorticoid-Related Genes and Fetal Neurobehavior.* Am J Psychiatry, 2016. **173**(7): p. 705-13.
23. Nagarajan, S., et al., *Adverse maternal exposures, methylation of glucocorticoid-related genes and perinatal outcomes: a systematic review.* Epigenomics, 2016. **8**(7): p. 925-44.
24. Kertes, D.A., et al., *Prenatal Maternal Stress Predicts Methylation of Genes Regulating the Hypothalamic-Pituitary-Adrenocortical System in Mothers and Newborns in the Democratic Republic of Congo.* Child Dev, 2016. **87**(1): p. 61-72.
25. Breton, C.V., et al., *Small-Magnitude Effect Sizes in Epigenetic End Points are Important in Children's Environmental Health Studies: The Children's Environmental Health and Disease Prevention Research Center's Epigenetics Working Group.* Environ Health Perspect, 2017. **125**(4): p. 511-526.