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DNA methylation in Children with Prenatal Methamphetamine Exposure and Environmental Adversity

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Abstract

Background—Methamphetamine (MA) use during pregnancy is a significant public health concern in the United States and affects long term brain and behavioral development in children. We hypothesized that prenatal MA exposure would be related to greater DNA methylation of *HSD11B2* and postnatal environmental stress.

Methods—The Infant Development, Environment, and Lifestyle Study (IDEAL), a longitudinal study of Prenatal MA exposure enrolled mother-infant dyads in California, Hawaii, Iowa, and Oklahoma. Prenatal exposure was defined by maternal self-report and/or meconium toxicology

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screening. At ages 10–11 years, 100 children were assessed for drug exposure and DNA methylation of *HSD11B2*. Hierarchical linear models were used to determine the association between Prenatal MA exposure and methylation of *HSD11B2* at 4 CpG sites.

Results—Prenatal MA exposure (1.4% vs 0.31%, P<0.01) and early childhood adversity (3.0 vs 2.0, P<0.01) were associated with greater DNA methylation of *HSD11B2* at the CpG2 site. The statistically significant effects of early childhood adversity (B=0.11, P<0.01) and Prenatal MA exposure (B=0.32, P=0.03) on DNA methylation remained after adjusting for covariates.

Conclusions—Prenatal MA exposure is related to postnatal childhood adversity and epigenetic alterations in *HSD11B2*, an important gene along the stress response pathway suggesting prenatal and postnatal programming effects.

Introduction

Recent reports on the public health risks of drugs have shown a surge in the abuse of methamphetamine (MA) in the United States (US) and worldwide.(1, 2) Current reports suggest methamphetamine is second only to heroin in its threat to public health in the US.(2) MA use during pregnancy continues to be a significant public health concern in the United States (3, 4) and affects long term brain and behavioral development in children. Further, in utero methamphetamine exposure is a form of toxic stress.(5) Toxic stress can affect development in children through biological embedding, including epigenetic pathways and as cumulative damage over time or allostatic load. We investigated the effects of Prenatal MA exposure on epigenetic changes in 10–11-year-old children. Based on previous work,(6) and consistent with the notion of toxic stress, we reasoned that Prenatal MA exposure could act as an intrauterine stressor that affects gene expression particularly of stress related genes such as hydroxysteroid 11-Beta dehydrogenase type 2 (*HSD11B2*) that converts cortisol to its inert form, cortisone, thereby reducing levels of circulating cortisol.(7) We have previously demonstrated alterations in placental *HSD11B2* methylation following adverse intrauterine exposures.(8) We hypothesized that Prenatal MA exposure would be related to greater DNA methylation of *HSD11B2* in children exposed to methamphetamine antenatally, and that because of the developmental nature of the exposure that this increased methylation would be persistent, and be evident in middle childhood. We further hypothesized that greater DNA methylation of *HSD11B2* would be related to postnatal environmental stress measured by an environmental adversity index and hair cortisol/cortisone.

METHODS

Subjects

Subjects included 10–11-year-old children; 55 with Prenatal MA exposure and a comparison group (COMP) of 45 children without Prenatal MA exposure from 2 of the 4 original recruitment sites (Hawaii and California) for the Infant Development Environment and Lifestyle (IDEAL) study. Detailed recruitment methods for the IDEAL study have been reported previously.(9) Mothers were recruited for the study between delivery and discharge. The study was approved by the institutional review boards at all participating sites including a Certificate of Confidentiality from the National Institute on Drug Abuse and all child

participants in this study provided written informed assent in addition to parental consent. Prenatal MA exposure was determined by self-reported MA use during this pregnancy and/or a positive meconium screen with gas chromatography/mass spectroscopy confirmation. Children in the COMP group were born to mothers who denied methamphetamine use during pregnancy and their meconium screened negative for amphetamines and opiates. A matched case-control study design was used. Mothers in the control group were matched to those in the MA exposure group on maternal race, private versus public insurance, maternal education (completed high school versus not completed), and infant birth weight category (< 1500, 1500– 2500, >2500 g).

