NEW RESEARCH

Stage 2 Registered Report: Epigenetic Intergenerational Transmission: Mothers' Adverse Childhood Experiences and DNA Methylation

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Objective: Individual differences in risk for mental disorders over the lifespan are shaped by forces acting before the individual is born—in utero, but likely even earlier, during the mother's own childhood. The environmental epigenetics hypothesis proposes that sustained effects of environmental conditions on gene expression are mediated by epigenetic mechanisms. Recent human studies have shown that adversities in childhood are correlated with DNA methylation (DNAm) in adulthood. In the current study, we tested the following pre-registered hypotheses: Mothers' adverse childhood experiences (ACEs) are correlated with DNAm in peripheral blood during pregnancy (hypothesis 1) and in cord blood samples from newborn infants (hypothesis 2), and women's depression and anxiety symptoms during pregnancy mediate the association between mothers' ACE exposure and prenatal/neonatal DNA methylation (hypothesis 3).

Method: Data were from the Avon Longitudinal Study of Parents and Children Accessible Resource for Integrated Epigenomic Studies substudy. Women provided retrospective self-reports during pregnancy of ACE exposure. We conducted an epigenome-wide association study testing whether mothers' ACE exposure, cumulative score (0-10), was associated with DNAm in maternal antenatal blood and infant cord blood in more than 450,000 CpG (point on DNA sequence where cytosine and guanine base pairs are linked by a phosphate, where methylation usually occurs) sites on the Illumina 450K BeadChip. Analyses for cord blood were separated by infant sex, a pre-registered analysis.

Results: Hypothesis 1: In 896 mother−infant pairs with available methylation and ACE exposure data, there were no significant associations between mothers' ACE score and DNAm from antenatal peripheral blood, after controlling for covariates. Hypothesis 2: In infant cord blood, there were 5 CpG sites significantly differentially methylated in relation to mothers' ACEs (false discovery rate [FDR] $< .05$), but only in male offspring. Effect sizes were medium, with partial eta squared values ranging from 0.060 to 0.078. CpG sites were in genes related to mitochondrial function and neuronal development in the cerebellum. Hypothesis 3: There was no mediation by maternal anxiety/depression symptoms found between mothers' ACEs score and DNAm in the significant CpG sites in male cord blood. Mediation was not tested in antenatal peripheral blood, because no direct association between mothers' ACE score and antenatal peripheral blood was found.

Conclusion: Our results show that mothers' ACE exposure is associated with DNAm in male offspring, supporting the notion that DNAm could be a marker of intergenerational biological embedding of mothers' childhood adversity.

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Key words: DNA methylation; adverse childhood experiences; intergenerational transmission; longitudinal cohort; ALSPAC

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Individual differences in risk for mental disorders over the lifespan are shaped by forces acting before the individual is born—in utero, and likely even earlier, during the mother's own childhood. Understanding the biological mechanisms by which the environment programs future generations for mental health risk would expand the possibilities for designing and evaluating interventions to prevent and ameliorate the negative impact of adversities on future generations' mental health. The environmental epigenetics hypothesis proposes that sustained effects of environmental conditions on gene expression are influenced by epigenetic mechanisms, which can be transmitted across generations. Epigenetic mechanisms can include small non-coding RNA and other histone modifications such as acetylation; however, the most well-studied epigenetic mechanism is DNA methylation, which will be the focus of this article. Abundant animal data support intergenerational epigenetic transmission through DNAm,[1-6] yet human studies rigorously addressing this question are rare.[7]

Human studies provide evidence that within 1 generation, adverse childhood experiences (ACEs) are correlated with DNA methylation in saliva and peripheral blood later in childhood. A study of 96 maltreated children removed from their parents because of abuse or neglect and 96 demographically matched control children found that the 2 groups had significantly different methylation at 2868 CpG sites, many in genes relevant to neurodevelopmental processes.[8] Using a sample from the Bucharest Early Intervention Study, institutionalization in the first 3 years of life in Romanian orphanages was associated with DNA methylation in 2 stress-related genes (FKBP5 and SLC6A4) based on buccal swab samples at age 12 years.[9] Similarly, in another study, a methylome-wide analysis of 50 adoptees from Russia and Eastern Europe and 33 nonadopted youth, 30 CpG sites in 19 genes were found to be more highly methylated in the adopted group at age 15 years; functional analysis revealed that these differentially methylated sites were clustered in 223 genes related to neurodevelopmental processes.[10] A more recent study analyzed DNAm from blood (n = 92) and buccal cells (n = 124) from a socioeconomically diverse sample of 3-month-old infants from the Alberta Pregnancy Outcomes and Nutrition study, and found preliminary associations suggesting that infant DNAm patterns may relate to maternal ACEs.[11]

However, these and similar studies are limited by the following: (1) candidate-gene approaches, which look only at specific genes and CpG sites, precluding a broader understanding of methylation across the genome; or (2) methylome-wide approaches with small sample sizes (eg, <200), increasing both type I and type II errors. One recent exception is a methylome-wide analysis in 691 children followed longitudinally since birth in the Avon Longitudinal Study of Parents and Children (ALSPAC). In this genome-wide study, 38 CpG sites were differentially methylated in children at 7 years of age following exposure to adversities from birth to age 7 years, as reported prospectively by their parents.[12] Most of these differentially methylated sites were specific to the timing of adversity, predominantly associated with exposure before 3 years of age, indicating a potential early period of higher susceptibility. Genes annotated to differentially methylated CpG sites in this study relate to axon development and neuron apoptotic processes, suggesting relevance to neurodevelopment and future neuropsychiatric disorders.

To date, most studies have examined ACEs and DNA methylation within 1 generation and during a relatively brief time span, often during childhood.[8-10,12-17] However, studies that examine associations between ACEs exposure and DNA methylation decades later in adulthood provide evidence that these effects endure, with associations detected years after childhood adversities occur. Notably, a study in postmortem brain tissues of patients who died by suicide found that those with a history of early life adversity (n = 25) had 248 hypermethylated and 114 hypomethylated regions compared with individuals without a history of early life adversity (n = 16) and brains of patients who died not by suicide (n = 20).[18] As described in 2 recent systematic reviews, studies examining DNA methylation in peripheral blood in adults after exposure to childhood trauma also detect differential methylation.[19,20] However, many of these studies are limited by candidate gene approaches and/or small sample sizes, and findings have been difficult to replicate because of methodological differences, as the systematic reviews note, precluding robust conclusions about DNA methylation arising from childhood trauma and persisting into adulthood.

If ACEs contribute to DNA methylation in genes relevant to neurodevelopment and future neuropsychiatric disorders, and if methylation persists throughout the life course, the next question is whether methylation changes are passed on across generations. In adult offspring of Holocaust survivors, trauma before conception is related to methylation of FKBP5 in parents and in their adult offspring.[21] It remains unclear whether DNAm associated with parents' early life adversity is present at birth or whether it is acquired in offspring through adversity-related parenting and other factors over the life course. As the embryo develops, its DNA methylation marks are largely erased, making transmission seem unlikely.[22] However, it recently has been demonstrated that there is incomplete erasure in the oocyte and placenta, making across-generation ACE-associated DNAm transmission a possibility.[23,24] Another way that DNAm could be transmitted across generations is through alterations in the in utero environment. Evidence suggests that the in utero environment in women who have experienced adversities in childhood could produce DNAm changes that could program their offspring to have neurodevelopmental alterations.[25] Despite the theoretical possibility of intergenerational transmission of DNAm, no human methylome−wide studies to date have tested whether maternal ACEs are correlated with DNAm in their newborn offspring.

