

Sensitive Periods for the Effect of Childhood Adversity on DNA Methylation: Results from a Prospective, Longitudinal Study

Supplement I

Sample and Procedures

Data came from the Avon Longitudinal Study of Parents and Children (ALSPAC), a prospective, longitudinal birth cohort of children born to mothers who were living in the county of Avon, England (120 miles west of London) with estimated delivery dates between April 1991 and December 1992 (1-3). ALSPAC was designed to increase knowledge of the pathways to health across the lifespan, with an emphasis on genetic and environmental determinants. Approximately 85 percent of eligible pregnant women agreed to participate (N=14,541), and 99% of eligible live births (n=14,062) who were alive at one year of age (n=13,988 children) were enrolled. Response rates to data collection have been good (75% have completed at least one follow-up). Ethical approval for the study was obtained from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committee. More details are available on the ALSPAC website, including a fully searchable data dictionary: <http://www.bristol.ac.uk/alspac/researchers/access/>. The ARIES mother-child pairs were randomly selected out of those with complete data across at least five waves of data collection.

The ALSPAC sample is comprised of predominately White (94.6%) children; the ARIES subsample used in this study is racially homogenous (97.23% White in the analytic sample). As genetic data were not available for one-eighth of the analytic sample, we inferred ancestry information using an epigenome-wide DNAm data based principal component analysis (4), which has been shown to reliably capture population structure even in the absence of genetic data. After adjusting for sex and cell counts, we found no apparent outlier or pattern of population

stratification (**Figure S10**). In light of these findings, adjustment for self-reported race/ethnicity as a covariate should be sufficient to address issues with respect to population stratification and allow us to maximize the statistical power of the analyses.

Measures

Exposure to Adversity

Caregiver physical or emotional abuse. Exposure to physical or emotional abuse was determined through mailed questionnaires administered separately to the mother and the mother's partner. Children were coded as having been exposed to physical or emotional abuse if the mother, partner, or both responded affirmatively to any of the following items assessed over six time-points (8 months, 1.75 years, 2.75 years, 4 years, 5 years, and 6 years): 1) your partner was physically cruel to your children; 2) you were physically cruel to your children; 3) your partner was emotionally cruel to your children; 4) you were emotionally cruel to your children. Participants were informed that all of their responses were confidential, and reports of caregiver physical or emotional abuse were not reported to child welfare agencies, consistent with the lack of mandatory reporting laws in the UK (5, 6).

Sexual or physical abuse. Exposure to sexual or physical abuse was determined through an item asking the mother to indicate whether or not the child had been exposed to either sexual or physical abuse from anyone. This question was included at six time-points: child ages 1.5 years, 2.5 years, 3.5 years, 4.75 years, 5.75 years, and 6.75 years. As noted above, reports of sexual or physical abuse were not reported to child welfare agencies.

Maternal psychopathology. Maternal psychopathology was determined using data from: 1) the Crown-Crisp Experiential Index (CCEI), which includes separate subscales for anxiety and

depression (7, 8) ; 2) the Edinburgh Postnatal Depression Scale (EPDS) (9); and 3) a question asking about suicide attempts in the past 1.5 years. These measures were collected from mothers at five time-points: child ages 8 months, 1.75 years, 2.75 years, 5 years, and 6 years of age. Consistent with prior ALSPAC studies (10) and previous cut-points established in the literature (see below), we coded children as exposed to maternal psychopathology if one or more of the following criteria occurred: 1) the mother had a CCEI depression score greater than 9 (8); 2) mother had a CCEI anxiety score greater than 10 (8); 3) mother had an EPDS score greater than 12 (9); or the 4) mother reported a suicide attempt since the time of the last interview.

One adult in the household. Mothers indicated the number of adults (>18 years of age) living in the household at five time-points: when the child was 8 months, 1.75 years, 2.75 years, 4 years, and 7 years. Children were coded as exposed if there were fewer than two adults in the household.

Family instability. Mothers indicated whether the child had: 1) been taken into care; 2) been separated from their mother for two or more weeks; 3) been separated from their father for two or more weeks; or 4) acquired a new parent. These items were completed at six time-points: when children were ages 1.5 years, 2.5 years, 3.5 years, 4.75 years, 5.75 years, and 6.75 years. Children were coded as exposed if at least two of these events occurred at a single time point. Although being placed in foster care versus being separated from parents could reflect fundamentally different experiences of family instability, these four events were combined to create a binary measure of exposure because: 1) the prevalence of being taken into care or acquiring a new parent was too low for these experiences to be examined as separate measures; 2) separation from caregivers, especially in early life, can result in behavioral changes (11) and has been found to have a profound effect on development (12).

Financial stress. Mothers indicated the extent to which the family had difficulty affording the following: 1) items for the child; 2) rent or mortgage; 3) heating; 4) clothing; 5) food. Each of the 5 items was coded on a Likert-type scale (1=not difficult; 2=slightly difficult; 3=fairly difficult; 4=very difficult). These items were completed at five time-points: when children were ages 8 months, 1.75 years, 2.75 years, 5 years, and 7 years. Children were coded as exposed if their mothers reported at least fair difficulty for three or more items at each time point; this cut-point corresponds to response option 3 on a 4-point scale, with a higher score reflecting more difficulty.

Neighborhood disadvantage. At four time-points, when children were 1.75 years, 2.75 years, 5 years, and 7 years of age, mothers indicated the degree to which the following were problems in their neighborhood: 1) noise from other homes; 2) noise from the street; 3) garbage on the street; 4) dog dirt; 5) vandalism; 6) worry about burglary; 7) mugging; and 8) disturbance from youth. Response options to each item were: 2=serious problem, 1=minor problem, 0=not a problem or no opinion. Items were summed, yielding scores ranging from 0-16. Children with scores of eight or greater, which generally corresponded to the 95th percentile, were classified as exposed to neighborhood disadvantage.

DNA Methylation

As described elsewhere (13), DNAm was measured at 485,000 CpG dinucleotide sites across the genome using the Illumina Infinium Human Methylation 450K BeadChip microarray, which captures DNAm variation at 99% of RefSeq genes (17 CpG sites per gene, on average). Bisulfite treatment of DNA extracted from cord blood and peripheral blood leukocytes was performed using the Zymo EZ DNA MethylationTM kit. The arrays were scanned using an Illumina iScan and initial quality review was assessed using GenomeStudio (version 2011.1).

The proportion of molecules methylated at each interrogated CpG site on the array was detected using the Illumina 450K BeadChip assay. The estimated level of DNA methylation at each CpG site was expressed as a ‘beta’ value (β), defined as the ratio of the intensity measured by the methylated probe and the sum of the overall intensity and a recommended offset value $\alpha = 100$ ($\beta = \text{intensity of the Methylated allele (M)} / \text{intensity of the Unmethylated allele (U) + intensity of the Methylated allele (M) + 100}$). The β value ranges from 0 (no methylated dinucleotides observed) to 1 (all dinucleotides methylated). The preprocessing analyses were performed using R (version 3.0.1). Background correction and subset quantile normalization within each time point were applied to the raw methylation β -values following the pipeline developed by Touleimat and Tost (14) to remove or minimize the effects of variation due to technical artifacts. Additionally, a post-hoc correction for white blood cell heterogeneity was performed, as cell heterogeneity may confound DNA methylation measurement yet whole blood cell counts were not obtained for the majority of ALSPAC samples. The estimateCellCounts function in the minfi Bioconductor package implemented in R (15) was used to estimate the fraction of different cell types (CD8 T cells, CD4 T cells, NK cells, B cells, monocytes, and granulocytes).

To minimize potential confounding by batch effects, all samples in ARIES were distributed across slides semi-randomly (to represent all time points on each array). A laboratory information management system (LIMS) was built to record the batch variables as well as the quality control (QC) metrics from the standard control probes for each sample. The QC procedure consisted of excluding samples with average probe P-value ≥ 0.01 from further analysis, scheduling repeat assay for those failed samples, and comparing genotype probes with the same individual’s SNP-chip data to correct any sample mismatches. For the last step, if no genome-wide SNP data were

available for that individual yet a sex-mismatch based on X-chromosome methylation was present, the sample was flagged.

Data Analysis

Overview of the Structured Life Course Modeling Approach (SLCMA)

Our analyses were based on a structured life course modeling approach (SLCMA), which was originally developed by Mishra (16) and later extended by Smith (17, 18) to analyze repeated, binary exposure data across the life course. The goal of the SLCMA is to identify the single life course theoretical model (or potentially more than one life course theoretical model working in combination) that explains the most outcome variation (R^2). **Table S8** summarizes the life course theoretical models tested in this study, using exposure to abuse as an example.

As summarized in text, the SLCMA is performed in two stages. In the first stage, a set of encoded variables are entered into the LARS variable selection procedure (19). Thus, for each subject, exposure to the i^{th} adversity ($i = 1, 2, \dots, 7$, denoting the seven types of adversity mentioned in **Measures**) was encoded based on three theoretical models:

Sensitive period. The sensitive period hypothesis tests if the presence of exposure at a specific time point explains the most variance in the outcome. Formally, for the j^{th} time point of assessment ($j = 1, 2, \dots, J_i$, $J_i \geq 4$, the value of J is dependent on the type of adversity as described in the **Measures** section above),

$$H_{SP,ij} = b_{ij}, \text{ where } b_{ij} = \begin{cases} 0, & \text{no exposure to the } i^{th} \text{adversity at the } j^{th} \text{ timepoint} \\ 1, & \text{exposure to the } i^{th} \text{adversity at the } j^{th} \text{ timepoint} \end{cases}$$

Accumulation. The accumulation hypothesis tests whether the total impact of the i^{th} adversity reported across all time periods explains the most variance in the outcome. The variable is formally defined as:

$$H_{\text{accumulation},i} = \sum_{j=1}^{J_i} b_{ij}$$

Recency. The recency hypothesis is defined by a weighted sum of exposure across all time periods. It tests if temporal proximity to the adverse events explains the most variance in the outcome. The variable is formally defined as:

$$H_{\text{recency},i} = \sum_{j=1}^{J_i} b_{ij} \times \text{age}_{ij}$$

Covariates

Beyond the technical adjustments described earlier, we additionally controlled for the following variables, measured at child birth: *child race/ethnicity* (0=non-White; 1=White); *child birth weight*; *number of previous pregnancies* (between 0-3+); *maternal age* (0=ages 15-19, 1=ages 20-35, 2=age>35); *parent social class* (i.e. the highest social class of either parent: 1=foreman; 2=manager; 3=supervisor; 4=lending hand; 5=self-employed; 6=none of these); and *sustained maternal smoking during pregnancy* (0=non-smoker; 1=smoker in two or more trimesters, including the third trimester) (20). Given that we were modeling maternal psychopathology explicitly as an adversity exposure, that polygenic risk scores for mood disorders have been found to poorly predict maternal depression in ALSPAC (21), and applications of polygenic risk scores have not yet been widely incorporated into epigenetic analyses, we did not adjust for maternal genomic liability to psychopathology in our analyses.

Correction for Multiple Testing

To assess the sensitivity of our results to a Bonferroni-correction threshold ($p < 1 \times 10^{-7}$), we additionally used a more liberal false discovery rate threshold (FDR $q < 0.05$). This allowed an

analysis of the distribution of theoretical models chosen across FDR-significant sites. With this larger number of sites, we sought to determine whether the distribution of theoretical models selected differed between these FDR-significant ($q < 0.05$) sites and the background, estimated as the non-FDR significant sites ($q > 0.05$). Additionally, an expanded set of genes annotated to all sites surpassing a more liberal threshold (FDR $q < 0.05$) increased our power to test for enrichment of regulatory elements and biological processes (Gene Ontology (GO) terms).

Sensitivity Analyses

To evaluate the sensitivity of our results to specific analytic strategies, we conducted four sensitivity analyses. First, we evaluated the LARS variable selection procedure by examining later steps of the LARS procedure (additional theoretical models chosen) for the top CpG sites. For each top site, we calculated the variance explained by additional steps, and assessed the significance of the increase with a covariance test at each step.