Measurements

DNA Collection.—Buccal derived DNA from saliva samples were collected from children using the Oragene-DNA saliva collection system. Children were asked to provide a saliva sample by expectorating into the provided collection tube up to a marked line. The collection tube was sealed, releasing a stabilizing solution into the collected sample to allow for processing of the sample at a later period. Sample collection tubes were bar-coded and labeled with ID numbers. DNA was isolated from the collection tubes following the Oragene methods, which reliably result in 50–150 μg of high-quality DNA for downstream analysis.

DNA Methylation Analysis.—A sodium bisulfite pyrosequencing approach was used to assess the methylation status of *HSD11B2*. Characteristics of the assay were assessed using dilution series of fully methylated referent DNA into fully unmethylated referent DNA. The PyroMark MD system (QIAGEN Inc., Germantown, MD) was used for all pyrosequencing assays. Quantitative assessment of the extent of DNA methylation at each of the 4 *HSD11B2* CpG sites was performed by using the integrated Pyro-Q-CpG software (QIAGEN Inc.) to analyze data using previously published methods described further in Supplementary Material (online).(10) Genomic coordinates are GRch37/hg19: 67464389, 67464395, 67464399, 67464412.Methylation variables for the CpG sites were winsorized and natural log transformed and analyzed as a continuous measure based on percent of methylation at each CpG site.

Early Adversity.—Early adversity was coded using a single index score similar to those used in previous studies.(11,12) The early adversity index accounted for cumulative measures of adversity from birth through age 5 years by calculating the sum of a set of binary indicators (Table 1).

Hair Collection.—A proximal crown head hair sample was obtained from the child at 10–11 years of age. Hair was collected from 87 of the 100 participants. There were 5 refusals, 1 hair specimen was too short, and the reason not collected was unknown for 7 children. The 3-cm hair segment was analyzed by the United States Drug Testing Laboratories (USDTL) using a new liquid chromatography-tandem mass spectrometry assay to measure cortisol and cortisone.(13) Specimens were analyzed according to a slightly modified version of a previously published procedure.(14) Of the 87 hair specimens, 63 had a value of “0” for cortisol indicating that cortisol was not detected. However of the 87 samples, 72 had positive cortisone values, therefore cortisone data was used in the analysis. Raw cortisone values

(pg/mg) were right skewed and normalized using a natural log transformation. Outliers 3 *SD* or more from the mean were winsorized by replacing their value with the nearest value less than 3 *SD* from the mean. Cortisone is used as a biological marker of chronic stress in this study.

Data Analysis

One-way analysis of variance (ANOVA) was used for analysis of continuous measures and Chisquare for categorical measures. Hierarchical multiple regression using SPSS (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.) examined the effects of Prenatal MA exposure, early adversity, and chronic stress on DNA methylation of *HSD11B2* after controlling for covariates. The models were adjusted for the following covariates: gestational age at birth, quantity of self-reported use of tobacco, alcohol, and marijuana use. Covariates were selected based on maternal and child characteristics that significantly differed between mothers in the MA exposure and comparison groups ($P<0.05$) yet were not highly correlated with other covariates ($r>0.70$), trends in the literature, and conceptual reasons.

RESULTS

Included mothers reported greater prenatal alcohol use and differed by race with a higher number of white and Hispanic included in the study relative to those who were not included in this sample. There were no differences in newborn characteristics between children who were included in the study and those not included (Table 2). Children with prenatal MA exposure were exposed to more tobacco and marijuana, were more likely to be born to single mothers, had lower socioeconomic status (SES) and were less likely to have received any prenatal care relative to the COMP group (Table 3). The children exposed to methamphetamine weighed on average 267 g less, measured 2.9 cm shorter, had 0.7 cm smaller head circumference, and were 1.36 weeks younger than children in the COMP group.