In the current study, we tested whether adverse experiences in mothers' childhoods are correlated with DNAm in the following: (1) mothers' peripheral blood during pregnancy, and (2) cord blood samples from their newborn infants. We use data from ALSPAC, in which 27% of participants reported experiencing at least 1 type of childhood adverse experiences such as physical or emotional abuse or parental death, and 17% reported more than 2.[26]

We also used a subset of ALSPAC, the Accessible Resource for Integrated Epigenetic Studies (ARIES).[27] This unique dataset includes genome-wide methylation data based on Illumina 450K bead chips using DNA from peripheral blood samples from more than 1,000 pregnant women and from umbilical cord blood from their infants collected at birth, as well as linked reports of mothers' childhood ACEs from ALSPAC.

We expected to find differentially methylated CpG sites in pregnant women and in their newborn infants associated with mothers' ACEs. We also hypothesized that the differentially methylated regions would at least partially overlap between mothers and infants, indicating enduring and transmitted impacts of mothers' ACEs above and beyond the accumulation of environmental influences that could change the mothers' methylomes across the life course.

As a secondary analysis, we tested prenatal depression and anxiety symptoms as a possible mediator of the association between mothers' ACE exposure and prenatal/neonatal DNA methylation. We specifically tested this mediation pathway for the following reasons: (1) ACEs have consistently been shown to be associated with perinatal mood and anxiety disorders,[28,29] and (2) depression and anxiety have been correlated with DNAm in adults,[30] and prenatal anxiety and depression have been associated with DNAm in infant offspring.[31,32]

We expected the associations between pregnant women's ACEs and pregnant women's and newborns' DNAm to be partially but not fully mediated by mothers' prenatal depression and anxiety symptoms, demonstrating a pathway from ACEs to DNAm above and beyond ACEs' impact on prenatal mood symptoms.

This analysis tested an extended timeframe for the impact of childhood adversity, namely, mothers' childhood affecting DNAm in her newborn offspring, and could provide evidence for a novel biological pathway for the enduring impact of childhood adversity across generations.

METHOD

We used an intergenerational longitudinal study design to test the association between ACEs and DNAm in pregnant women and their newborn offspring, and we examined prenatal maternal depression and anxiety symptoms as a possible mediator.

Sample

Data are from the ALSPAC ARIES substudy.[27] Pregnant women resident in Avon, UK, with expected dates of delivery between April 1, 1991, and December 31, 1992, were invited to take part in the study. A total of 20,248 pregnancies have been identified as being eligible, and the initial number of pregnancies enrolled was 14,541. Of the initial pregnancies, there was a total of 14,676 fetuses, resulting in 14,062 live births. An estimated 85% to 90% of the eligible population participated, and participating families resemble those in the United Kingdom, although ethnic minority representation is lower (3% vs 7.6%) (D. Baker, S. Morris, and H. Taylor, unpublished data, 1997). Women answered 4 questionnaires during pregnancy, at approximately 8 weeks' gestation, 12 weeks' gestation, 18 weeks gestation,' and 32 weeks' gestation, as well as at additional timepoints after birth. Details on the methodology of the ALSPAC study have been published previously.[33–35] We used data from the subset of 1,018 mother−offspring pairs included in the ARIES subsample, which was based on availability of DNA samples. Mothers in ARIES were slightly older, more likely to have a non-manual occupation, and less likely to have smoked throughout pregnancy compared to the overall ALSPAC sample.[27] Approval for data access from ALSPAC was provided by the ALSPAC Executive Committee. Please note that the study website contains details of all the data that are available through a fully searchable data dictionary and variable search tool and reference the following webpage: http://www.bristol.ac.uk/alspac/researchers/our-data/.

Ethical approval for the study was obtained from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committees. Consent for biological samples has been collected in accordance with the Human Tissue Act (2004). Informed consent for the use of data collected via questionnaires and clinics was obtained from participants following the recommendations of the ALSPAC Ethics and Law Committee at the time.

Measures

Maternal Adverse Childhood Experiences. For consistency with the literature, we chose to analyze a cumulative count of the 10 ACEs studied in the flagship CDC−Kaiser ACE study[36]: emotional abuse, physical abuse, sexual abuse, emotional neglect, physical neglect, loss of a parent, domestic violence, family member with addiction, family member with depression or another mental illness, and family member incarcerated. Rather than being asked as 1 "Adverse Childhood Adversities" questionnaire, ACE items were included separately in different questionnaires at different time points. Some ACEs were asked during pregnancy at approximately 18 weeks' gestation, others were asked at 32 weeks' gestation, and others were asked when the participant's child was approximately 3 years old. If the ACE item was ever endorsed when asked, that ACE was counted as present. Table 1 displays the 10 questions in ALSPAC that we used to create binary variables of each type

TABLE 1 Avon Longitudinal Study of Parents and Children Questions Used to Measure Adverse Childhood Experiences

Adverse childhood experiences domain	Question in Avon Longitudinal Study of Parents and Children
Emotional abuse	Parent emotionally cruel, affected me moderately or a lot
Physical abuse	Physically abused (eg, beaten), severely or somewhat
Sexual abuse	Sexually abused, affected me moderately or a lot
Emotional neglect	Neglected emotionally during my childhood, severely or somewhat
Physical neglect	Physically neglected as a child (eg, not fed or clothed properly), severely or somewhat
Loss of parent	Parent died or parents separated, affected me moderately or a lot
Domestic violence	When parents were together, they were violent, sometimes, frequently, or always
Family member with addiction	Mother or father known to have an alcohol problem
Family member with mental illness	Mother or father known to have depression OR parents mentally ill, affected me moderately or a lot
Family member incarcerated	Parent imprisoned, affected me moderately or a lot

of adversity. We then generated an ordinal variable summing the number of types of adversities reported (0-10). Although a cumulative risk score of ACEs has limitations, it is parsimonious and has been associated with multiple types of adult health problems.[36-38]

DNA Methylation Data. DNA samples were extracted from umbilical cord blood samples drawn upon delivery for infants and peripheral blood samples drawn at an antenatal clinic visit for mothers, at an average of 25.7 weeks' gestation (SD = 9.5). Following extraction, DNA was bisulfite converted using the Zymo EZ DNA Methylation kit, and genome-wide methylation status of more than 485,000 CpG sites was measured using the Illumina Infinium HumanMethylation450K BeadChip assay. The arrays were scanned using an Illumina iScan.

Quality Control and Data Processing Steps. Initial quality review was assessed by the ARIES study team using Illumina GenomeStudio (version 2011.1). Samples from all participant ages in ARIES were distributed across chips using a semi-random approach to minimize the possibility of confounding by batch effects. Batch variables were also recorded, as were quality control (QC) metrics from the standard control probes on the array for each sample. Samples failing QC were excluded from further analysis, and assays were repeated for those samples. As an additional QC step, genotype probes were compared with single nucleotide polymorphism (SNP)-chip data from the same individual to identify and remove any sample mismatches. For individuals with no genome-wide SNP data, samples were flagged if there was a sex mismatch based on X-chromosome methylation.