Second, because some adversities exist prenatally and could affect methylation *in utero*, we assessed methylation at birth in umbilical cord blood at the top CpG sites. Sample collection, laboratory procedures, and quality control are described elsewhere (13). Methylation beta values were normalized (14), corrected for cell count heterogeneity (22), and Winsorized (23) to remove outliers following the quality control for age 7 DNAm as described above. At each top CpG site, we tested the predictive value of the theoretical model chosen at age 7 on methylation at birth with linear regression, controlling for the same covariates as described previously. We used a Bonferroni correction to adjust the alpha level for multiple testing.

Third, because methylation can be influenced by genetic variation, we assessed whether any of our top sites were affected by methylation quantitative trait loci (mQTLs), using a recently

published database of mQTLs of the ARIES dataset (mQTLdb: (24)). We downloaded the list of mQTLs at age 7, and filtered the data to our top CpG sites. Children were genotyped using the Illumina HumanHap550 quad chip; imputation was performed to the 1000 Genomes (phase 1, version 3, release Dec 2013) reference population using IMPUTE v2.2.2 (25). Variants were filtered by minor allele frequency ($\text{MAF} > 0.01$), Hardy-Weinberg equilibrium ($\text{HWE} > 5 \times 10^{-7}$), and imputation quality ($\text{info} > 0.8$); subjects were filtered by missing genotype rate (missingness $< 3\%$) and cryptic relatedness ($r < 0.1$). For each top CpG site with 5 or fewer associated SNPs, we included minor allele dosages as additional covariates in a linear regression testing the theoretical model chosen, controlling for the same covariates as described previously. For each top CpG site with more than 5 associated SNPs, we filtered SNPs by call rate ($> 97\%$) and ran a principal components analysis among all SNPs associated with each CpG. The top 5 principal components were used as covariates to represent genetic variation in downstream analyses.

Fourth, as not all CpG sites on the epigenome are variable, we restricted the analyses to variable CpG sites using an empirical data reduction approach (26). We removed CpG sites with less than 5% change in beta between the 10th and 90th percentile and were left with 292,686 variable probes, resulting in a more liberal Bonferroni corrected p-value threshold of $p < 1.71 \times 10^{-7}$. The new threshold would allow us to identify 10 additional probes, all of which were already included in the list of 380 probes after FDR correction as presented in **Table S3**. We have added a footnote in Table S3 to highlight the 10 additional hits passing the less stringent p-value threshold.

Epigenome-Wide Association Study (EWAS) with Exposed vs. Unexposed to Adversity

To evaluate the loss or gain of information when using a simpler versus more complex analytic approach, we also performed seven EWASs (one for each type of adversity) to evaluate

the association between lifetime exposure to adversity before age 7 (coded as ever versus never exposed) and DNAm across all CpG sites. The EWAS results were then compared to the SLCMA to determine if the two approaches yielded similar or distinct conclusions regarding the number of significant loci detected.

Analyses that compare the outcome of DNAm between exposed and unexposed groups assume that the true relationship between exposure and outcome does not depend on the timing or amount of exposure. When this assumption is not valid, for example under a true sensitive period, accumulation or recency model, then such analyses will be underpowered when compared with the analyses presented in the main paper. To illustrate this, we will first present a summary of the proof showing how regression of the outcome on exposed vs. unexposed suffers when the true underlying relationship is a sensitive period model, accompanied by explanations in the context of the current study. The summary is followed by a mathematical proof that shows in details how the test statistics are derived.

Suppose that the outcome Y depends on the exposures X_1, X_2, \dots, X_J through the sensitive period linear model

$$Y_i = \beta_0 + \beta_1 X_{si} + \varepsilon_i, \quad \varepsilon_i \sim N(0, \sigma^2).$$

Regression of Y on X_s (i.e., fitting the correct sensitive period model) will give an average regression coefficient of β_1 .

Now let X_{any} be the variable indicating exposure at any of the measurement occasions, so

$$X_{any} = \begin{cases} 0 & \text{if } X_1 = X_2 = \dots = X_J = 0 \\ 1 & \text{otherwise.} \end{cases}$$

Regression of Y on X_{any} (i.e. fitting an exposed vs. unexposed model) will give an average regression coefficient of

$$\frac{p_s}{p_{any}} \beta_1$$

where p_s and p_{any} are the prevalences of X_s and X_{any} respectively. Since $p_{any} \geq p_s$, this average regression coefficient will be smaller than that found by fitting the correct sensitive period model.

As an example, family instability had a prevalence of 4% in very early childhood, but an overall prevalence of 16%. The size of the regression coefficient from an exposed vs. unexposed analysis will be, on average, 0.25 times the size of the regression coefficient estimated for the very early childhood sensitive period model.

The average R^2 resulting from regression of Y on X_{any} will be

$$R_s^2 \frac{p_s/(1-p_s)}{p_{any}/(1-p_{any})}$$

where R_s^2 is the average R^2 resulting from regression of Y on X_s . The above odds ratio will always be smaller than 1, since the odds of X_s will be smaller than the odds of X_{any} .

For family instability in very early childhood, where the odds were 0.04 and 0.19 respectively, the R^2 from the exposed vs. unexposed will be 0.21 times that of the R^2 for the very early childhood sensitive period model.

The average standardized test statistic resulting from regression of Y on X_{any} will be

$$z_s \sqrt{\frac{p_s/(1-p_s)}{p_{any}/(1-p_{any})}} \sqrt{\frac{\sigma^2}{\sigma^2 + \beta_1^2 p_s (p_{any} - p_s) / p_{any}}}$$

where z_s is the average standardized test statistic resulting from regression of Y on X_s . Note that both the fractions inside the square roots will always be smaller than 1.

For the family instability in very early childhood sensitive period, we estimated $\beta_1 = 0.08$ and $\sigma^2 = 0.0003$, leading to a test statistic of $z_s = 4.71$ and a p-value of 2.5×10^{-6} . However, the

test statistic for the exposed vs. unexposed model drops to 2.06, with an associated p-value of approximately 0.04.

Simulation studies (17) have shown that LARS can select the correct sensitive period on 80% of occasions, in samples smaller than ours with greater correlation between exposures. The power lost through having to choose the correct sensitive period is less substantial than the drop in regression coefficient, test statistic, and R² typically associated with fitting an exposed vs. unexposed model instead of the correct sensitive period model.

Theorem:

Let X_1, X_2, \dots, X_J denote the J exposure variables, Y denote the outcome that depends on the exposure through the sensitive period linear model X_s . Let X_{any} be the variable indicating exposure at any of the measurement occasions, so

$$X_{any} = \begin{cases} 0 & \text{if } X_1 = X_2 = \dots = X_J = 0 \\ 1 & \text{otherwise.} \end{cases}$$

The average standardized test statistic resulting from regression of Y on X_{any} (z_{any}) will be larger than the standardized test statistic resulting from the true sensitive period model (z_s), i.e., the Exposed vs. Unexposed analysis will be underpowered.

Proof:

We assume that the true underlying model is

$$Y_i = \beta_0 + \beta_1 X_{si} + \varepsilon_i, \quad \varepsilon_i \sim N(0, \sigma^2).$$

Fitting the ever exposed vs. unexposed model,

$$\hat{Y}_i = \hat{\beta}_{0,any} + \hat{\beta}_{any} X_{any_i},$$

On average,

$$\begin{aligned}
\sum_i X_{any i} Y_i &= 0 \beta_0 n P(X_{any} = 0) + 1 \beta_0 n P(X_{any} = 1 \& X_s = 0) + 1 (\beta_0 + \beta_1) n P(X_s = 1) \\
&= 0 \beta_0 n(1 - p_{any}) + 1 \beta_0 n(p_{any} - p_s) + 1 (\beta_0 + \beta_1) np_s \\
&= n(p_{any}\beta_0 + p_s\beta_1).
\end{aligned}$$

Therefore on average,

$$\begin{aligned}
\hat{\beta}_{any} &= \frac{\sum_i X_{any i} Y_i / n - (\sum_i X_{any i} / n)(\sum_i Y_i / n)}{\sum_i X_{any i}^2 / n - (\sum_i X_{any i})^2} \\
&= \frac{p_{any}\beta_0 + p_s\beta_1 - p_{any}(\beta_0 + \beta_1)}{p_{any} - p_{any}^2} \\
&= \frac{p_s}{p_{any}}\beta_1.
\end{aligned}$$

The residuals resulting from this regression are given by

$$\begin{aligned}
Y_i - \hat{Y}_i &= \beta_0 + \beta_1 X_{si} + \varepsilon_i - \beta_0 - \frac{p_1}{p_{any}}\beta_1 X_{any i} \\
&= \varepsilon_i + \beta_1 \left(X_{si} - \frac{p_s}{p_{any}}X_{any i} \right).
\end{aligned}$$

The sum of squares of residuals will average

$$\begin{aligned}
\sum_i (Y_i - \hat{Y}_i)^2 &= \sum_i \left(\varepsilon_i^2 + 2\varepsilon_i\beta_1 \left(X_{si} - \frac{p_s}{p_{any}}X_{any i} \right) + \beta_1^2 \left(X_{si} - \frac{p_s}{p_{any}}X_{any i} \right)^2 \right) \\
&= n\sigma^2 + \beta_1^2 \sum_i \left(X_{si} - \frac{p_s}{p_{any}}X_{any i} \right)^2 \\
&= n\sigma^2 + \beta_1^2 \left(0^2 n P(X_{any} = 0) + \frac{p_s^2}{p_{any}^2} n P(X_{any} = 1 \& X_s = 0) + \frac{(p_{any} - p_s)^2}{p_{any}^2} n P(X_s = 1) \right) \\
&= n\sigma^2 + \beta_1^2 \left(0 np_s + \frac{p_s^2}{p_{any}^2} n(p_{any} - p_s) + \frac{(p_{any} - p_s)^2}{p_{any}^2} np_s \right) \\
&= n\sigma^2 + n\beta_1^2 \frac{p_s(p_{any} - p_s)}{p_{any}}.
\end{aligned}$$

Hence the average R² will be

$$\begin{aligned}
& 1 - \frac{\sum_i (Y_i - \hat{Y}_i)^2}{\sum_i (Y_i - \bar{Y}_i)^2} \\
&= 1 - \frac{n\sigma^2 + n\beta_1^2 p_s (p_{any} - p_s)/p_{any}}{n\sigma^2 + n\beta_1^2 p_s (1 - p_s)} \\
&= \frac{\beta_1^2 p_s (1 - p_{any})/p_{any}}{\beta_1^2 p_s (1 - p_{any})/p_{any} + \sigma^2}
\end{aligned}$$

The average standard error of the regression coefficient will be

$$\begin{aligned}
& \sqrt{\frac{\sum_i (Y_i - \hat{Y}_i)^2 / n}{n p_{any} (1 - p_{any})}} \\
&= \sqrt{\frac{\sigma^2 + \beta_1^2 p_s (p_{any} - p_s)/p_{any}}{n p_{any} (1 - p_{any})}}.
\end{aligned}$$

Leading to the average standardized test statistic of

$$\begin{aligned}
z_{any} &= \frac{p_s}{p_{any}} \beta_1 \sqrt{\frac{\sigma^2 + \beta_1^2 p_s (p_{any} - p_s)/p_{any}}{n p_{any} (1 - p_{any})}} \\
&= \beta_1 p_s \sqrt{\frac{1 - p_{any}}{p_{any}}} \sqrt{\frac{n}{\sigma^2 + \beta_1^2 p_s (p_{any} - p_s)/p_{any}}}.
\end{aligned}$$

For comparison, the residuals resulting from regression of Y on X_s are ε_i , which have sum of squares $n\sigma^2$, leading to an average R^2 of

$$R_s^2 = \frac{\beta_s^2 p_s (1 - p_s)}{\beta_s^2 p_s (1 - p_s) + \sigma^2},$$

an average standard error of $\sqrt{\sigma^2/n}$, and an average standardized test statistic of

$$z_s = \beta_1 \sqrt{\frac{np_s(1 - p_s)}{\sigma^2}}.$$

Therefore,

$$z_{any} = z_s \sqrt{\frac{p_s/(1 - p_s)}{p_{any}/(1 - p_{any})}} \sqrt{\frac{\sigma^2}{\sigma^2 + \beta_1^2 p_s (p_{any} - p_s)/p_{any}}}$$

Since $\frac{p_s/(1-p_s)}{p_{any}/(1-p_{any})} < 1$ and $\frac{p_s/(1-p_s)}{p_{any}/(1-p_{any})} < 1$, we have shown that $z_{any} < z_s$.