To determine if Prenatal MA exposure use, early adversity, and chronic stress predicts greater DNA methylation, hierarchical linear regression was conducted with DNA methylation as the dependent variable. In an initial model, Prenatal MA exposure children had higher levels of DNA methylation at the *HSD11B2* CpG site 2 than children in the COMP group (B=0.34, 95% CI:0.11, 0.57, P=<0.01) (Table 4, Model 1). In Model 2, the early adversity index was added to the model. Higher scores on the early adversity index related to higher levels of DNA methylation at CpG site 2 (B=0.11, 95% CI:0.03, 0.19, P<0.05), however Prenatal MA exposure was no longer statistically significant. However, while the addition of cortisone was not related to amount of DNA methylation at CpG2 (B= −0.13, 95% CI: −0.36, 0.09, P=0.23) the inclusion of cortisone resulted in significant effects for early adversity (B=0.11, 95% CI: 0.03, 0.19, P<0.01) and Prenatal MA exposure (B=0.24, 95% CI:0.002, 0.48, P<0.05). In the final model (Model 4), covariates were added, including: gestational age at birth, and count variables reflecting the quantity of tobacco, alcohol, and marijuana consumed prenatally. None of the covariates were related to DNA methylation at the *HSD11B2* CpG2 site, but the statistically significant effects of early

adversity (B=0.11, 95% CI: 0.02, 0.19, P<0.05) and Prenatal MA exposure (B=0.32, 95% CI: 0.04, 0.60, P=0.03) remained. There was no significant interaction between Prenatal MA exposure and early adversity.

DISCUSSION

This is the first study to measure epigenetic effects of Prenatal MA exposure on a stress-related gene. It is also the first study to measure the effects of postnatal early adversity on a stress-related gene in Prenatal MA exposure children aged 10–11 years. We found that Prenatal MA exposure and the postnatal early adversity index was associated with greater DNA methylation of *HSD11B2* with adjustment for a biological marker of chronic stress (hair cortisone). Critically, these epigenetic effects represent 2 distinct pathways, a biological pathway due to *in utero* drug exposure and a pathway related to adversity in the postnatal environment. These findings provide evidence for the biological embedding of early experiences during sensitive periods that could have long term physical and mental health implications.(5)

We measured DNA methylation in the *HSD11B2* promoter region because this region regulates the neuroendocrine system, including the HPA axis and stress response. The specific epigenetic effects that we observed involved increases in DNA methylation of *HSD11B2*. Increased methylation at CpG sites in the promoter and first exon of *HSD11B2* inhibits the activity of *HSD11B2* and thereby reduces the capacity to inactivate cortisol.(15) The resulting increased levels of circulating cortisol can be harmful as stress hormones are widely implicated in the development of mental and physical disorders, especially when related to chronic, toxic stress.(5) Toxic stress disrupts brain circuitry and normal organ and metabolic systems during sensitive developmental periods. These disruptions can produce physiological changes leading to learning and behavior impairments, as well as increased risks for developing physical and mental illness.(16) Toxic stress and early adversity are potential drivers of health disparities.(5) The Adverse Childhood Experiences Study found that parental substance abuse is a stressor that can yield toxic stress in children.(17) We have documented toxic stress both at the biological (Prenatal MA exposure) and environmental (early adversity) levels.

Epigenetic effects are highly tissue specific. We have previously measured changes in DNA methylation of *HSD11B2* in placenta in several settings. We have shown that prenatal socioeconomic adversity affects placental *HSD11B2* methylation.(18) There was a sex related difference. We have compared placental *HSD11B2* methylation to measures of fetal growth and neural behavioral assessment.(19) We showed a higher level of methylation was greatest in infants with low birth weight. This increase in *HSD11B2* methylation was associated with reduced neurodevelopmental scores on quality of movement. These results suggest that the programming effects of *in utero* exposures are among the biological pathways may be mediated by alterations in the epigenetic alterations in this important gene. We note that these are small magnitude effects. Small-magnitude epigenetic effect sizes are common findings in environmental epigenetic studies resulting from such exposures. A small difference in methylation means that a small fraction of the cells exhibits this difference at a particular CpG site. Depending on the identity and nature of that cell, small

differences could substantially affect functioning of that cell and the functioning of that cell's progeny because of mitotic heritability of DNA methylation. (20) Future work examining the functional implications of these small differences would be warranted.