Additional QC steps were also performed by the ARIES team. Functional normalization was implemented in the R package meffil to normalize the data.[37] Functional normalization is a between-array normalization method for the Illumina Infinium HumanMethylation450 platform and an extension to quantile normalization. It removes unwanted technical variation by regressing out variability explained by the control probes present on the array. A dye bias correction was applied, and a detection p value of .01 was used to exclude probes with weak signals compared to background noise. Chip effects were regressed on the raw betas before normalization and on the control matrix. Each sample was normalized individually to a cell type reference dataset using meffil.cell.count.estimates.[38,39]

Covariates

The variables from ALSPAC that we included as covariates were measured as follows. Maternal age was calculated from maternal self-report of date of birth during the 8-week pregnancy interview; parity (number of previous live or still births) was self-reported by mothers at 18 weeks' gestation. For maternal smoking during pregnancy, the number of cigarettes smoked per day during the first trimester of pregnancy was self-reported around 18 weeks' gestation and was used as a continuous variable. Maternal education was self-reported at approximately 32 weeks' gestation and was categorized as follows: less than high school, high school but no continuing education, some college or technical training, or completed university. Gestational age at birth in weeks was taken from obstetrics records. Maternal pre-pregnancy body mass index (BMI) was calculated using maternal self-reports from 12 weeks' gestation of her weight and height before pregnancy. After receiving the ARIES data, we observed a mix of sample types in both the maternal antenatal blood and cord blood. Antenatal samples consisted of whole blood (56%) and white cells (44%). Cord blood samples consisted of white

cells (82%) and blood spots (18%). White cells were buffy coats (comprising lymphocytes, monocytes, and granulocytes) and were collected with heparin or ethylenediaminetetraacetic acid (EDTA) tubes. Blood spots were from cord blood samples taken at birth, not from heel prick. Therefore, we included sample type as an additional covariate in all analyses. Maternal ACE score was not significantly correlated with sample type (Wilcoxon test $p = .35$ in cord blood and $p = .61$ in antenatal blood).

Mediation

The Edinburgh Postnatal Depression Scale (EPDS)[40] was used to measure depression and anxiety symptoms in pregnant women at 18 and 32 weeks' gestation in ALSPAC. Although the EPDS is meant to measure depression and does not specifically measure anxiety disorders, items that do measure anxiety symptoms include item 4 ("I have been anxious or worried for no good reason") and item 5 ("I have felt scared or panicky for no very good reason").[41] Therefore, we used the summary score of the EPDS at 18 weeks' gestation as a single variable to test maternal depression/anxiety symptoms (EPDS score as a single variable) as a mediator in differentially methylated CpG sites.

Data Analysis

To test our first hypothesis—namely, that mothers' ACE exposure is associated with methylation in DNA from maternal peripheral blood during pregnancy—we used generalized linear regression models with a beta distribution. For each sample, the estimated proportion of molecules methylated at each CpG site was expressed as a beta value (β), which is the ratio of the methylated probe intensity and overall intensity ranging from 0 (no cytosine methylation) to 1 (complete cytosine methylation). Beta values for the $\sim$450,000 probes on the Illumina bead chip that pass QC checks were the outcome variables. The 0-10 ACE summary score was the main predictor variable. We considered the following variables to be potential confounders because of their known association with the exposure (maternal ACEs): maternal age at pregnancy, parity, maternal smoking during pregnancy, pre-pregnancy BMI, and maternal education. We therefore adjusted for those variables in all models, as well as the sample type that was used for the methylation analysis (ie, whole blood or white cells), as this could bias the DNA methylation results. Still, the potential for unmeasured confounding exists. We calculated the lambda statistic for each set of models[42] to estimate genomic inflation, that is, the extent of the effect of potential confounding on the observed associations.

To test our second hypothesis that mothers' ACE exposure is associated with methylation in DNA from newborn cord blood samples, we again used generalized linear regression models with a beta distribution, this time using DNA from cord blood. The outcome, predictor, and covariates were the same as in the previous analysis, except for the addition of gestational age at birth as a covariate. We conducted the analysis with newborn DNA separately in male and female newborns, based on evidence of sex differences in prenatal programming, including epigenetic alterations.[43,44]

For the ACE items forming the 0-10 ordinal variable, we pro-rated missing items with the average of the non-missing items when fewer than 3 items were missing. We treated individuals' scores with more than 2 ACE items missing as missing ACE scores. We planned to exclude covariates with more than 20% missing; however, all covariates had less than 20% of missing values (0%-4.1%). We used multiple imputation using all covariates via PcAux.[45,46]

Biological Significance

We examined the potential biological significance of the findings by the following: (1) examining the correlation in methylation between blood and brain tissue for the differentially methylated CpG sites using the online Blood Brain DNA Methylation Comparison Tool developed by Hannon *et al.*,[47] which reports correlations between DNAm in blood vs prefrontal cortex, entorhinal cortex, superior temporal gyrus, and cerebellum; and (2) performing a functional clustering analysis of all gene ontology terms for genes annotated to false discovery rate (FDR)-significant sites using the DAVID 6.8 database.[48] We report gene ontology clustering analyses only for significance at the FDR $q < 0.05$ level.

Mediation Analysis

Maternal depression and anxiety symptoms score (based on the EPDS) was tested as a mediator using linear structural equation models. Beta values at CpG sites found to be significant in the primary analyses were the outcome variables. The independent variable and covariates were the same as in the primary analyses. The EPDS total score was tested as the mediator. For mediation analysis, the mediation package in R statistical software was used with bootstrapping to estimate standard errors of the estimates.

Sensitivity Analyses

Although we expected little confounding from genetics, SNP-associated CpG sites were removed with the DMRcate package in R.[49]

We examined significant findings against the ARIES methylation quantitative trait loci (mQTL) catalogue as an additional sensitivity step to identify the possibility of confounding via genetic associations. Cell type composition was significantly different between sample types. We therefore added cell type composition as a covariate and compared in a Q-Q plot the p values of the results with and without controlling cell type composition.

Because different ACE items were asked at 3 different time points (18 and 32 weeks' gestation, and approximately 2 years 9 months after birth), there exists the possibility that questionnaire administration time may have an impact on ACE responses. To examine this issue, we tested whether the report of physical abuse was significantly different when it was asked at 32 weeks' gestation ("Hurt by parents?" Yes/No) and at 2 years 9 months after birth ("[Mother] physically abused as a child").

To address a recent report using the ARIES child cohort findings in which adversities occurring before age 3 in mothers had a particularly marked association with DNA methylation,[12] we had planned to repeat our analyses using adversities occurring before age 3 years in the mother as exposure. Because age of ACE occurrence was included only for some of the adversities in ALSPAC, we planned to include only those ACEs in the sensitivity analysis: namely, sexual abuse, physical abuse, and parental divorce or death. Reporting 1 or more of these adversities before age 3 years was coded as 1, and not experiencing any was coded as 0. We hypothesized that methylation associated with adverse experiences occurring before the mothers were 3 years of age would be stronger than the results from the primary analyses using ACE score before age 18.

RESULTS

The ARIES sample with available methylation data for both antenatal blood and cord blood was 1,006 mother−infant pairs. Of those, 896 (89%) had non-missing ACE scores (defined as having less than 3 missing ACE items). Thus, n = 896 mother−infant pairs was the final sample for our analysis. Average ACE score in the mothers was 1.27, and 10.5% of women reported having experienced 3 or more ACEs. The average EPDS score was 6.26 (SD = 4.55). Of the women, 17.97% reached the cutoff of above 10, which is recommended for detecting a probable case of major depression in pregnant women.[50] Women with higher EPDS scores were more likely to have higher ACE scores and to smoke during pregnancy. Descriptive statistics are provided in Tables 2 and 3.

Mothers' ACEs and DNA Methylation

There were no significant associations between a summary score of mothers' ACEs and DNA methylation from peripheral blood in pregnancy, after controlling for covariates.

TABLE 2 Avon Longitudinal Study of Parents and Children Descriptive Statistics (n = 896)

	Mean	SD	Min	Max
ACE score	1.27	1.53	0	8
Prenatal anxiety/depression (EPDS)	6.26	4.55	0	25
Maternal age, y	29.73	4.27	16	42
Parity	0.74	0.83	0	5
Smoking during pregnancy, cigarettes/day	1.02	3.74	0	30
Gestational age at birth, wk	39.59	1.51	30	44
Pre-pregnancy BMI, kg/m^2	22.7	3.63	14.23	45.24
Smoking during pregnancy, yes, %	12.09			
Maternal education, %				
Less than high school	7.25			
Highschool	34.15			
Tech/ Some college	37.50			
University	21.09			
Infant sex, %				
Female	51.39			
Male	48.61			

Note: ACE = adverse childhood experience; BMI = body mass index; EPDS = Edinburgh Postnatal Depression Scale.