Sensitivity Analysis Examining Baseline Parent Social Class as a Confounder

In the current study, baseline parent social class was included as a covariate in the primary analysis. Parent social class, which captures job industry and rank, is related to other indicators of socioeconomic status, but likely has distinct effects on health across the life course (27). In the current sample, parent social class was only modestly correlated ($r \leq 0.45$) with other aspects of socioeconomic status, such as financial stress and neighborhood disadvantage. Inclusion of parent social class thus allowed us to control for potential confounding effects of the social class into which children are born.

As there is concern that adjusting for baseline parent social class as a covariate may not be appropriate given that it conceptually overlaps with some of the childhood adversity types in the current study (in particular, the measure of financial stress and neighborhood disadvantage), we report here on results from: 1) our investigation into the definition of confounding from the causal inference literature, 2) our investigation in the theoretical and empirical literature to understand the nature of socioeconomic status and its effects on childhood adversity and DNA methylation, and 3) additional statistical analyses to compare results with and without adjusting for baseline parent social class. In the narrative below, we summarize what we learned through these processes. We hope that these insights will be useful to make explicit our thinking and help guide future research efforts, including attempts to replicate these study findings.

The Definition of Confounding

A confounder is traditionally defined as a variable that meets the following three criteria, as determined through either bivariate or multivariate tests of association: 1) it is associated with the exposure; 2) it is associated with the outcome given the exposure; 3) it does not lie on the causal pathway between the exposure and the outcome.

In the past decade, researchers in the field of causal inference (see for example: (28-30)) have questioned whether relying purely on these three associational criteria is sufficient to evaluate confounding. These concerns have been raised following instances when a true confounder has failed to satisfy the three associational criteria noted above, or when a variable meets these three associational criteria should not be adjusted for. Causal inference experts have therefore proposed alternative strategies for determining the extent to which a third variable could be a potential confounder, which are intended to be used alongside the three associational criteria highlighted above. Some of these alternative strategies draw from things that cannot be directly tested through association analyses, such as greater use of causal diagrams and critical examination of theoretical evidence. Other alternative definitions are based on evaluating bias before and after adjustment for a potential confounding variable (29).

Related to this last strategy, another property central to the concept of confounding is *collapsibility*. In other words, when a potential confounder is removed from the analysis, does the association between the outcome and exposure remain the same? Or, is the exposure-outcome relationship invariant to the inclusion of the potential confounder? Whenever collapsibility fails, meaning where the results are not the same before and after adjusting for the potential confounder, it suggests that the exposure-outcome relationship may be confounded.

As summarized in the sections that follow, we considered the theoretical evidence regarding whether parent social class should be treated as a confounder and investigated whether the results were collapsible before and after the inclusion of baseline parent social class as a covariate.

Theoretical Evidence

Theoretical evidence is critical to justify the inclusion of covariates. Here, we briefly review the literature on links between socioeconomic status (SES) and exposure to childhood adversity as well as the associations between SES and DNA methylation. As shown below, the major take-home from this in-depth literature review is that baseline SES, including indicators of parent social class – as it is commonly measured in UK-based sample and was examined here (31, 32), is a plausible suspect for confounding the relationship between exposure to other types of childhood adversity and DNAm and that the estimate of these types of adversity on DNAm may be biased without adjusting for baseline SES. Furthermore, not all measures of SES perform the same in terms of their association with DNAm, suggesting that each different facet of the construct of SES needs to be considered on its own.

First, it is known from decades of literature that different dimensions of SES, including parent social class, are associated with childhood adversity. This literature has documented that children who experience adversity – including child maltreatment, parental psychopathology, parental substance use, or family disruption – are more likely to be poor, and to be raised by mothers who have less education, receive public assistance, and live in disadvantaged neighborhoods. Moreover, some dimensions of child SES that are linked to these specific types of childhood adversity, such as parental education or parent social class (as defined by parent

employment), tend to be more fixed or stable across time. Other dimensions tend to be less stable, such as indicators of financial stress or neighborhood disadvantage, which varies as a function of access to specific resources at different time-points in life or the occurrence of major life events leading to change in individual circumstances. It has been argued (33-35) that this temporal variation requires the separate consideration of different domains of SES, as they each could have different links to health outcomes. In the current study, controlling for baseline parental social class would help tease apart the effects of subjective levels of poverty or neighborhood disadvantage experienced by the participants throughout development from a less variable status of social disadvantage as captured by baseline social class. Therefore, there is theoretical ground for suspecting the existence of an SES-adversity exposure relationship.

Secondly, there has been growing evidence documenting associations between different indicators of SES and DNA methylation. Specifically, Swartz et al. (36) found that methylation marks associated with SES (defined as a composite score of education levels and income) may be an underlying mechanism for changes in depression-related brain functions. Several studies also found differential methylation patterns for individuals with lower geographical index of deprivation or education levels (37, 38). Furthermore, Stringhini et al. (39) showed that indicators of SES (parental occupational position) were associated with DNAm of genes involved in inflammation. These findings suggest that this relatively fixed aspect of SES (distinctive from the perceived economic or environmental hardship as measured by financial stress or neighborhood disadvantage in our sample) may induce DNAm changes, thereby supporting the potential SES-outcome link. In fact, prior longitudinal studies examining the effects of SES on DNAm also adjusted for baseline SES to control for risk factors prior to exposure or a more stable dimension of SES (40).

Results Before and After Adjusting for Baseline SES

To evaluate the collapsibility principle, we examined the magnitude of change in our primary results (meaning of the 38 identified loci) before and after the inclusion of parent social class as a covariate. As presented in the main analyses (**Table 1**), methylation differences at 38 CpG sites were found to be associated with exposure to childhood adversity ($p < 1 \times 10^{-7}$).

After removing baseline parent social class as a covariate, 38 CpG sites were again identified (**Table S7**). However, they were not identical sites to those 38 that were originally identified. Specifically, 31 CpGs were shared between the two sets of results and the same life course hypotheses were identified for these. Moreover, the Stage 2 beta estimates, corresponding standard errors, and R^2 values were also effectively unchanged (relative difference, as defined by $\frac{(\theta_{SES} - \theta_{no\ SES})}{\theta_{no\ SES}}$, was under 6% for all sites for all three parameters: β , SE, and R^2). This comparison indicated that for these 31 sites, the results were largely consistent before and after including parent social class, thus the results were largely collapsible.

Importantly, however, the results overall were not entirely *collapsible*. There were seven loci dropped from the original results, and another seven new sites added after no longer adjusting for parent social class. These 14 sites were dispersed across different adversity types. For example, the seven sites that only appeared after adjusting for parent social class were capturing DNAm differences resulting from five different types of adversity (physical or sexual abuse, maternal psychopathology, one adult in the household, family instability, and financial stress/poverty). The finding that 20% of the identified sites in each analysis did not overlap suggests that baseline parent social class may potentially confound the relationships between childhood adversity and DNAm differences at some loci.

We then dug deeper into the pattern of findings related to the 14 loci that were not shared by the two sets of results (i.e., the seven hits that were dropped from the original analysis and the seven hits that were added in the revised analysis). The discrepancy in results appears to be attributed to the potential positive and negative confounding by baseline parent social class. Positive confounding refers to a scenario where the observed association is biased *away from* the null in the presence of an unadjusted confounder, whereas negative confounding refers to the opposite: the unadjusted association is biased *towards* the null. Whether the confounding is positive or negative depends on the directions of the confounder-exposure and confounder-outcome associations. As SES may be associated with both hyper- and hypo- methylation, both types of confounding are possible in epigenetic studies. When the unadjusted estimate is biased away from the null (positive confounding), including the confounder may result in those CpG sites being dropped as significant. When the unadjusted estimate is biased towards the null (negative confounding), the inclusion of the confounder may lead to new discoveries. Since adjusting for baseline parent social class led to both new additional hits being identified and unadjusted hits being removed, both types of confounding may be present in our analyses given that the directions of effects between parent social class and DNAm are CpG site-specific.

To better understand the specific pattern of these associations, we additionally examined the associational criteria presented earlier. Of the 14 sites that were not shared by the results before and after adjusting for parent social class, two of these CpG sites (thus 15% of the loci) were associated with baseline parent social class (cg15577126, family instability, $F=3.21$, $p=0.007$; cg01370449, sexual or physical abuse, $F=4.28$, $p=0.0007$). However, the effect estimates in epigenetic studies are known to be small and the models may be under powered to detect such associations, thus there could be even more significant loci linked to parent social class at baseline.

We are hoping to replicate the analyses in larger studies where we are more sufficiently powered to test whether the small, albeit important, effects of parent social class on DNAm exist or not. Testing the associations between the life course hypotheses encoding childhood adversity identified at these 14 loci and baseline parent social class, we found that 7 of the 12 (58%) unique life course hypotheses were associated with baseline parent social class (chi-squared test $p < 0.05$). Taken together, these association tests may provide evidence for the presence of confounding induced by baseline SES. However, as we discussed above, confounding cannot be determined purely based on associational criteria and the results should be interpreted with this notion in mind.

Based on a careful review of the theoretical evidence for SES being a confounder as well as an investigation of differences in results before and after including SES, we concluded that the more conservative approach would be to adjust for baseline parent social class as a covariate. This decision is supported based on prior research literature and our finding that the results shifted with the exclusion of this variable. However, results at most of the identified hits (more than 80% among both the Bonferroni corrected 38 loci and 380 FDR corrected loci) remained invariant, suggesting that the inclusion of SES did not cause a substantial change in the findings. While some loci are sensitive to potential bias induced by SES and should not be neglected, the patterns of results are largely stable. The fact that the same number of top hits were identified in these two sets of analyses is reassuring and shows that we did not intentionally overfit the model and include parent social class purely based on its impact on the statistical significance of findings.

Exploring the Biological Significance of the Findings

Correlation Between Blood and Brain Tissue

To examine the relevance of methylation at our top sites to psychopathology, we examined the correlation between methylation in peripheral blood tissue and that of the brain using a publicly available database of methylation in 122 adults (42). We retrieved Pearson r correlation values between methylation in blood and four brain regions: prefrontal cortex (PFC), entorhinal cortex (EC), superior temporal gyrus (STG), and cerebellum (CER).

Enrichment of Regulatory Elements

To assess potential functional relevance of methylation changes at CpG sites associated with exposure to adversity, we examined the enrichment of regulatory elements annotated to FDR-significant loci. We obtained annotations of gene promoters, enhancers, and CpG Islands (CGIs) for all CpG sites from the *IlluminaHumanMethylation450kanno.ilmn12.hg19* package in R/Bioconductor. We compared the proportion of annotations between the FDR-significant sites and all autosomal sites tested with chi-squared goodness-of-fit tests. We also tested for enrichment of DNase I hypersensitivity sites (DHS) and histone marks (H3K27ac, H3K4me3, H3K4me1, H3K9ac, and H3K36me3) for FDR-significant sites using data from all tissues and cell types in the Roadmap Epigenomics Project (43) and ENCODE (44) using eFORGE 1.2 (45). eFORGE performs an overlap analysis by selecting 1000 sets of CpGs matched for gene relationship and CpG island relationship annotation and calculating a confidence interval of expected enrichment. The resulting p-values for each tissue and cell type were then corrected with Benjamini-Yekutieli multiple testing correction (45).

Biological Processes Potentially Affected by Adversity

To identify common biological processes shared by these genes, we performed a functional clustering analysis in DAVID 6.8 (46), which identifies Gene Ontology (GO) biological process terms that are enriched for genes annotated to the FDR-significant sites. CpG sites were annotated to the nearest gene (located in the gene body or within 300 kb of a transcription start site, TSS) using the *FDb.InfiniumMethylation.hg19* package in R/Bioconductor (46). DAVID calculates an enrichment score for each functional cluster, which is the negative log of the geometric mean of the p-values of all GO terms within the cluster. The p-value for each GO term is derived from a modified Fisher's exact test, which tests whether the GO term is overrepresented among genes in the gene set as compared to a background of all autosomal genes tagged by the Illumina Human Methylation 450K BeadChip microarray.