The absence of cortisol in the hair was an unexpected finding. Unlike plasma in which concentrations of cortisol are significantly higher than that of cortisone, in human hair cortisone concentrations are higher than cortisol.(14) Higher concentrations of cortisone than cortisol in hair may be due to increased activity of *HSD11B2* in the hair bulb.(21) Thus hair cortisone is thought to provide a more reliable measure of HPA axis activity than cortisol.(22) Alternatively, the lack of measured cortisol in the hair samples may be secondary to allostatic load which would be consistent with previous work showing cortisol blunting in children with prenatal methamphetamine(23) or cocaine exposure. (24)

This study is limited by its small sample size. However, this novel study can make a significant contribution to the field of epigenetics despite the small sample size because the findings provide an impetus for further study with additional subjects. Another possible limitation of this study is that DNA samples were collected from peripheral tissue. Although it is unlikely that patterns of DNA methylation in buccal cells will exactly match those of neural tissue, buccal cells and neural cells are both derived from the same germ layer, the ectoderm, during fetal development, and recent reports suggest that there are similarities in early epigenetic patterning as well as in susceptibility to epigenetic change during the developmental period.(25),(26) We have also demonstrated relationships between buccal cell derived DNA methylation and infant neurobehavioral outcomes.(27) We also acknowledge that due to the cell type specificity of DNA methylation, what we have observed as differential methylation may represent changes in the underlying cell type proportions, although this is still a biologically interesting phenomenon and is worthy of further examination. Finally, although our findings clearly have implications for the development of mental health and physical disorders we do not have measures of these outcomes.

CONCLUSION

Our findings provide a new insight into the study of prenatal and postnatal programming models in children with prenatal substance exposure which is somewhat surprising given the known relationship between maternal drug use during pregnancy and toxic stress. Intrauterine substances could act as stressors, or challenges that disrupt fetal-placental homeostasis, through DNA methylation, thus prompting the fetus to make compensatory adjustments. These homeostatic adjustments could, in turn, trigger the reprogramming, or recalibration, of physiological systems. Early adversity in the postnatal environment could produce a second order reprogramming of physiological systems that can be expected to have long term developmental and physical consequences.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. Hedegaard H, Warner M, & Minino AM Drug overdose deaths in the United States, 1999–2015. Hyattsville, MD: National Center for Health Statistics. 2017.
2. World Drug Report 2018. Vienna: United Nations Office on Drugs and Crime. 2018.
3. Center for Behavioral Health Statistics and Quality. 2014 National Survey on Drug Use and Health: Detailed Tables. Substance Abuse and Mental Health Services Administration, Rockville, MD. 2015.
4. Center for Behavioral Health Statistics and Quality. 2015 National Survey on Drug Use and Health: Detailed Tables. Substance Abuse and Mental Health Services Administration, Rockville, MD. 2016.
5. Shonkoff JP, Boyce T, & McEwen BS Neuroscience, molecular biology, and the Childhood roots of health disparities: building a new framework for health promotion and disease prevention. JAMA. 2009; 301(21):2252–2259. [PubMed: 19491187]
6. Lester BM & Padbury J Third pathophysiology of prenatal cocaine exposure. Dev Neurosci. 2009; 31, 23–35. [PubMed: 19372684]
7. Seckl JR & Walker BR Minireview: 11beta-hydroxysteroid dehydrogenase type 1- a tissue-specific amplifier of glucocorticoid action. Endocrinology. 2001 4; 142 (4): 1371–6. [PubMed: 11250914]
8. Appleton AA, Lester BM, Armstrong DA, Lesseur C, & Marsit CJ Examining the joint contribution of placental NR3C1 and HSD11B2 methylation for infant neurobehavior. Psychoneuroendocrinology. 2015 2; 52:32–42. [PubMed: 25459891]
9. Smith LM, et al. Developmental and behavioral consequences of prenatal methamphetamine exposure: a review of the Infant Development, Environment, and Lifestyle (IDEAL) study. Neurotoxicol Teratol. 2015 Sep-Oct; 51:35–44. [PubMed: 26212684]
10. Marsit CJ, Maccani MA, Padbury JF& Lester BM Placental 11-beta hydroxysteroid dehydrogenase methylation is associated with newborn growth and a measure of neurobehavioral outcome. PLoS One. 2012;7(3):e33794. [PubMed: 22432047]
11. Abar B, et al. Examining the relationships between prenatal methamphetamine exposure, early adversity, and child neurobehavioral disinhibition. Psychol Addict Behav. 2013 9;27(3):662–73. [PubMed: 23067308]
12. Fisher PA, et al. The combined effects of prenatal drug exposure and early adversity on neurobehavioral disinhibition in childhood and adolescence. Dev Psychopathol. 2011 8; 23(3):777–788. [PubMed: 21756431]
13. Himes SK, et al. Risk of neurobehavioral disinhibition in prenatal methamphetamine-exposed young children with positive hair toxicology results. Ther Drug Monit. 2014 8;36(4):535–43. [PubMed: 24518561]
14. Raul JS, Cirimele V, Ludes B, & Kintz P Detection of physiological concentrations of cortisol and cortisone in human hair. Clin Biochem. 2004; 37:1105–1111. [PubMed: 15589817]
15. Jensen Peña C, Monk C, & Champagne FA Epigenetic effects of prenatal stress on 11beta-hydroxysteroid dehydrogenase-2 in the placenta and fetal brain. PLoS One. 2012; 7(6):e39791. [PubMed: 22761903]
16. Shonkoff JP & Garner AS & Committee on Psychosocial Aspects of Child and Family Health. The Lifelong Effects of Early Childhood Adversity and Toxic Stress. Pediatrics. 2012. 129 (1) e232–46. [PubMed: 22201156]
17. Felitti VJ, et al. Relationship of childhood abuse and household dysfunction to many of the leading causes of death in adults. The Adverse Childhood Experiences (ACE) Study. Am J Prev Med. 1998;14(4):245–258. [PubMed: 9635069]
18. Appleton AA, et al. Patterning in Placental 11-B Hydroxysteroid Dehydrogenase Methylation According to Prenatal Scocioeconomic Adversity. PLoS ONE 8(9): e74691. [PubMed: 24040322]