TABLE 3 Correlations Between Edinburg Postnatal Depression Score in the Second Trimester of Pregnancy and Other Variables

Variable	Pearson correlation	*p*
Continuous variables		
ACE score	0.1653	<.0001
Maternal age	−0.0504	.1399
Parity	0.0604	.0794
Smoking during pregnancy, cigarettes/d	0.1560	<.0001
GA at birth	−0.0613	.163
Pre-pregnancy BMI	0.0207	.556
	R^2 (GLM)	*p*
Categorical variables		
Smoking during pregnancy (yes/no)	0.0405	<.0001
Education	0.009	.04964
Sex	0.002	.3645

Note: ACE = adverse childhood experience; BMI = body mass index; GA = gestational age; GLM = generalized linear model.

In terms of DNAm in infant cord blood, there were no significant associations between mothers' ACE score and DNAm in infant cord blood in the overall sample. However, when dividing analyses by infant sex, in relation to mothers' ACEs there were 5 CpG sites significantly differentially methylated in male cord blood DNA after controlling for covariates and controlling the FDR at 0.05. Of these, 4 were hyper-methylated, and 1 was hypomethylated. Effect sizes were medium, with partial eta squared values ranging from 0.06 to 0.078. The lambda statistic to measure genomic inflation was 2.21. These results are depicted in Figures 1 and 2 and Table 4. These sites were not differentially methylated overall by infant sex independent of maternal ACEs (*p* values = .08-.68).

Biological Significance

To evaluate potential neurological implications of our findings, we tested correlations between blood and brain DNAm using the online Blood Brain DNA Methylation Comparison Tool developed by Hannon *et al.*[47] Two of the significantly differentially methylation CpG sites in male cord blood (cg03425217 and cg19447686) showed a positive, significant correlation between blood and entorhinal cortex ($r = .33$, $p = .005$, and $r = 0.28$, $p = .017$), and 2 sites (cg19447686 and cg00279392) showed a positive, significant correlation between blood and cerebellum ($r = 0.26$, $p = .028$, and $r = 0.23$, $p = .057$).

The DAVID database did not return any significantly enriched gene ontology biological process terms common to the genes annotated to the significant hits in our analysis, likely because of the small number of genes represented. Figure S1, available online, provides a functional annotation table of genes annotated to significant CpG sites in our analyses.

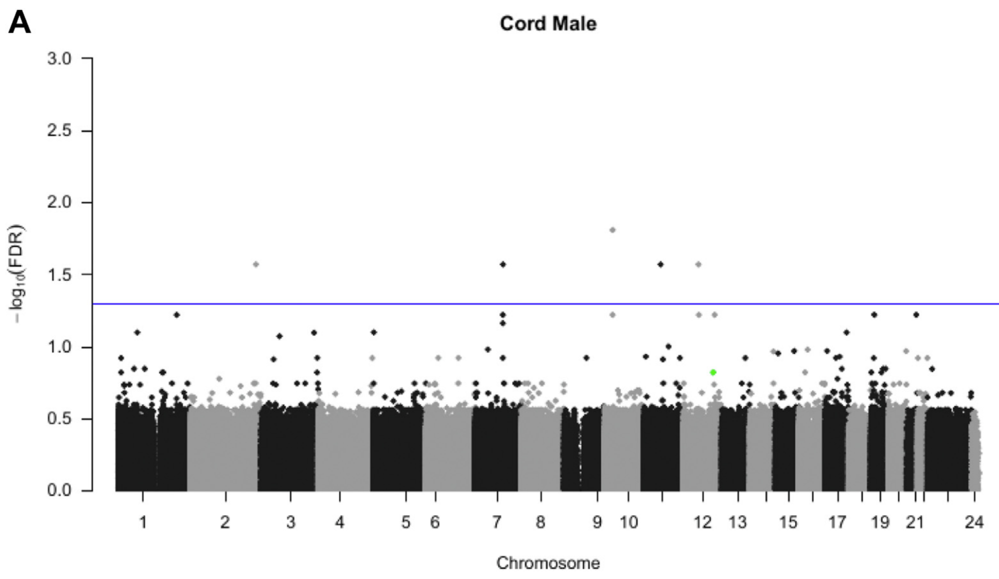
In terms of the specific genes and gene locations of the significant CpG sites, YME1L1 (cg03425217) and TIMM10 (cg00279392) code proteins important for mitochondrial function. The CpG sites are also on the promoter regions of these genes and are associated with transcription start sites. The YME1L1 differentially methylated site (cg03425217) is also located on a CpG island, a dense concentration of primarily unmethylated CpG sites near promoter regions of genes. The protein encoded by TENC1 (cg19447686) is a focal adhesion molecule that binds to actin filaments and participates in signaling pathways. This protein plays a role in regulating cell migration. The CpG site differentially methylated on TENC1 is on an island, and it is in a promoter region of this gene. DOCK10 (cg04318855) is essential for dendritic spine morphogenesis in hippocampal neurons and has been identified as a biomarker for predicting depression.[51] The differentially methylated CpG site on this gene (cg04318855) is associated with a promoter, potentially signaling important functional implications for gene expression. It is also located on an island shore, regions up to 2 kilobase pairs away from a CpG island. DNAm in CpG islands and island shores is considered more likely to affect gene expression.

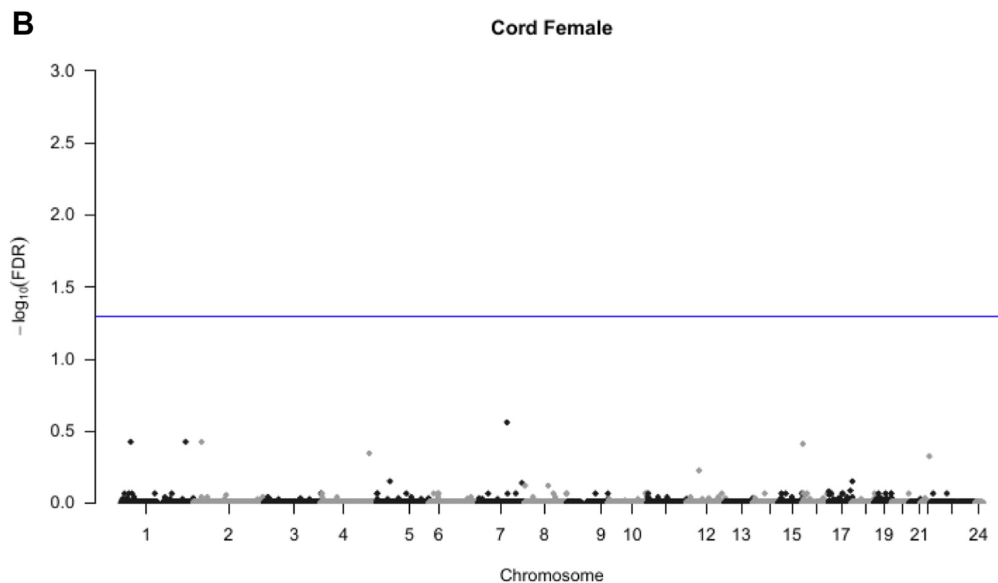
Analyses

Mediation Analysis. Maternal depression and anxiety symptoms (EPDS score) did not mediate the association between mothers' ACEs and methylation at the 5 significant CpG sites in male cord blood DNA. The *p* values for average causal mediation effects ranged from .22 to .84. Because we did not find significant associations between mothers' ACEs and methylation in maternal antenatal blood, we did not carry out the maternal ACEs→EPDS→DNAm in maternal antenatal blood mediation analysis.