To assess the selective constraint of these genes, we downloaded the gene constraint metrics from the Exome Aggregation Consortium (ExAC) and calculated the difference in the probability of intolerance to Loss-of-Function variation (pLI) in genes annotated to the FDR-significant loci as compared to genes annotated to the rest of the autosomal loci. The significance of this difference was tested with a permutation test. The FDR-significant gene label was permuted among all genes 10000 times and the difference in pLI was calculated; the number of permutations in which the absolute value of the difference in means was greater than the absolute value of the observed difference in means was recorded as the empirical p-value.

Supplementary Tables and Figures

Table S1. Distribution of covariates in the total sample (N=971) and among those exposed to any adversity (N=650)

	Total Sample		Exposure to any adversity		chi-squared	p-value
	%	N	%	N		
Sex					0.562	0.453
Males	49.85	484	48.92	318		
Females	50.15	487	51.08	332		
Race					4.811	0.028
White	2.78	26	3.7	23		
Non-White	97.22	909	96.3	599		
Age of Mother at Child's Birth					4.52	0.104
Ages 15-19	0.93	9	1.38	9		
Ages 20-35	89.54	865	88.92	578		
Age 36+	9.52	92	9.69	63		
Parental Social Class					13.327	0.021
Foreman	17.92	174	17.23	112		
Manager	38.83	377	37.38	243		
Supervisor	20.91	203	20	130		
Lending Hand	5.56	54	5.54	36		
Self-Employed	1.85	18	2.15	14		
None of these	14.93	145	17.69	115		
Number of Previous Pregnancies					4.703	0.195
0	46.8	439	46.26	291		
1	36.67	344	35.61	224		
2	12.69	119	13.51	85		
3+	3.84	36	4.61	29		
Birth Weight (g)					0.697	0.874
<3000	13.33	127	13.84	89		
3000 - 3499	36.31	346	35.61	229		
3500 - 3499	35.15	335	35.15	226		
≥ 4000	15.22	145	15.4	99		
Sustained Smoking During Pregnancy					10.522	0.001
Yes	89.23	820	86.81	533		
No	10.77	99	13.19	81		

Note. The chi-squared statistics and p-values in bold indicate that the tests reached statistical significance at $\alpha = 0.05$.

Table S2. Tetrachoric correlations among time-points within adversities

Caregiver physical or emotional abuse (N=787)							Maternal psychopathology (N=760)						
Age	8 mo	1.75	2.75	4	5	6	Age	8 mo	1.75	2.75	5	6	
8 mo	1	---	---	---	---	---	8 mo	1	---	---	---	---	
1.75	0.82	1	---	---	---	---	1.75	0.67	1	---	---	---	
2.75	0.69	0.77	1	---	---	---	2.75	0.56	0.67	1	---	---	
4	0.62	0.7	0.78	1	---	---	5	0.61	0.6	0.65	1	---	
5	0.58	0.58	0.69	0.66	1	---	6	0.44	0.53	0.57	0.71	1	
6	0.45	0.46	0.5	0.56	0.67	1							
Sexual or physical abuse (by anyone) (N=769)							Family instability (N=769)						
Age	1.5	2.5	3.5	4.75	5.75	6.75	Age	1.5	2.5	3.5	4.75	5.75	6.75
1.5	1	---	---	---	---	---	1.5	1	---	---	---	---	---
2.5	0.44	1	---	---	---	---	2.5	0.74	1	---	---	---	---
3.5	0.02	0.32	1	---	---	---	3.5	0.48	0.6	1	---	---	---
4.75	0.33	0.44	0.69	1	---	---	4.75	0.27	0.41	0.28	1	---	---
5.75	0.4	0.51	0.61	0.49	1	---	5.75	0.24	0.21	0.34	0.52	1	---
6.75	0.28	0.42	0.25	0.4	0.56	1	6.75	0.28	0.37	0.11	0.61	0.58	1
One adult in the household (N=726)							Financial stress (N=846)						
Age	8 mo	1.75	2.75	4	7		Age	8 mo	1.75	2.75	5	7	
8 mo	1	---	---	---	---		8 mo	1	---	---	---	---	
1.75	0.9	1	---	---	---		1.75	0.71	1	---	---	---	
2.75	0.78	0.93	1	---	---		2.75	0.59	0.67	1	---	---	
4	0.64	0.82	0.91	1	---		5	0.53	0.54	0.59	1	---	
7	0.54	0.75	0.81	0.79	1		7	0.36	0.4	0.38	0.56	1	
Neighborhood disadvantage (N=771)													
Age	1.75	2.75	5	7									
1.75	1	---	---	---									
2.75	0.74	1	---	---									
5	0.71	0.8	1	---									
7	0.67	0.75	0.89	1									

Cell entries are correlation values indicating the strength of each pairwise association between exposure at two time points, with 0=unexposed and 1=exposed

See Supplemental Table S3 in Supplement 2.

Table S4. Results of sensitivity analysis examining differential methylation at birth for all Bonferroni-significant CpG sites

CpG site	Adversity	First hypothesis chosen by LARS procedure	Birth DNAm in unexposed group (beta)	Birth DNAm in exposed group (beta)	Beta	SE	P	Directions of effect (birth, age 7)
cg10713431	Caregiver physical or emotional abuse (N=643)	middle childhood (age 6)	0.117	0.121	0.00497	0.0031	0.1116	++
cg12023170		middle childhood (age 6)	0.058	0.057	-0.00132	0.0032	0.6787	-+
cg19825600		middle childhood (age 6)	0.283	0.246	-0.03488	0.0214	0.1037	--
cg01370449	Sexual or physical abuse (by anyone) (N=630)	very early childhood (age 2.5)	0.2	0.225	0.01823	0.0217	0.4006	++
cg06430102		very early childhood (age 2.5)	0.902	0.902	0.00043	0.0226	0.9848	+-
cg19170021		early childhood (age 4.75)	0.767	0.759	0.00015	0.0277	0.9958	++
cg05072819		early childhood (age 5.75)	0.041	0.051	0.01205	0.004	0.0029	++
cg05936516		middle childhood (age 6.75)	0.105	0.1	-0.00009	0.008	0.9911	0+
cg04583813	Maternal psychopathology (N=618)	very early childhood (age 8 mo.)	0.866	0.871	0.00359	0.0101	0.7228	+-
cg08171937		very early childhood (age 2.75)	0.016	0.017	0.00051	4.00E-04	0.2503	++
cg10666628		very early childhood (age 2.75)	0.019	0.019	-0.00012	4.00E-04	0.7789	-+
cg17806989		early childhood (age 5)	0.971	0.97	-0.00157	0.0049	0.7487	--
cg08337366	One adult in the household (N=638)	very early childhood (age 8 mo.)	0.926	0.914	-0.01153	0.013	0.3744	--
cg10192047		very early childhood (age 8 mo.)	0.016	0.015	-0.00111	0.0017	0.5249	-+
cg26990406		very early childhood (age 8 mo.)	0.827	0.835	0.00992	0.0449	0.8251	+-
cg24468070		very early childhood (age 1.75)	0.026	0.024	-0.00154	0.0054	0.7734	-+
cg03397307		very early childhood (age 2.75)	0.026	0.035	0.01011	0.0025	1.00E-04	++
cg11631610	Financial stress (N=694)	very early childhood (age 8 mo.)	0.94	0.943	0.00514	0.0105	0.623	+-
cg06783003		very early childhood (age 1.75)	0.865	0.865	0.00321	0.0102	0.7528	++
cg01050704		early childhood (age 5)	0.018	0.019	0.0011	6.00E-04	0.0496	++
cg02006977		early childhood (age 5)	0.015	0.015	-0.00034	6.00E-04	0.553	-+
cg21299458		early childhood (age 5)	0.097	0.113	0.01361	0.0077	0.0795	++
cg19219503		middle childhood (age 7)	0.878	0.879	0.00496	0.0154	0.7482	+-
cg11714846		accumulation	0.896	0.896	-0.00013	0.0022	0.9515	--
cg21924472		recency	0.73	0.746	0.00062	9.00E-04	0.4711	++
cg24996440		recency	0.575	0.597	0.00343	0.0014	0.0129	++

CpG site	Adversity	First hypothesis chosen by LARS procedure	Birth DNAm in unexposed group (beta)	Birth DNAm in exposed group (beta)	Beta	SE	P	Directions of effect (birth, age 7)
cg00928478	Neighborhood disadvantage (N=629)	very early childhood (age 1.75)	0.021	0.02	-0.00085	6.00E-04	0.1744	--
cg01954337		very early childhood (age 1.75)	0.053	0.055	0.00322	0.0023	0.1639	++
cg04996689		very early childhood (age 1.75)	0.032	0.032	0.00072	0.0018	0.6794	++
cg12069925		very early childhood (age 1.75)	0.042	0.042	-0.00014	0.0016	0.9303	-+
cg14522055		very early childhood (age 1.75)	0.031	0.031	0.00047	0.0012	0.7035	++
cg19157140		very early childhood (age 1.75)	0.014	0.014	0.00064	5.00E-04	0.2422	++
cg21740964		very early childhood (age 1.75)	0.15	0.152	0.00508	0.0042	0.2262	++
cg24826892		very early childhood (age 1.75)	0.016	0.016	0.00018	7.00E-04	0.7923	++
cg08546016		early childhood (age 5)	0.048	0.047	-0.00254	0.003	0.4041	-+
cg12412390		middle childhood (age 7)	0.029	0.03	0.00069	0.0017	0.6822	++
cg18311384	Family instability (N=630)	very early childhood (age 2.5)	0.019	0.019	-0.00067	9.00E-04	0.4595	-+
cg27637303		very early childhood (age 2.5)	0.195	0.209	0.0114	0.0182	0.5308	++

To assess the degree of differential methylation present at birth, we performed regression analysis on methylation in umbilical cord blood at the top CpG sites. The hypothesis associated with DNAm at age 7 was significantly associated with DNAm at birth for one CpG site (bold value, $p < 0.05/38 = 0.00132$). The direction of the effect of exposure to adversity on DNAm at birth was the same as that on DNAm at age 7 in the majority of CpG sites (24 of 37 sites in which the first hypothesis chosen was not significantly associated with methylation at birth), suggesting that there may be insufficient power to detect effects of later exposure to adversity on DNAm at birth. Birth DNAm = unadjusted DNA methylation (beta values) in umbilical cord blood averaged within group; Beta, SE, P = parameter estimate, standard error, and p-value of regression coefficient of first hypothesis chosen; Directions of effect = sign of regression coefficient for the effect of the first hypothesis chosen on methylation in blood from the umbilical cord and from age 7. "0" indicates that the magnitude of effect (absolute value of the beta coefficient) was below 0.0001.

See Supplemental Table S4-extension in Supplement 2.