19. Marsit CJ, Maccini MA, Padbury JF, & Lester BM Placental 11-B Hydroxysteroid Dehydrogenase Methylation is associated with Newborn Growth and a Measure of Neurobehavioral Outcome. PLoS ONE 7(3): e33794. [PubMed: 22432047]
20. Breton CV., 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:–526.
21. Tiganescu A, Walker EA, Hardy RS, Mayes AE, & Stewart PM Localization, age- and site dependent expression, and regulation of 11beta-hydroxysteroid dehydrogenase type 1 in skin. J Invest Dermatol. 2011; 131(1):30–6. [PubMed: 20739946]
22. Maurer N, et al. Salivary and hair glucocorticoids and sleep in very preterm children during school age. Psychoneuroendocrinology. 2016; 72:166–174. [PubMed: 27434634]
23. Kirlic N, et al. Cortisol reactivity in two-year-old children prenatally exposed to methamphetamine. J Stud Alcohol Drugs. 2013 5; 74(3):447–51. [PubMed: 23490574]
24. Lester B, et al. Prenatal Cocaine Exposure Alters Cortisol Stress Reactivity in 11 Year Old Children. J Pediatr. 2010 8; 157(2): 288–295. [PubMed: 20400094]
25. Berko ER, et al. Mosaic Epigenetic Dysregulation of Ectodermal Cells in Autism Spectrum Disorder. PLoS Genet. 2014; 10(5): e1004402. [PubMed: 24875834]
26. Lowe R, et al. Buccals are likely to be a more informative surrogate tissue than blood for epigenome-wide association studies. Epigenetics. 2013, 8(4): 445–454. [PubMed: 23538714]
27. Everson TM, et al. Epigenome-wide Analysis Identifies Genes and Pathways Linked to Neurobehavioral Variation in Preterm Infants. Sci Rep. 2019;9(1):6322. [PubMed: 31004082]
28. Derogatis LR Brief Symptom Inventory(BSI): Administration, Scoring and Procedures Manual, Third Edition. 1993. Minneapolis, MN: National Computer Systems, Inc.
29. Beck AT, Steer RA, & Brown GK The Beck Depression Inventory manual, 2nd edition. Psychological Corporation, San Antonio, 1996.
30. Caldwell BM, & Bradley RH HOME Inventory Administration Manual(Standard Edition). Little Rock, AK: University of Arkansas, 2003.
31. Hollingshead A Four Factor Index of Social Status. New Haven, CT: Yale University, Department of Sociology, 1975.
32. LaGasse LL, et al. The Maternal Lifestyle Study (MLS): The Caretaking Environment of Infants Exposed to Cocaine/Opiates. Pediatr Res. 1999;45 (No. 4. Part 2).