Sensitivity Analyses. Significant CpG sites were checked against the ARIES mQTL catalog and were not among the mQTLs. Adding cell type composition as a covariate did not meaningfully change the results. The Q-Q plot of *p* values for cord blood is provided in Figure S2, available online. Because not all individual ACE questions were asked at the same time point, we tested whether maternal report of physical abuse (1 ACE that was asked at 2 different timepoints) was consistent when it was asked at 32 weeks' gestation ("Were your parents physically cruel?" Yes/No) and at 2 years 9 months after birth ("Were you physically

FIGURE 1 Manhattan Plots of Epigenome-Wide Association Study of Mothers' Adverse Childhood Experiences in Relation to Methylation in Male and Female Cord Blood



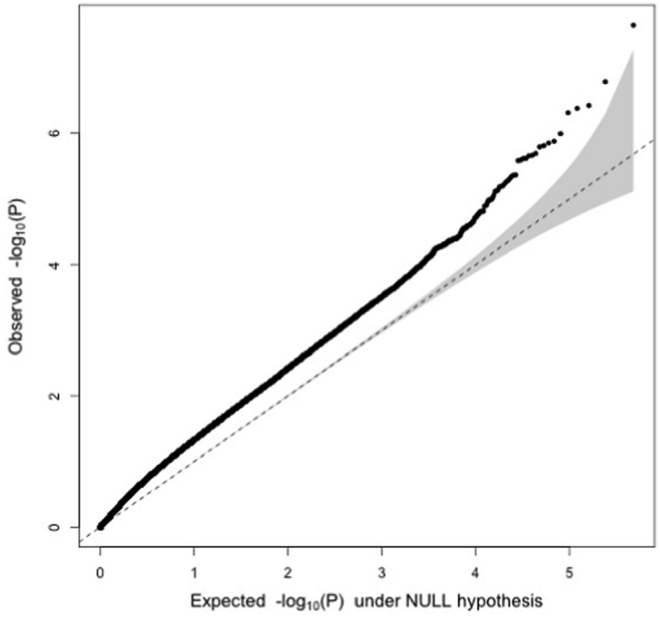


Note: Horizontal line represents a false discovery rate p *value of .05.*

abused [eg, beaten] as a child?"). The kappa coefficient was 0.47 (95% CI = 0.32-0.62), showing moderate reliability over time,[52] even though the question was not asked in the exact same way. We attempted to repeat our analyses using only adversities that occurred before age 3 years in the mother as the exposure. However, only 34 women (3.96% of the sample) endorsed any of the 3 ACEs with age data before age 3 years (physical abuse, sexual abuse, and loss of a parent), and we were therefore underpowered to perform the sensitivity analysis.

Exploratory Analysis. In an additional analysis, we removed the maternal smoking covariate. ACEs are significantly associated with smoking behavior in adulthood,[53] and smoking is strongly associated with DNAm across the genome.[54] We therefore reasoned that smoking could mediate an association between ACEs and DNAm in maternal blood. Excluding smoking as a covariate, maternal ACE score was significantly correlated with DNAm in 2 CpG sites in maternal antenatal blood. The Manhattan plot for these 2 CpG sites is shown in Figure 3. The gene

FIGURE 2 Q-Q Plot Showing Lambda Statistic to Measure Genomic Inflation (2.21)



annotated to 1 of these sites, AHRR (cg05575921), is highly associated with smoking status.[55] The other (cg21566642) is on a H3K27Ac mark, a histone modification tied to transcription.

DISCUSSION

To our knowledge, this is the first study to report the association between mothers' ACE exposure and genome-wide DNAm in newborn offspring. Our results show that mothers' ACE exposure is associated with differential methylation, primarily hyper-methylation, in 5 CpG sites in male umbilical cord blood. These CpG sites are in gene areas likely to affect gene expression—promoters, CpG islands, and CpG island shores. They are in genes related to mitochondrial function and a gene, DOCK10, that has been proposed as a biomarker for depression. These results support the notion that DNAm may be a biological substrate of intergenerational effects of mothers' childhood adversity. However, although the functions of the genes with differentially methylated CpG sites may suggest biological pathways of intergenerational transmission, these results show 5 significant CpG sites on disparate genes across the genome and therefore do not in themselves provide strong evidence of specific biological processes. Still, the locations of these CpG sites could be used to suggest hypotheses for potential mechanisms for how adversity becomes embedded biologically across generations. To our knowledge, this is the first epigenome-wide association study in humans of the association between maternal ACEs and newborn methylation.

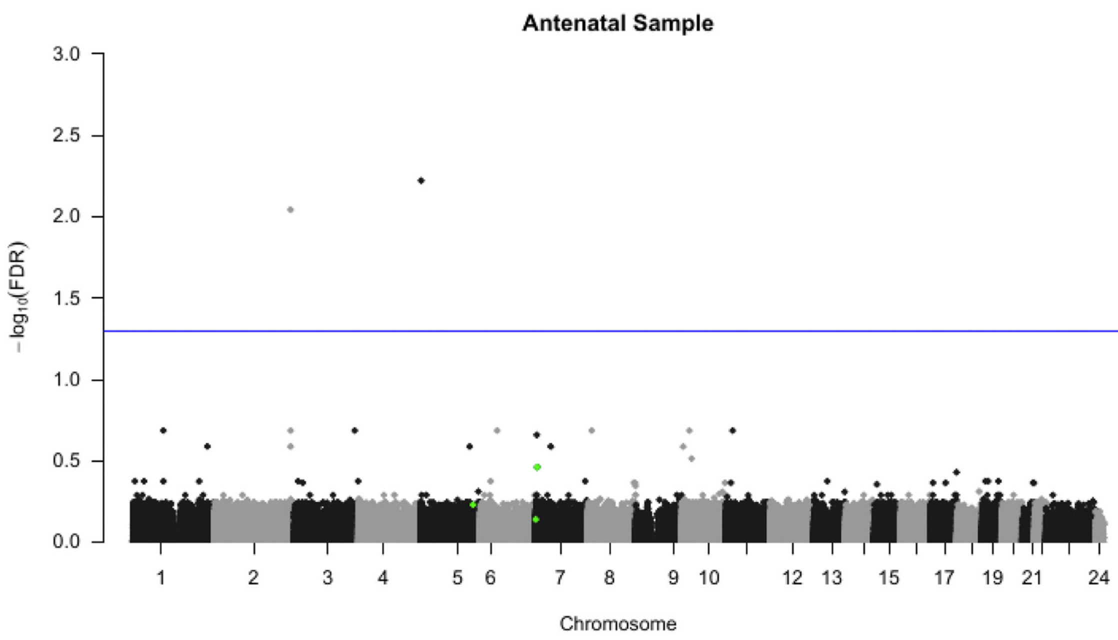
Findings on mothers' childhood adversity and DNAm in maternal antenatal blood were less clear. In our covariate-adjusted analysis, we did not find any CpG sites with methylation significantly correlated with mothers' ACE scores. Two recent systematic reviews have described the existing literature on childhood maltreatment and DNAm in adulthood.[19,20] Although the 2 reviews interpret the evidence as overall supportive of an association, the authors agreed that conclusions are limited by low comparability across studies and other methodological limitations. The majority of existing studies use maltreatment, or specific types of maltreatment, as the exposure, as opposed to an overall adversity score, limiting direct comparison with our analysis. Furthermore, the few studies that use a genome-wide approach, rather than a candidate-gene approach, examine primarily a composite DNAm measure (such as epigenetic age) or regional analyses, rather than reporting site-specific DNAm across a methylation array. However, 1 previous study has examined ACEs and site-specific DNAm in women from the ALSPAC dataset. Using a summary score of 7 ACEs (rather than the 10 in our analysis), the study also found no significantly