Table S5. Results of sensitivity analysis examining differential methylation at age 7 after controlling for genotypes, for all Bonferroni-significant CpG sites linked to mQTLs

CpG	Adversity	First hypothesis chosen by LARS procedure	Number of mQTL SNPs	N*	Beta	SE	P	Directions of effect (age 7, age 7 controlling for genotype)
cg10713431	Caregiver physical or emotional abuse (N=719)	middle childhood (age 6)
cg12023170		middle childhood (age 6)	95	559	0.0107	0.0025	1.89E-05	++
cg19825600		middle childhood (age 6)
cg01370449	Sexual or physical abuse (by anyone) (N=703)	very early childhood (age 2.5)	101	510	0.0775	0.0187	3.95E-05	++
cg06430102		very early childhood (age 2.5)
cg19170021		early childhood (age 4.75)	8	632	0.0833	0.0216	1.30E-04	++
cg05072819		early childhood (age 5.75)	218	423	0.009	0.0036	1.30E-02	++
cg05936516		middle childhood (age 6.75)
cg04583813	Maternal psychopathology (N=691)	very early childhood (age 8 mo.)	6	632	-0.025	0.0048	2.76E-07	--
cg08171937		very early childhood (age 2.75)
cg10666628		very early childhood (age 2.75)
cg17806989		early childhood (age 5)
cg08337366	One adult in the household (N=710)	very early childhood (age 8 mo.)	1	644	-0.0337	0.0065	2.57E-07	--
cg10192047		very early childhood (age 8 mo.)
cg26990406		very early childhood (age 8 mo.)
cg24468070		very early childhood (age 1.75)	40	600	0.0231	0.0044	2.64E-07	++
cg03397307		very early childhood (age 2.75)	1	625	0.0048	0.001	3.09E-06	++
cg18311384	Family instability (N=703)	very early childhood (age 2.5)
cg27637303		very early childhood (age 2.5)	27	612	0.0669	0.0174	1.36E-04	++
cg11631610	Financial stress (N=774)	very early childhood (age 8 mo.)	1	580	-0.0174	0.0068	1.05E-02	--
cg06783003		very early childhood (age 1.75)
cg01050704		early childhood (age 5)	1	712	0.0023	5.00E-04	5.52E-06	++
cg02006977		early childhood (age 5)	1	617	0.0019	5.00E-04	2.17E-04	++
cg21299458		early childhood (age 5)	2	522	0.0461	0.008	1.49E-08	++
cg19219503		middle childhood (age 7)
cg11714846		accumulation
cg21924472		recency
cg24996440		recency
cg00928478	Neighborhood disadvantage (N=702)	very early childhood (age 1.75)	1	608	-0.0021	5.00E-04	1.04E-05	--
cg01954337		very early childhood (age 1.75)	2	612	0.0094	0.0019	5.06E-07	++
cg04996689		very early childhood (age 1.75)
cg12069925		very early childhood (age 1.75)
cg14522055		very early childhood (age 1.75)
cg19157140		very early childhood (age 1.75)
cg21740964		very early childhood (age 1.75)	5	614	0.014	0.003	5.02E-06	++
cg24826892		very early childhood (age 1.75)
cg08546016		early childhood (age 5)	6	627	0.0061	0.0013	2.65E-06	++
cg12412390		middle childhood (age 7)

To assess the degree of differential methylation attributable to genetic variation, we conducted a sensitivity analysis testing the effect of the hypothesis chosen by the first stage of the LARS on DNA methylation after controlling for known mQTLs. After controlling for genotypes at mQTL SNPs, the direction of the effect of exposure to adversity on DNA methylation did not change. Number of mQTL SNPs = number of SNPs associated with methylation at CpG site identified by Gaunt et al. 2015; N* = number of subjects included in analysis (i.e. with non-missing genotype data); Beta, SE, P = parameter estimate, standard error, and p-value of regression coefficient of first hypothesis chosen, after controlling for genotype; Directions of effect = sign of regression coefficient for the effect of the first hypothesis chosen on methylation in blood at age 7 (unadjusted) and at age 7 controlling for genotype (adjusted).

Table S6. Correlation of methylation between blood and four brain regions (data from Hannon et al. 2015)

CpG site	Adversity	First hypothesis chosen by LARS procedure	Correlation with brain methylation, by region			
			PFC	EC	STG	CER
cg10713431	Caregiver physical or emotional abuse (N=719)	middle childhood (age 6)	0.367	0.397	0.319	0.395
cg12023170		middle childhood (age 6)	0.389	0.385	0.508	0.598
cg19825600		middle childhood (age 6)	0.250	0.149	0.316	0.162
cg01370449	Sexual or physical abuse (by anyone) (N=703)	very early childhood (age 2.5)	0.402	0.409	0.413	0.090
cg06430102		very early childhood (age 2.5)	-0.131	-0.047	-0.025	-0.132
cg19170021		early childhood (age 4.75)	0.043	-0.188	-0.114	-0.038
cg05072819		early childhood (age 5.75)	0.740	0.744	0.833	0.754
cg05936516		middle childhood (age 6.75)	-0.014	0.049	0.003	-0.129
cg04583813	Maternal psychopathology (N=691)	very early childhood (age 8 mo.)	0.008	-0.153	0.044	0.033
cg08171937		very early childhood (age 2.75)	-0.169	0.204	-0.074	0.099
cg10666628		very early childhood (age 2.75)	-0.005	-0.015	0.103	-0.026
cg17806989		early childhood (age 5)	0.011	0.278	0.305	-0.017
cg08337366	One adult in the household (N=710)	very early childhood (age 8 mo.)	-0.068	0.120	0.180	0.028
cg10192047		very early childhood (age 8 mo.)	0.116	-0.079	-0.141	-0.020
cg26990406		very early childhood (age 8 mo.)	0.146	0.015	0.387	-0.114
cg24468070		very early childhood (age 1.75)	0.120	0.083	0.116	0.001
cg03397307		very early childhood (age 2.75)	-0.182	0.046	-0.122	0.048
cg18311384	Family instability (N=703)	very early childhood (age 2.5)	-0.054	0.086	-0.077	-0.104
cg27637303		very early childhood (age 2.5)	0.197	-0.045	0.033	0.174
cg11631610	Financial stress (N=774)	very early childhood (age 8 mo.)	-0.034	-0.037	0.071	-0.001
cg06783003		very early childhood (age 1.75)	-0.022	-0.196	0.010	-0.055
cg01050704		early childhood (age 5)	-0.023	-0.012	0.039	-0.081
cg02006977		early childhood (age 5)	0.044	0.179	-0.221	-0.019
cg21299458		early childhood (age 5)	0.293	0.251	0.252	-0.005
cg19219503		middle childhood (age 7)	-0.007	0.180	0.230	0.098
cg11714846		accumulation	-0.011	-0.272	-0.060	-0.024
cg21924472		recency	0.285	0.431	0.378	0.192
cg24996440		recency	0.118	0.174	0.148	-0.164
cg00928478	Neighborhood disadvantage (N=702)	very early childhood (age 1.75)	-0.084	0.051	0.139	-0.067
cg01954337		very early childhood (age 1.75)	0.008	-0.067	0.077	0.023
cg04996689		very early childhood (age 1.75)	0.057	0.042	-0.175	-0.172
cg12069925		very early childhood (age 1.75)	0.277	0.108	-0.061	0.256
cg14522055		very early childhood (age 1.75)	-0.107	0.031	0.022	-0.025
cg19157140		very early childhood (age 1.75)	0.088	0.153	-0.032	0.105
cg21740964		very early childhood (age 1.75)	0.410	0.455	0.449	0.445
cg24826892		very early childhood (age 1.75)	0.086	0.038	0.131	-0.074
cg08546016		early childhood (age 5)	-0.078	0.069	-0.249	-0.096
cg12412390		middle childhood (age 7)	0.158	0.295	-0.072	0.043

To examine the relevance of methylation at our top sites to psychopathology, we examined the correlation in methylation in peripheral blood with that of four brain regions: prefrontal cortex (PFC), entorhinal cortex (EC), superior temporal gyrus (STG), and cerebellum (CER).

Table S7. Sensitivity analysis results of the Structured Life Course Modeling Approach (SLCMA) in ARIES, with annotation to the closest gene, for the Bonferroni-significant CpG sites ($p < 1 \times 10^{-7}$), without adjusting for baseline social class

CpG site	Adversity	First hypothesis chosen by LARS procedure	DNAm in unexposed	DNAm in exposed group (beta)	Increases in R ²	P	Beta (effect estimate)	SE	Lower 95% CI	Upper 95% CI	Chr	Coordinate (bp)	Nearest gene	Distance to nearest gene (bp)
cg10713431	Caregiver physical or emotional abuse (N=719)	middle childhood (age 6)	0.132	0.139	0.024	5.91E-08	0.008	0.0019	0.004	0.012	20	43933204	MATN4	0
cg12023170 ^a		middle childhood (age 6)	0.074	0.086	0.038	2.86E-10*	0.013	0.0023	0.008	0.017	1	23751761	TCEA3	499
cg19825600 ^{a,b}		middle childhood (age 6)	0.458	0.384	0.028	1.77E-08	-0.073	0.0158	-0.104	-0.042	2	3704501	ALLC	1283
cg02106682 [†]	Sexual or physical abuse (by anyone)	very early childhood (age 2.5)	0.216	0.252	0.030	6.84E-08	0.033	0.0066	0.020	0.046	7	27184461	HOXA-	0
cg06430102	(N=703)	very early childhood (age 2.5)	0.926	0.862	0.039	4.13E-10*	-0.060	0.0103	-0.080	-0.039	19	1151960	SBNO2	0
cg16691821 ^{a,†}		early childhood (age 3.5)	0.089	0.124	0.028	9.12E-08	0.035	0.0074	0.020	0.049	1	2375627	PEX10	31616
cg19170021		early childhood (age 4.75)	0.734	0.827	0.028	6.28E-08	0.093	0.0209	0.052	0.134	17	79077169	BAIAP2	0
cg05072819 ^a	Maternal psychopathology (N=691)	early childhood (age 5.75)	0.040	0.053	0.031	2.54E-08	0.014	0.0027	0.009	0.019	3	20081367	KAT2B	155
cg05936516		middle childhood (age 6.75)	0.128	0.153	0.031	7.18E-08	0.025	0.0047	0.016	0.035	5	114507066	TRIM36	0
cg04583813		very early childhood (age 8 mo.)	0.900	0.878	0.032	3.57E-08	-0.023	0.0045	-0.032	-0.014	10	560323	DIP2C	0
cg08216050 ^{a,b,†}		very early childhood (age 8 mo.)	0.964	0.968	0.026	7.89E-08	0.004	0.0008	0.002	0.005	16	704013	WDR90	0
cg08171937		very early childhood (age 2.75)	0.016	0.017	0.032	6.79E-10*	0.001	0.0003	0.001	0.002	12	49454761	RHEBL1	3705
cg17806989		early childhood (age 5)	0.981	0.975	0.032	1.55E-08	-0.006	0.0012	-0.008	-0.004	13	25338287	RNF17	12
cg08337366 ^a	One adult in the household (N=710)	very early childhood (age 8 mo.)	0.934	0.906	0.031	2.45E-08	-0.032	0.0065	-0.045	-0.020	19	6371622	ALKB7	820
cg10192047		very early childhood (age 8 mo.)	0.016	0.019	0.029	1.12E-08*	0.003	0.0007	0.002	0.005	19	18722754	TMEM59	926
cg24468070		very early childhood (age 1.75)	0.038	0.058	0.031	7.94E-09*	0.022	0.0044	0.013	0.031	19	54976501	CDC42E	0
cg03397307		very early childhood (age 2.75)	0.025	0.030	0.030	8.42E-09*	0.005	0.0010	0.003	0.007	12	3862423	CRACR2	56
cg05502103 ^{a,b,†}	Family instability (N=703)	early childhood (age 3.5)	0.750	0.626	0.029	6.36E-08	-0.133	0.0283	-0.189	-0.078	7	588936	PRKAR1	0
cg15577126 [†]		early childhood (age 4.75)	0.227	0.291	0.029	7.68E-08	0.061	0.0124	0.037	0.086	2	218932178	RUFY4	0
cg11631610	Financial stress (N=774)	very early childhood (age 8 mo.)	0.949	0.923	0.028	5.75E-09*	-0.027	0.0056	-0.038	-0.016	19	11322739	DOCK6	0
cg01050704 ^a		early childhood (age 5)	0.017	0.019	0.027	1.92E-08	0.002	0.0005	0.001	0.003	19	59084995	MZF1-	0
cg21299458		early childhood (age 5)	0.110	0.147	0.035	1.55E-11*	0.038	0.0070	0.024	0.052	22	20779896	SCARF2	0
cg19219503		middle childhood (age 7)	0.922	0.889	0.029	1.05E-09*	-0.034	0.0070	-0.048	-0.020	10	37414802	ANKRD3	0
cg11714846		accumulation	0.923	0.915	0.023	4.44E-08	-0.005	0.0011	-0.007	-0.003	1	230419534	GALNT2	1658
cg21924472		recency	0.756	0.770	0.028	9.36E-09*	0.003	0.0006	0.002	0.004	4	139600734	LINC004	255235
cg24996440		recency	0.566	0.585	0.027	2.01E-08	0.005	0.0009	0.003	0.006	2	3583570	RNASEH	9119
cg00928478	Neighborhood disadvantage (N=702)	very early childhood (age 1.75)	0.020	0.018	0.028	1.22E-08*	-0.002	0.0005	-0.003	-0.001	10	99078824	FRAT1	196
cg01954337		very early childhood (age 1.75)	0.050	0.059	0.029	3.39E-08	0.008	0.0018	0.005	0.012	11	3819010	NUP98	0
cg04996689		very early childhood (age 1.75)	0.029	0.035	0.027	3.61E-08	0.006	0.0011	0.003	0.008	5	52285560	ITGA2	0
cg12069925		very early childhood (age 1.75)	0.042	0.048	0.030	2.34E-09*	0.007	0.0014	0.004	0.009	17	11900858	ZNF18	72
cg14522055		very early childhood (age 1.75)	0.030	0.035	0.028	4.63E-08	0.005	0.0011	0.003	0.007	15	64338757	DAPK2	235
cg19157140		very early childhood (age 1.75)	0.014	0.016	0.037	3.48E-11*	0.002	0.0004	0.001	0.003	7	766323	PRKAR1	0
cg21740964		very early childhood (age 1.75)	0.160	0.173	0.025	6.32E-08	0.014	0.0028	0.008	0.019	6	3849331	FAM50B	299
cg22396033 [†]	In potentially noisy probe list of Naeem et al. 2014 (i.e., cross-reactive probes, probes with SNPs/INDELs/repeat regions, probes affected by unknown factors).	very early childhood (age 1.75)	0.022	0.025	0.027	9.89E-08	0.003	0.0006	0.002	0.004	1	156862233	PEAR1	1288
cg24826892 ^a		very early childhood (age 1.75)	0.016	0.018	0.030	7.46E-09*	0.003	0.0006	0.002	0.004	11	71159390	DHCR7	0
cg08546016		early childhood (age 5)	0.050	0.056	0.028	1.12E-08*	0.006	0.0012	0.004	0.008	17	72776238	TMEM10	0
cg04007726 ^{a,†}	In potentially noisy probe list of Chen et al. 2013 (i.e., cross-reactive probes, probes with SNPs).	middle childhood (age 7)	0.883	0.858	0.029	5.35E-08	-0.025	0.0053	-0.036	-0.015	10	80981129	ZMZ1	0
cg12412390		middle childhood (age 7)	0.038	0.046	0.030	6.11E-08	0.008	0.0016	0.005	0.011	4	96469286	UNC5C	0