Impact Statement:

- Prenatal methamphetamine exposure has been associated with developmental issues in newborns, yet little is known about the stress pathophysiology of methamphetamine on neurobehavior.
- This is the first evidence that prenatal methamphetamine exposure acts as a stressor, confirming the third pathophysiology of methamphetamine exposure.

Table 1.

Early adversity index binary indicators

Binary indicators of early adversity
any self-reported maternal substance use/abuse during the first 3 years of life
any extreme poverty experienced between birth and 5 years (as indicated by annual income <$ 10,000, approximately 50% of the U.S. Department of Health and Human Services poverty line for families with two to five members at the time of data collection)
any transition to new primary caregiver from birth through 5 years
any maternal subscale score on the Brief Symptom Inventory(28) greater than the clinical cut-off point (Derogatis, 1993) through 3 years
maternal depression at least one standard deviation above the mean from birth through 3 years indicated by the Beck Depression Inventory(29) (M=9.44, SD=7.03; Beck, Steer & Brown, 1996)
quality of the living environment at least one standard deviation below the mean at 2.5 years as indicated by the Home Inventory(30) (M=37.69, SD=4.39; Caldwell & Bradley, 2003)
community violence at least one standard deviation above the mean from birth through 3 years as indicated by the Neighborhood Problems section of the Lifestyle Interview (M=1.70, SD=1.84)
social position at least one standard deviation below the sample mean from birth through 5 years as indicated by the Index of Social Position which reflects a weighted average of parental occupational status and education level(31,32) (M=31.32, SD=8.92; see Hollingshead, 1975; LaGasse et al., 1999)

Table 2.

Comparison of dyads included and not included in the analysis

N (%) or mean (SD)	Included N=100	Not included N=212	P
Maternal/demographic characteristics			
Race			<0.01
White	33 (33.0%)	49 (23.1%)	
Hispanic	35 (35.0%)	51 (24.1%)	
Hawaii & Pacific Islander	15 (15.0%)	56 (26.4%)	
Asian	12 (12.0%)	45 (21.2%)	
Black	3 (3.0%)	11 (5.2%)	
American Indian	1 (1.0%)	0 (0.0%)	
Other	1 (1.0%)	0 (0.0%)	
Low SES	27 (27.0%)	59 (28.1%)	0.84
Partner at birth	53 (53.0%)	118 (55.7%)	0.66
Education <12 years	48 (48.0%)	95 (45.0%)	0.62
Prenatal care	95 (95.0%)	196 (92.5%)	0.40
Prenatal tobacco use	50 (50.0%)	115 (54.2%)	0.48
Average number of cigarettes/day across pregnancy	3.20 (5.87)	4.20 (7.40)	0.24
Prenatal alcohol use	30 (30.0%)	41 (19.3%)	0.04
Average oz. absolute alcohol/day across pregnancy	0.05 (0.30)	0.07 (0.43)	0.71
Prenatal marijuana use	17 (17.0%)	31 (14.6%)	0.59
Average number of joints/day across pregnancy	0.03 (0.15)	0.09 (1.03)	0.53
Prenatal methamphetamine (MA) use	55 (55.0%)	102 (48.1%)	0.26
Maternal age (yr)	25.24 (6.21)	25.38 (5.78)	0.85
Neonatal characteristics			
Sex (Male)	52 (52.0%)	115 (54.2%)	0.71
Birth weight (g)	3229 (642)	3234 (580)	0.95
Length (cm)	50.0 (3.9)	51.0 (3.0)	0.17
Head Circumference (cm)	33.8 (1.9)	33.9 (1.8)	0.77
Gestational age (wks)	38.4 (2.6)	38.7 (1.8)	0.25
Small for gestational age	10 (10.0%)	30 (14.2%)	0.31

Table 3.