TABLE 4 Maternal Adverse Childhood Experiences Scores Associated With Five Differentially Methylated CpG Sites in Male Umbilical Cord Blood

CpG	Gene	β	FDR-adjusted *p* value	Partial eta squared	Partial eta- squared CI
cg03425217	YME1L1	0.004	.015	0.078	0.034-0.134
cg00279392	TIMM10	−0.005	.039	0.064	0.025-0.116
cg04318855	DOCK10	0.003	.045	0.064	0.025-0.117
cg08828819	PON3	0.003	.045	0.070	0.029-0.124
cg19447686	TENC1	0.001	.045	0.060	0.023-0.112

Note: *FDR = false discovery rate.*

FIGURE 3 Manhattan Plot of Epigenome-Wide Association Study of Methylation in Antenatal Blood Associated With Mothers' Adverse Childhood Experiences



differentially methylated CpG sites in a site-by-site analysis, which included smoking as a covariate.[56] However, in our analysis, mothers' ACE score was significantly correlated with DNAm in 2 CpG sites when smoking was not included as a covariate. Given high correlations between ACEs and smoking behavior,[53] and smoking and DNAm of AHRR[55] (1 of the genes displaying a differentially methylated CpG site), lifestyle factors related to childhood adversity—in this case, smoking—may be responsible for DNAm changes in adulthood.[54]

We aimed to test whether childhood adversity was related to DNAm in pregnant women and whether that adversity-related DNAm was passed on to newborn infants. We hypothesized that such intergenerational transmission could occur through germline inheritance or through changes to the in utero environment. Given that we did not find maternal ACEs to be correlated with DNAm in maternal antenatal blood when controlling for covariates, and in no analyses did we find ACE-associated DNAm in the same CpG sites or genes in mothers and infants, our results lend more support to the hypothesis that maternal childhood adversity has biological impacts on the next generation through changes in the in utero environment, rather than through germline transmission. Alternatively, germline transmission could be at play, but may not be detected in the present study if it is a more subtle effect. A broad set of factors related to mothers' childhood adversity could influence the in utero environment. These could include aspects of the external environment correlated with adversity (eg, pollution, chemical exposure, built environment, etc). They could include psychological factors related to long-term consequences of childhood trauma, including stress, social support, or relationship quality as well as lifestyle factors, including diet, exercise, smoking. In terms of smoking, the 5 CpG sites in cord blood correlated with maternal ACEs in our data were not among the more than 6,000 CpG sites in cord blood found to be differentially methylated in relation to maternal smoking in pregnancy in a meta-analysis,[57] although 2 of the genes represented were the same (DOCK 10 and PON3). However, a recent study in a large, multi-ethnic cohort in the United States ($n = 954$ mother−newborn pairs) found that cord blood DNAm significantly mediated the association between smoking during pregnancy and lower birth weight, and 2 significant CpG sites in that analysis were on the gene AHRR[58] (1 of the genes displaying a differentially methylated CpG site in maternal antenatal blood in relation to maternal ACEs). Interestingly, studies have shown sex-specific infant DNAm correlated with maternal smoking during pregnancy.[59-61]

Sex differences were clear in our analysis. Maternal ACE−associated methylation in infant cord blood was found only in male but not in female individuals. This is consistent with findings on vulnerability of male fetuses to

environmental insults.[44] Being male is a known risk factor for neurodevelopmental disorders, and DNAm is considered to be part of the mechanism conferring this risk.[62] Animal models have shown that females have higher levels of methylation of CpG sites throughout the genome, and expression of genes involved in immune regulation are strongly epigenetically suppressed.[63] Our results contribute to the findings on sex differences in DNAm in humans, and extend the timeframe to exposures that occur in the previous generation.

Several limitations should be noted. The prevalence of ACEs in the ALSPAC sample is relatively low, although this is the case for much existing literature on the topic. Studies in samples exposed to relatively low levels of adversity, even with large sample sizes, might fail to detect correlations, particularly those with small effect sizes, as is the case with psychosocial exposures and DNAm. Because our analysis was not performed in a population highly exposed to adversities, our findings might not generalize to such populations. Of note, only 3% of ALSPAC mothers self-identified as belonging to a racial/ethnic minority group. Unfortunately, populations highly exposed to adversity and minoritized are those most affected by intergenerational transmission of sequelae of childhood adversity. Marginalized racial and ethnic groups, including Black and Latinx populations, sustain a higher exposure to and experience more ACEs than their White counterparts.[64] Future research should therefore focus on populations with very high exposure to adversity.

Our analysis uses a summary score of ACEs because it is parsimonious and has been associated with multiple types of adult health problems.[37,38] A short-coming of this approach is that our analyses do not include other characteristics of adverse childhood experiences that could differentially affect DNAm, including specific type of adversity, chronicity, age of first occurrence, and, for abuse, the relationship to the perpetrator. As is the case with the majority of literature on maternal childhood adversities, measurement of mothers' ACEs was retrospective. A recent meta-analysis found that correlations between retrospective and prospective reports are very low ($k = 0.19$),[65] although this study may underestimate the correlation, as it relies on corroborated abuse via findings reported to police or social services, which may under-count significant abuse that goes under-reported. One possibility is that that depression during pregnancy may cause an over-reporting of childhood adversity.[66] However, subjective reports of childhood maltreatment more accurately predict the development of psychopathology than objective reports based on court records.[65-67] This suggests that it is more likely that childhood adversity causes higher depression symptomatology in pregnancy, rather than that depression symptomatology in pregnancy causes higher retrospective reports of childhood adversity. In terms of unmeasured confounding, maternal drug use (both psychotropic medications and illicit drugs) could have led to unmeasured confounding; however, we did not have access to those data. In terms of the biological samples, it would have been ideal to have methylation analyses performed on the same sample type for all participants, which was not the case in the ALSPAC AIRES methylation data. However, sample type was not significantly related to ACE score, and we controlled for sample type as a covariate.

Our results show that mothers' ACE exposure is associated with DNAm in male offspring, supporting the notion that DNAm could be a marker of intergenerational biological embedding of mothers' childhood adversity. Future work through sequencing and transcription analyses could examine whether maternal ACEs are related to expression of these genes, which are involved in cellular functioning, including mitochondrial functioning, neuronal development in the cerebellum, and cellular signaling. Given that we did not find overlapping ACE-associated DNAm in mothers and their offspring, our results support the idea that ACE-related factors cause changes to the in utero environment. If this is the case, research on perinatal and pre-conception interventions to support at-risk future mothers should examine whether improvements in women's lives brought about by these interventions can change DNAm in their offspring, and possibly interrupt the intergenerational transmission of risk for psychopathology.