DNAm = unadjusted DNA methylation (beta values) averaged within group; Increase in R² = increase in R² explained by first hypothesis chosen after accounting for covariates; P = p-value of covariance test assessing significance of increase in R² explained; Beta, SE, Lower 95% CI, Upper 95% CI = parameter estimate, standard error, and lower and upper limits of 95% confidence interval of regression coefficient of first hypothesis chosen; Chr, Coordinate = chromosome and position of CpG site; Nearest gene, Distance to nearest gene = Gene symbol of and distance in bases to nearest gene from CpG site (as measured from transcription start site).

^a In potentially noisy probe list of Naeem et al. 2014 (i.e., cross-reactive probes, probes with SNPs/INDELs/repeat regions, probes affected by unknown factors).

^b In potentially noisy probe list of Chen et al. 2013 (i.e., cross-reactive probes, probes with SNPs).

*significant at $p < 1.43 \times 10^{-8}$, a more stringent p-value threshold that accounted for the testing of seven types of adversity ($1 \times 10^{-7} / 7 = 1.43 \times 10^{-8}$).

[†] Not identified in the main analysis presented in Table 1.

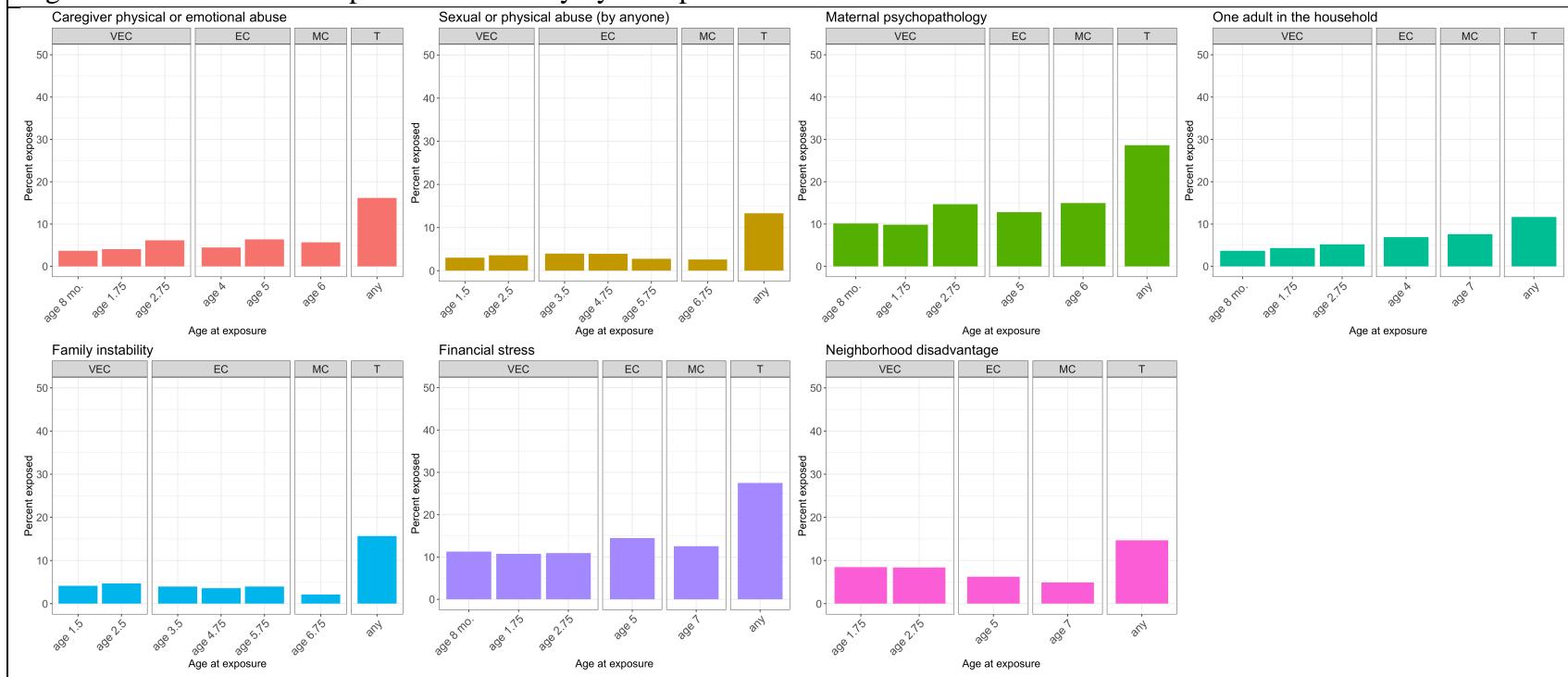
Table S8. Description of theoretical models used in the analysis, using exposure to abuse as an example

Lifecourse model tested	Definition	Variables	Specific variables entered into the LARs model
Accumulation	Sum of the total number of time periods of exposure to a specific adversity. To test whether the number of time periods of exposure explains the most variance in DNAm.	1	abuse_accumulation=count of the number of time periods exposed to abuse (range 0-6)
Sensitive period	A single time-point at which there can be exposure to adversity. To test if a single adversity experience at a specific time-point explains the most variance in DNAm.	6	abuse_period1=exposed (1) vs. unexposed (0) at time period 1 (8 months); abuse_period2= exposed (1) vs. unexposed (0) at time period 2 (1.75 years); abuse_period3= exposed (1) vs. unexposed (0) at time period 3 (2.75 years); abuse_period4= exposed (1) vs. unexposed (0) at time period 4 (4 years); abuse_period5= exposed (1) vs. unexposed (0) at time period 5 (5 years); abuse_period6= exposed (1) vs. unexposed (0) at time period 6 (6 years)
Recency	Sum of the total number of time periods of exposure to a specific type of adversity, with each time period weighted by the age in years of the child during exposure. To test if temporal proximity to adversity events explains the most variance in DNAm.	1	abuse_recency= abuse_period1 exposed (1) vs. unexposed (0)*(0.67) + abuse_period2 exposed (1) vs. unexposed (0) *(1.75) + abuse_period3 exposed (1) vs. unexposed (0) *(2.75) + abuse_period4 exposed (1) vs. unexposed (0) *(4) + abuse_period5 exposed (1) vs. unexposed (0) *(5) + abuse_period6 exposed (1) vs. unexposed (0) *(6)

In this study, *accumulation* was defined as the sum of the total number of time periods of exposure to a given adversity. Although accumulation is sometimes operationalized as the total number of distinct adversity types experienced (and in this case, is often referred to as “cumulative risk”), we defined accumulation in the manner we did for the following reasons. First, research on the effects of multiple adversities or “cumulative risks” in general has been well-covered by prior literature on “adverse childhood experiences” (e.g., 47, 48, 49). One of the unique contributions of the current study is its attention to differences between adversity types and their associations with DNAm changes. Secondly, accumulation models that do not account for adversity type or duration offer little promise for identifying optimal intervention targets, given that they treat all adverse experiences as equal. Finally, there is no unified definition of cumulative risk (50-52), and there have been multiple calls in the field for measures that capture exposure features like developmental timing and duration (50, 53). Our operationalization, then, represents one attempt at capturing accumulation through the lens of duration.

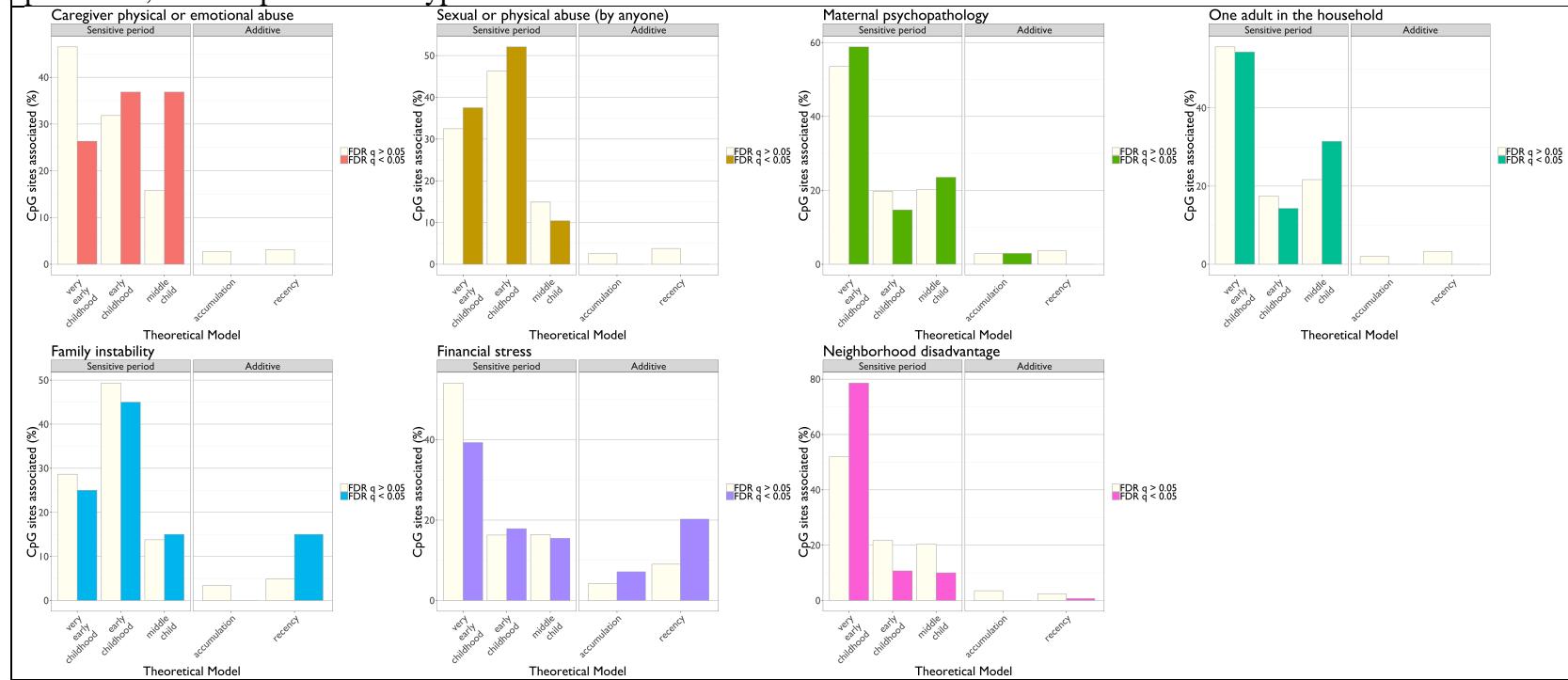
The *recency* hypothesis, in turn, assumes a linear increase in the effect of exposure over time, and weights more recent exposures more heavily than more distal ones (18). Unlike the last sensitive periods model, which captures only exposure to a given adversity within that specific time period, the recency model accounts for and weights all time periods of exposure.