Maternal and neonatal characteristics by MA exposure

N (%) or mean (SD)	PME N=55	Comparison N=45	P
Maternal/demographic characteristics			
Race			0.72
White	18 (32.7%)	15 (33.3%)	
Hispanic	18 (32.7%)	17 (37.8%)	
Hawaii & Pacific Islander	10 (18.2%)	5 (11.1%)	
Asian	7 (12.7%)	5(11.1%)	
Black	1 (1.8%)	2 (4.4%)	
American Indian	1 (1.8%)	0 (0.0%)	
Other	0 (0.0%)	1 (2.2%)	
Low SES	23 (41.8%)	4 (8.9%)	<0.01
Partner at birth	24 (43.6%)	29 (64.4%)	0.04
Education <12 years	31 (56.4%)	17 (37.8%)	0.06
Prenatal care	50 (90.9%)	45 (100.0%)	0.04
Prenatal tobacco use	40 (72.7%)	10 (22.2%)	<0.01
Average number of cigarettes/day across pregnancy	5.50 (7.06)	0.44 (1.50)	<0.01
Prenatal alcohol use	18 (32.7%)	12 (26.7%)	0.51
Average oz. absolute alcohol/day across pregnancy	0.09 (0.40)	0.01 (0.03)	0.18
Prenatal marijuana use	15 (27.3%)	2 (4.4%)	<0.01
Average number of joints/day across pregnancy	0.03 (0.14)	0.02 (0.15)	0.75
Maternal age (yr)	25.11 (5.79)	25.40 (6.75)	0.82
GA at 1st prenatal visit	13.6 (8.04)	8.2 (5.77)	<0.01
Neonatal characteristics			
Sex (Male)	31 (56.4%)	21 (46.7%)	0.33
Birth weight (g)	3109 (687)	3376 (554)	0.04
Length (cm)	48.7 (4.1)	51.6 (2.8)	<0.01
Head Circumference (cm)	33.5 (2.0)	34.2 (1.6)	0.05
Gestational age (wks)	37.75 (3.12)	39.11 (1.60)	<0.01
Small for gestational age	6 (10.9%)	4 (8.9%)	0.74
DNA methylation ofHSBUB2			
CpG site 1	1.3 (1.7)	1.6 (1.6)	0.52
CpG site 2	1.4 (1.7)	0.3 (0.6)	<0.01
CpG site 3	0.5 (0.7)	0.6 (0.8)	0.80
CpG site 4	3.8 (2.2)	3.6 (2.0)	0.69

Table 4.

Summary of multivariable analysis for predicting increased methylation at CpG2 of *HSD11B2*

	Model 1			Model 2			Model 3			Model 4		
Variable	***Estimate****	***95% CI***	***P***	***Estimate***	***95% CI***	***P***	***Estimate***	***95% CI***	***P***	***Estimate***	***95% CI***	***P***
Step 1 PME Exposure	0.34	0.11, 0.57	<0.01	0.23	−0.01, 0.46	0.06	0.24	0.002, 0.48	<0.05	0.32	0.04, 0.60	0.03
Step 2 Early adversity				0.11	0.03, 0.19	<0.05	0.11	0.03, 0.19	<0.01	0.11	0.02, 0.19	<0.05
Step 3 Cortisone							−0.13	−0.36, 0.09	0.23	−0.13	−0.36, 0.09	0.24
Step 4												
Average number of cigarettes/day										−0.01	−0.03, 0.02	0.57
Average absolute alcohol oz./day										−0.21	−0.60, 0.19	0.30
Average number of joints/day										0.39	−0.41, 1.2	0.34
Gestational age										0.02	0.02	0.38

*Estimate is unstandardized regression coefficient for percent change in DNA methylation at CpG site 2