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REFERENCES

1. Bock J, Poeschel J, Schindler J, *et al*. Transgenerational sex-specific impact of preconception stress on the development of dendritic spines and dendritic length in the medial prefrontal cortex. Brain Struct Funct. 2016;221(2):855-863. https://doi.org/10.1007/s00429-014-0940-4
2. Zaidan H, Leshem M, Gaisler-Salomon I. Prereproductive stress to female rats alters corticotropin releasing factor type 1 expression in ova and behavior and brain corticotropin releasing factor type 1 expression in offspring. Biol Psychiatry. 2013;74(9):680-687. https://doi.org/10.1016/j.biopsych.2013.04.014
3. Modir F, Elahdadi Salmani M, Goudarzi I, Lashkarboluki T, Abrari K. Prenatal stress decreases spatial learning and memory retrieval of the adult male offspring of rats. Physiol Behav. 2014;129:104-109. https://doi.org/10.1016/j.physbeh.2014.02.040
4. Pisu MG, Garau A, Olla P, *et al*. Altered stress responsiveness and hypothalamic-pituitary-adrenal axis function in male rat offspring of socially isolated parents. J Neurochem. 2013;126(4):493-502. https://doi.org/10.1111/jnc.12273
5. Rompala GR, Finegersh A, Homanics GE. Paternal preconception ethanol exposure blunts hypothalamic-pituitary-adrenal axis responsivity and stress-induced excessive fluid intake in male mice. Alcohol. 2016;53:19-25. https://doi.org/10.1016/j.alcohol.2016.03.006
6. Yao Y, Robinson AM, Zucchi FC, *et al*. Ancestral exposure to stress epigenetically programs preterm birth risk and adverse maternal and newborn outcomes. BMC Med. 2014;12(1):121. https://doi.org/10.1186/s12916-014-0121-6
7. Weaver IC, Cervoni N, Champagne FA, *et al*. Epigenetic programming by maternal behavior. Nat Neurosci. 2004;7(8):847-854. https://doi.org/10.1038/nn1276
8. Yang BZ, Zhang H, Ge W, *et al*. Child abuse and epigenetic mechanisms of disease risk. Am J Prev Med. 2013;44(2):101-107. https://doi.org/10.1016/j.amepre.2012.10.012
9. Non AL, Hollister BM, Humphreys KL, *et al*. DNA methylation at stress-related genes is associated with exposure to early life institutionalization. Am J Phys Anthropol. 2016;161(1):84-93. https://doi.org/10.1002/ajpa.23010
10. Esposito EA, Jones MJ, Doom JR, MacIsaac JL, Gunnar MR, Kobor MS. Differential DNA methylation in peripheral blood mononuclear cells in adolescents exposed to significant early but not later childhood adversity. Dev Psychopathol. 2016;28(4pt2):1385-1399. https://doi.org/10.1017/S0954579416000055
11. Moore SR, Merrill SM, Sekhon B, *et al*. Infant DNA methylation: an early indicator of intergenerational trauma? Early Hum Dev. 2022;164:105519. https://doi.org/10.1016/j.earlhumdev.2021.105519
12. Dunn EC, Soare TW, Zhu Y, *et al*. Sensitive periods for the effect of childhood adversity on DNA methylation: results from a prospective, longitudinal study. Biol Psychiatry. 2019;85(10):838-849. https://doi.org/10.1016/j.biopsych.2018.12.023
13. Cicchetti D, Hetzel S, Rogosch FA, Handley ED, Toth SL. An investigation of child maltreatment and epigenetic mechanisms of mental and physical health risk. Dev Psychopathol. 2016;28(4pt2):1305-1317. https://doi.org/10.1017/S0954579416000869
14. Naumova OY, Lee M, Koposov R, Szyf M, Dozier M, Grigorenko EL. Differential patterns of whole-genome DNA methylation in institutionalized children and children raised by their biological parents. Dev Psychopathol. 2012;24(1):143-155.
15. Hecker T, Radtke KM, Hermenau K, Papassotiropoulos A, Elbert T. Associations among child abuse, mental health, and epigenetic modifications in the proopiomelanocortin gene (POMC): a study with children in Tanzania. Dev Psychopathol. 2016;28(4pt2):1401-1412.
16. Kumsta R, Marzi SJ, Viana J, *et al*. Severe psychosocial deprivation in early childhood is associated with increased DNA methylation across a region spanning the transcription start site of CYP2E1. Transl Psychiatry. 2016;6(6):e830. https://doi.org/10.1038/tp.2016.95
17. Tyrka AR, Ridout KK, Parade SH, Paquette A, Marsit CJ, Seifer R. Childhood maltreatment and methylation of FK506 binding protein 5 gene (FKBP5). Dev Psychopathol. 2015;27(4 Pt 2):1637-1645. https://doi.org/10.1017/S0954579415000991
18. Labonte B, Suderman M, Maussion G, *et al*. Genome-wide epigenetic regulation by early-life trauma. Arch Gen Psychiatry. 2012;69(7):722-731. https://doi.org/10.1001/archgenpsychiatry.2011.2287
19. Cecil CAM, Zhang Y, Nolte T. Childhood maltreatment and DNA methylation: a systematic review. Neurosci Biobehav Rev. 2020;112:392-409. https://doi.org/10.1016/j.neubiorev.2020.02.019
20. Parade SH, Huffhines L, Daniels TE, Stroud LR, Nugent NR, Tyrka AR. A systematic review of childhood maltreatment and DNA methylation: candidate gene and epigenome-wide approaches. Transl Psychiatry. 2021;11(1):134. https://doi.org/10.1038/s41398-021-01207-y
21. Yehuda R, Daskalakis NP, Bierer LM, *et al*. Holocaust exposure induced intergenerational effects on FKBP5 methylation. Biol Psychiatry. 2016;80(5):372-380. https://doi.org/10.1016/j.biopsych.2015.08.005
22. Guo H, Zhu P, Yan L, *et al*. The DNA methylation landscape of human early embryos. Nature. 2014;511(7511):606-610. https://doi.org/10.1038/nature13544
23. Lee HJ, Hore TA, Reik W. Reprogramming the methylome: erasing memory and creating diversity. Cell Stem Cell. 2014;14(6):710-719. https://doi.org/10.1016/j.stem.2014.05.008
24. Hanna CW, Penaherrera MS, Saadeh H, *et al*. Pervasive polymorphic imprinted methylation in the human placenta. Genome Res. 2016;26(6):756-767. https://doi.org/10.1101/gr.196139.115
25. Buss C, Entringer S, Moog NK, *et al*. Intergenerational transmission of maternal childhood maltreatment exposure: implications for fetal brain development. J Am Acad Child Adolesc Psychiatry. 2017;56(5):373-382. https://doi.org/10.1016/j.jaac.2017.03.001
26. Collishaw S, Dunn J, O'Connor TG. Golding J, Avon Longitudinal Study of Parents and Children Study Team. Maternal childhood abuse and offspring adjustment over time. Dev Psychopathol. 2007;19(2):367-383. https://doi.org/10.1017/S0954579407070186
27. Relton CL, Gaunt T, McArdle W, *et al*. Data resource profile: Accessible Resource for Integrated Epigenomic Studies (ARIES). Int J Epidemiol. 2015;44(4):1181-1190. https://doi.org/10.1093/ije/dyv072
28. Choi KW, Sikkema KJ. Childhood maltreatment and perinatal mood and anxiety disorders: a systematic review. Trauma Violence Abuse. 2016;17(5):427-453. https://doi.org/10.1177/1524838015584369
29. Hughes K, Bellis MA, Hardcastle KA, *et al*. The effect of multiple adverse childhood experiences on health: a systematic review and meta-analysis. Lancet Public Health. 2017;2(8):e356-e366. https://doi.org/10.1016/S2468-2667(17)30118-4