Figure S1. Prevalence of exposure to adversity by time period



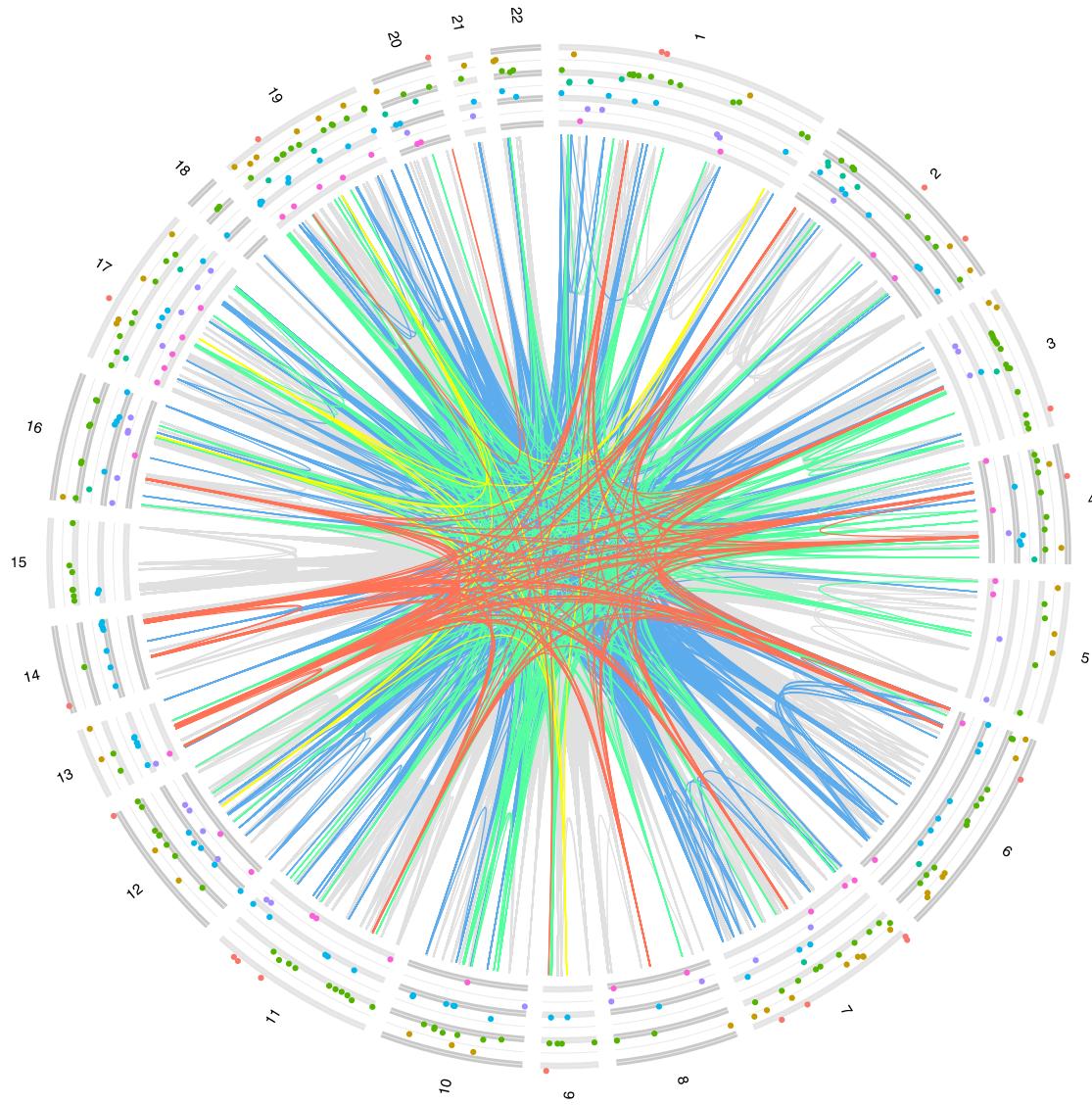
Time periods are very early childhood (I, before 3), early childhood (PS, ages 3-5), middle childhood (MC, ages 6-7), and total exposed at any time (T). As shown, age at exposure to adversity varied by the type of adversity. Family instability and neighborhood disadvantage were more common in very early childhood and early childhood (before age 4), whereas one adult in the household and financial stress were more common later in middle childhood

Figure S2. Frequency each theoretical model (sensitive period or additive) was chosen first by the LARS variable selection procedure, for all CpG sites and types of adversities tested



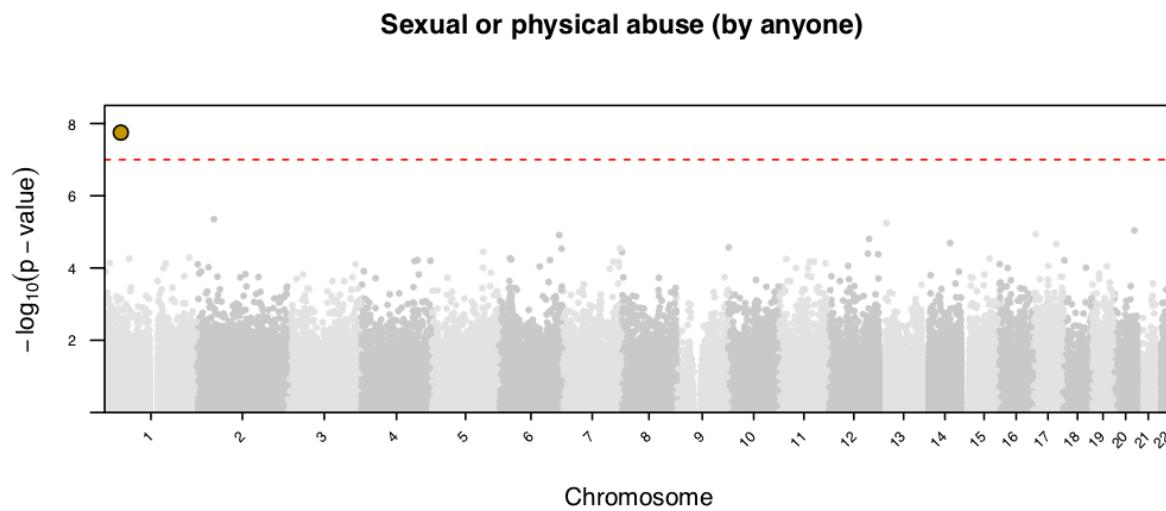
These figures display the percent of CpG sites for which methylation was best predicted by each of the theoretical models, after controlling for covariates. The distribution of hypotheses for FDR-significant CpG sites ($FDR\ q < 0.05$) was significantly different than that for the remaining CpG sites tested ($FDR\ q > 0.05$) for financial stress ($\chi^2=16.92$, $p=0.002$), and neighborhood disadvantage ($\chi^2=40.79$, $p<0.0001$). Sensitive period models were more often selected than additive models for family instability and neighborhood disadvantage, while the opposite was true for financial stress.

Figure S3. Circos plot of 380 FDR-significant sites



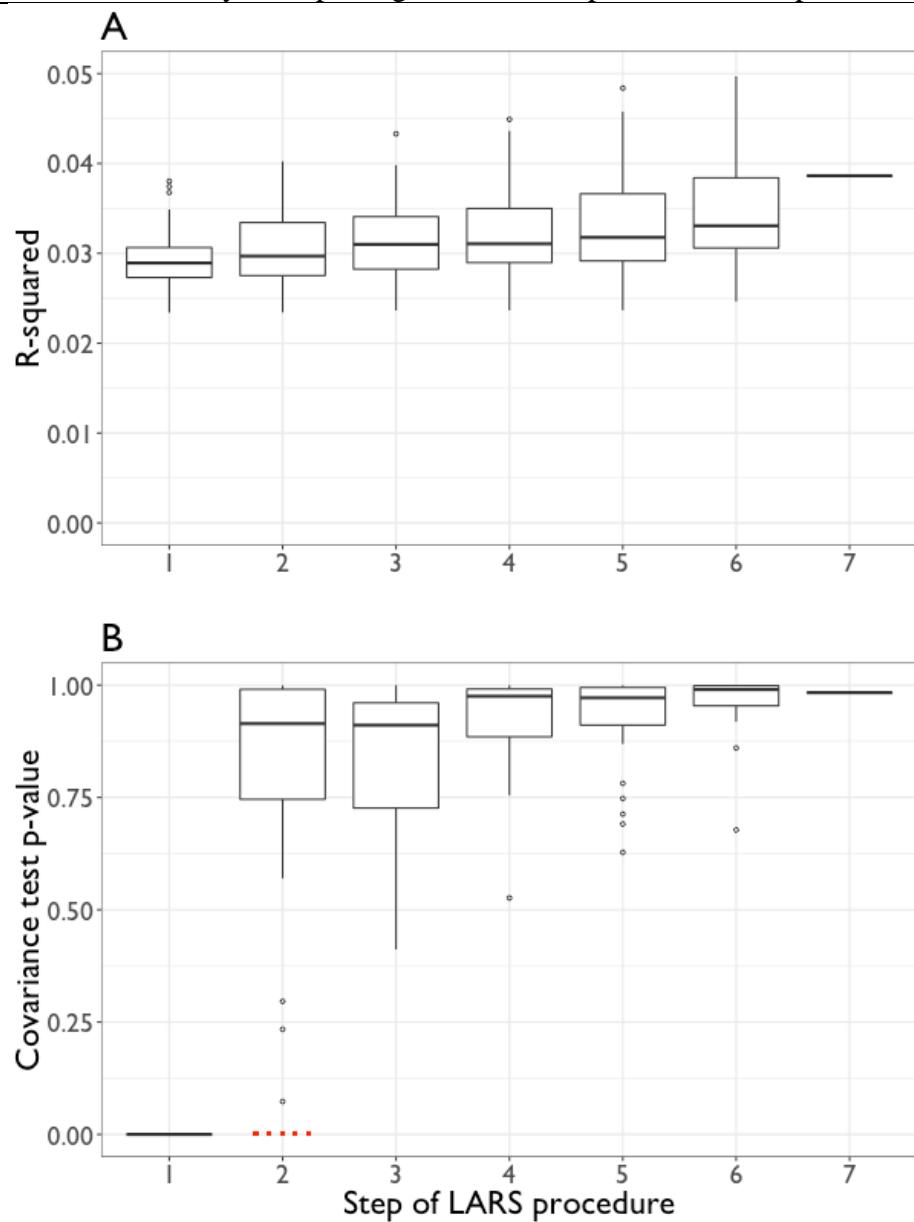
The effects of adversity on methylation were distributed throughout the genome. Outer rings: points represent genomic locations of all FDR-significant CpG sites, colored by adversity type (as above). Inner links: lines connect loci associated with the same adversity type and theoretical model, colored by theoretical model (grey=very early childhood, blue=early childhood, green=middle childhood, yellow=accumulation, red=recency).