30. Jovanova OS, Nedeljkovic I, Spieler D, *et al*. DNA methylation signatures of depressive symptoms in middle-aged and elderly persons: meta-analysis of multiethnic epigenome-wide studies. JAMA Psychiatry. 2018;75(9):949-959. https://doi.org/10.1001/jamapsychiatry.2018.1725
31. Sosnowski DW, Booth C, York TP, Amstadter AB, Kliewer W. Maternal prenatal stress and infant DNA methylation: a systematic review. Dev Psychobiol. 2018;60(2):127-139. https://doi.org/10.1002/dev.21604
32. Czamara D, Eraslan G, Page CM, *et al*. Integrated analysis of environmental and genetic influences on cord blood DNA methylation in new-borns. Nat Commun. 2019;10(1):2548. https://doi.org/10.1038/s41467-019-10461-0
33. Golding J, Pembrey M, Jones R, Team AS. ALSPAC—the Avon Longitudinal Study of Parents and Children. I. Study methodology. Paediatr Perinat Epidemiol. 2001;15(1):74-87. https://doi.org/10.1046/j.1365-3016.2001.00325.x
34. Boyd A, Golding J, Macleod J, *et al*. Cohort profile: the 'children of the 90s'—the index offspring of the Avon Longitudinal Study of Parents and Children. Int J Epidemiol. 2013;42(1):111-127.
35. Fraser A, Macdonald-Wallis C, Tilling K, *et al*. Cohort profile: the Avon Longitudinal Study of Parents and Children: ALSPAC mothers cohort. Int J Epidemiol. 2013;42(1):97-110.
36. Felitti VJ, Anda RF, Nordenberg D, *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. 2019;56(6):774-786. https://doi.org/10.1016/j.amepre.2019.04.001
37. Evans GW, Li D, Whipple SS. Cumulative risk and child development. Psychol Bull. Nov. 2013;139(6):1342-1396. https://doi.org/10.1037/a0031808
38. Rasmussen LJH, Moffitt TE, Eugen-Olsen J, *et al*. Cumulative childhood risk is associated with a new measure of chronic inflammation in adulthood. J Child Psychol Psychiatry. 2019;60(2):199-208. https://doi.org/10.1111/jcpp.12928
39. Min JL, Hemani G, Davey Smith G, Relton C, Suderman M. Meffil: efficient normalization and analysis of very large DNA methylation datasets. Bioinformatics. 2018;34(23):3983-3989.
40. Cox JL, Holden JM, Sagovsky R. Detection of postnatal depression. Development of the 10-item Edinburgh Postnatal Depression Scale. Br J Psychiatry. 1987;150(6):782-786. https://doi.org/10.1192/bjp.150.6.782
41. Brouwers EP, van Baar AL, Pop VJ. Does the Edinburgh Postnatal Depression Scale measure anxiety? J Psychosom Res. 2001;51(5):659-663.
42. Guintivano J, Shabalin AA, Chan RF, *et al*. Test-statistic inflation in methylome-wide association studies. Epigenetics. 2020;15(11):1163-1166.
43. Rosenfeld CS. Sex-specific placental responses in fetal development. Endocrinology. 2015;156(10):3422-3434. https://doi.org/10.1210/en.2015-1227
44. Bale TL. Sex differences in prenatal epigenetic programing of stress pathways. Stress. 2011;14(4):348-356. https://doi.org/10.3109/10253890.2011.586447
45. Howard WJ, Rhemtulla M, Little TD. Using principal components as auxiliary variables in missing data estimation. Multivariate Behav Res. 2015;50(3):285-299.
46. Lang K, Little T. PcAux Development Team. PcAux: automatically extract auxiliary features for simple, principled missing data analysis. R package version 0.0. 0.9010. R Foundation for Statistical Computing; 2017.
47. Hannon E, Lunnon K, Schalkwyk L, Mill J. Interindividual methylomic variation across blood, cortex, and cerebellum: implications for epigenetic studies of neurological and neuropsychiatric phenotypes. Epigenetics. 2015;10(11):1024-1032. https://doi.org/10.1080/15592294.2015.1100786
48. da Huang W, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc. 2009;4(1):44-57. https://doi.org/10.1038/nprot.2008.211
49. Peters TJ, Buckley MJ, Statham AL, *et al*. De novo identification of differentially methylated regions in the human genome. Epigenetics Chromatin. 2015;8:6.
50. Levis B, Negeri Z, Sun Y, Benedetti A, Thombs BD. Accuracy of the Edinburgh Postnatal Depression Scale (EPDS) for screening to detect major depression among pregnant and postpartum women: systematic review and meta-analysis of individual participant data. BMJ. 2020;371.
51. Le-Niculescu H, Roseberry K, Gill SS, *et al*. Precision medicine for mood disorders: objective assessment, risk prediction, pharmacogenomics, and repurposed drugs. Mol Psychiatry. 2021;26(7):2776-2804. https://doi.org/10.1038/s41380-021-01061-w
52. McHugh ML. Interrater reliability: the kappa statistic. Biochem Med (Zagreb). 2012;22(3):276-282.
53. Anda RF, Croft JB, Felitti VJ, *et al*. Adverse childhood experiences and smoking during adolescence and adulthood. JAMA. 1999;282(17):1652-1658. https://doi.org/10.1001/jama.282.17.1652
54. Hillemacher T, Frieling H, Moskau S, *et al*. Global DNA methylation is influenced by smoking behaviour. Eur Neuropsychopharmacol. 2008;18(4):295-298.
55. Philibert R, Dogan M, Beach SR, Mills JA, Long JD. AHRR methylation predicts smoking status and smoking intensity in both saliva and blood DNA. Am J Med Genet Part B Neuropsychiatr Genet. 2020;183(1):51-60.
56. Houtepen LC, Hardy R, Maddock J, *et al*. Childhood adversity and DNA methylation in two population-based cohorts. Transl Psychiatry. 2018;8(1):266. https://doi.org/10.1038/s41398-018-0307-3
57. Joubert BR, Felix JF, Yousefi P, *et al*. DNA methylation in newborns and maternal smoking in pregnancy: genome-wide consortium meta-analysis. Am J Hum Genet. 2016;98(4):680-696. https://doi.org/10.1016/j.ajhg.2016.02.019
58. Xu R, Hong X, Zhang B, *et al*. DNA methylation mediates the effect of maternal smoking on offspring birthweight: a birth cohort study of multi-ethnic US mother-newborn pairs. Clin Epigenetics. 2021;13(1):47. https://doi.org/10.1186/s13148-021-01032-6
59. King DE, Sparling AC, Lloyd D, *et al*. Sex-specific DNA methylation and associations with in utero tobacco smoke exposure at nuclear-encoded mitochondrial genes. Epigenetics. 2022;17(12):1573-1589. https://doi.org/10.1080/15592294.2022.2043591
60. Drake AJ, O'Shaughnessy PJ, Bhattacharya S, *et al*. In utero exposure to cigarette chemicals induces sex-specific disruption of one-carbon metabolism and DNA methylation in the human fetal liver. BMC Med. 2015;13(1):18. https://doi.org/10.1186/s12916-014-0251-x
61. Zhang B, Hong X, Ji H, *et al*. Maternal smoking during pregnancy and cord blood DNA methylation: new insight on sex differences and effect modification by maternal folate levels. Epigenetics. 2018;13(5):505-518. https://doi.org/10.1080/15592294.2018.1475978
62. McCarthy MM. Sex differences in neuroimmunity as an inherent risk factor. Neuropsychopharmacology. 2019;44(1):38-44.
63. Nugent BM, Wright CL, Shetty AC, *et al*. Brain feminization requires active repression of masculinization via DNA methylation. Nat Neurosci. 2015;18(5):690-697. https://doi.org/10.1038/nn.3988
64. Maguire-Jack K, Lanier P, Lombardi B. Investigating racial differences in clusters of adverse childhood experiences. Am J Orthopsychiatry. 2020;90(1):106-114. https://doi.org/10.1037/ort0000405
65. Baldwin JR, Reuben A, Newbury JB, Danese A. Agreement between prospective and retrospective measures of childhood maltreatment: a systematic review and meta-analysis. JAMA Psychiatry. 2019;76(6):584-593. https://doi.org/10.1001/jamapsychiatry.2019.0097
66. Dalgleish T, Werner-Seidler A. Disruptions in autobiographical memory processing in depression and the emergence of memory therapeutics. Trends Cogn Sci. 2014;18(11):596-604. https://doi.org/10.1016/j.tics.2014.06.010
67. Danese A, Widom CS. Objective and subjective experiences of child maltreatment and their relationships with psychopathology. Nat Hum Behav. 2020;4(8):811-818.