Figure S4. Manhattan plots displaying the only significant CpG site (cg02431672) associated with exposure to abuse identified by the EWAS approach



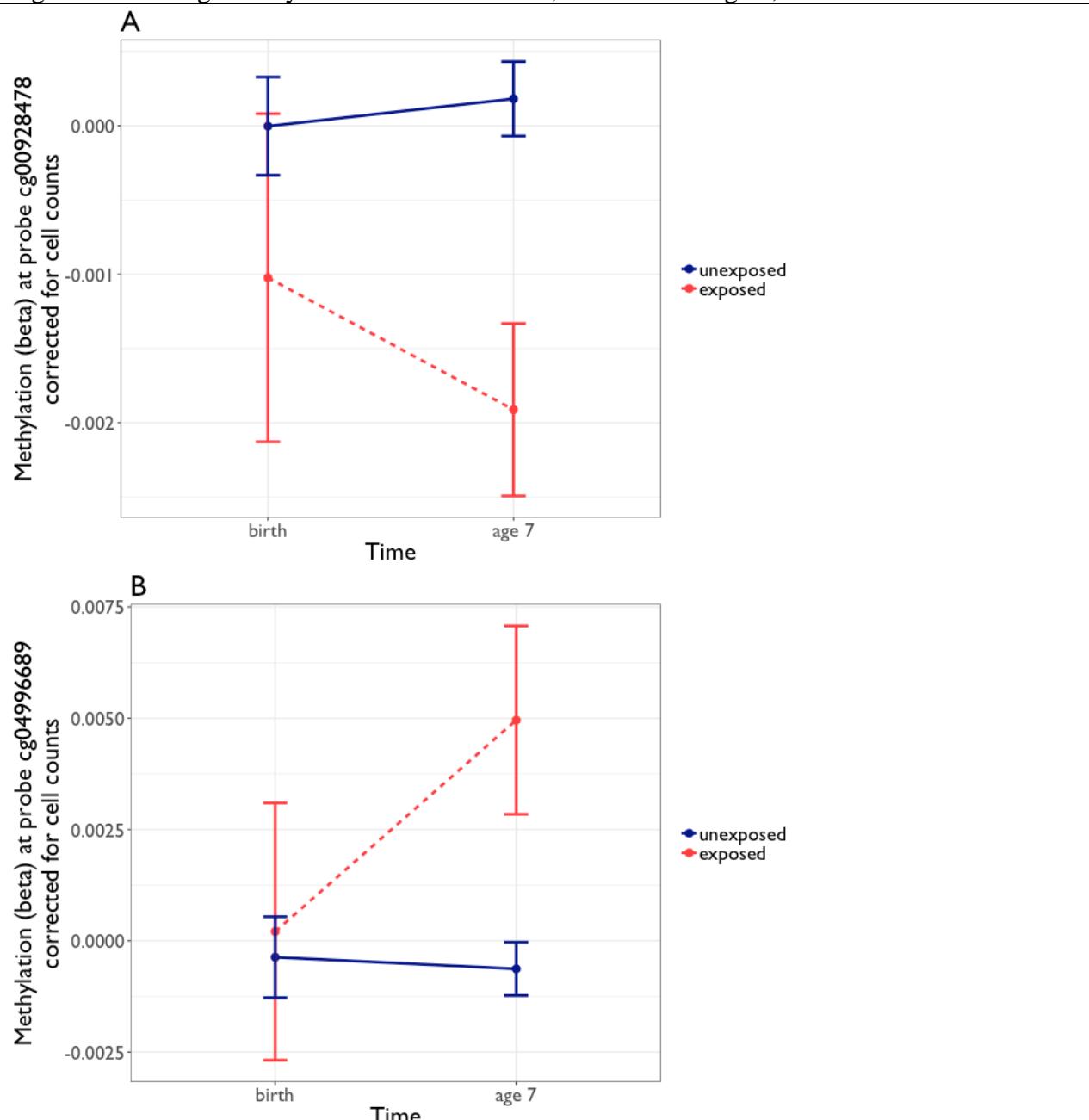
In this Manhattan plot, the x-axis is the chromosomal position for each CpG site and the y-axis is the $-\log_{10}$ p-value for the association between exposure to adversity and DNAm values at each CpG site. The dashed line shows the epigenome-wide significance level, with each CpG site above the line representing a statistically significant association ($p < 1 \times 10^{-7}$). As shown, only one CpG site was identified by the EWAS approach to be significantly affected by exposure to sexual or physical abuse. No locus was identified to be affected by other types of adversity.

Figure S5. Results of analyses exploring additional steps of the LARS procedure



The CpG sites associated with adversity were detected by examining the first step of the LARS variable selection procedure. The first step of the LARS identified the *single* theoretical model that explained the most variation in DNA methylation at a given CpG site. However, it is possible that additional theoretical models could have been chosen by the LARS at the second step and beyond. We therefore evaluated this possibility by calculating the variance explained by additional steps of the LARS and assessed the significance of the increase with a covariance test at each step. **Panel A:** Additional steps of the LARS procedure explained marginally more variance in methylation (R^2). **Panel B:** However, the significance of the increase in variance explained (covariance test p-value) did not surpass a nominal significance threshold (red dotted line: $p=0.05$) for any of the 38 top CpG sites, suggesting that there was little evidence that examining more than the first step of the LARS procedure would add more information.

Figure S6. Average methylation values over time, from birth to age 7, in ARIES



Because some adversities could have been present prenatally and could affect methylation *in utero*, we assessed methylation at birth in umbilical cord blood at the top CpG sites. At each top CpG site, we tested the predictive value of the theoretical model chosen at age 7 on methylation at birth with linear regression, controlling for the same covariates as described previously. We used a Bonferroni correction to adjust the alpha level for multiple testing. These plots display two illustrative examples of DNAm values over time. **Panel A:** Methylation that was different at birth among those exposed vs. unexposed to postnatal adversity. **Panel B:** Methylation that was not different at birth among those exposed vs. unexposed to postnatal adversity.

Figure S7. Genomic locations of FDR-significant CpG sites (n=380) as compared to all other autosomal CpG sites tested

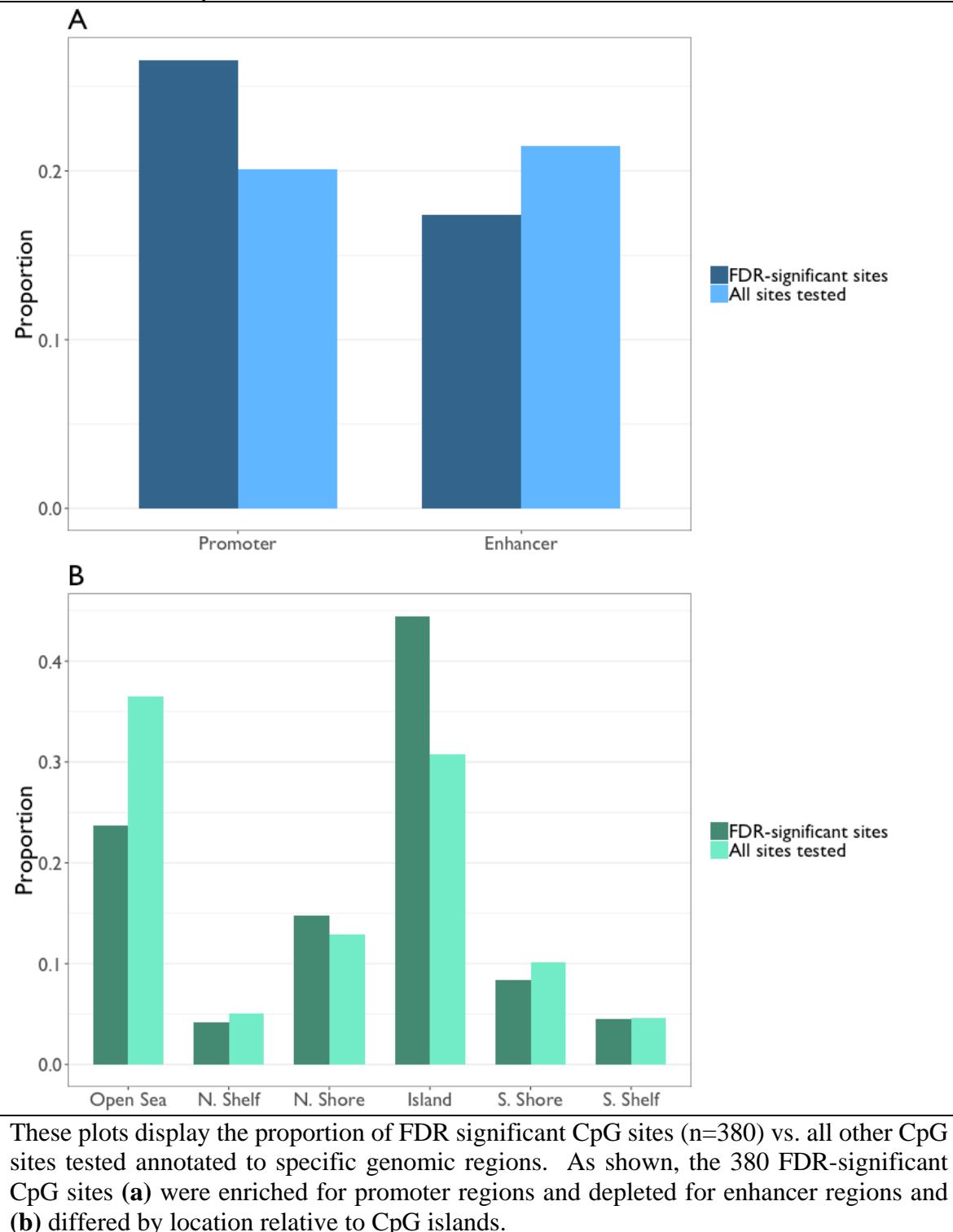
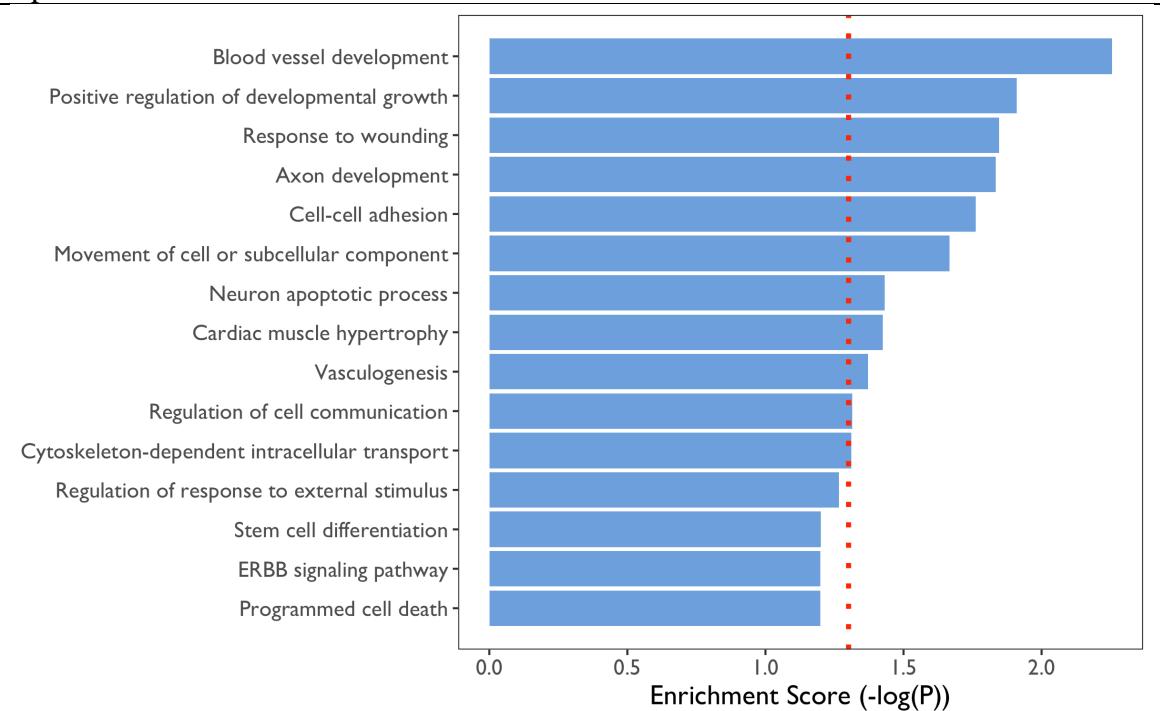
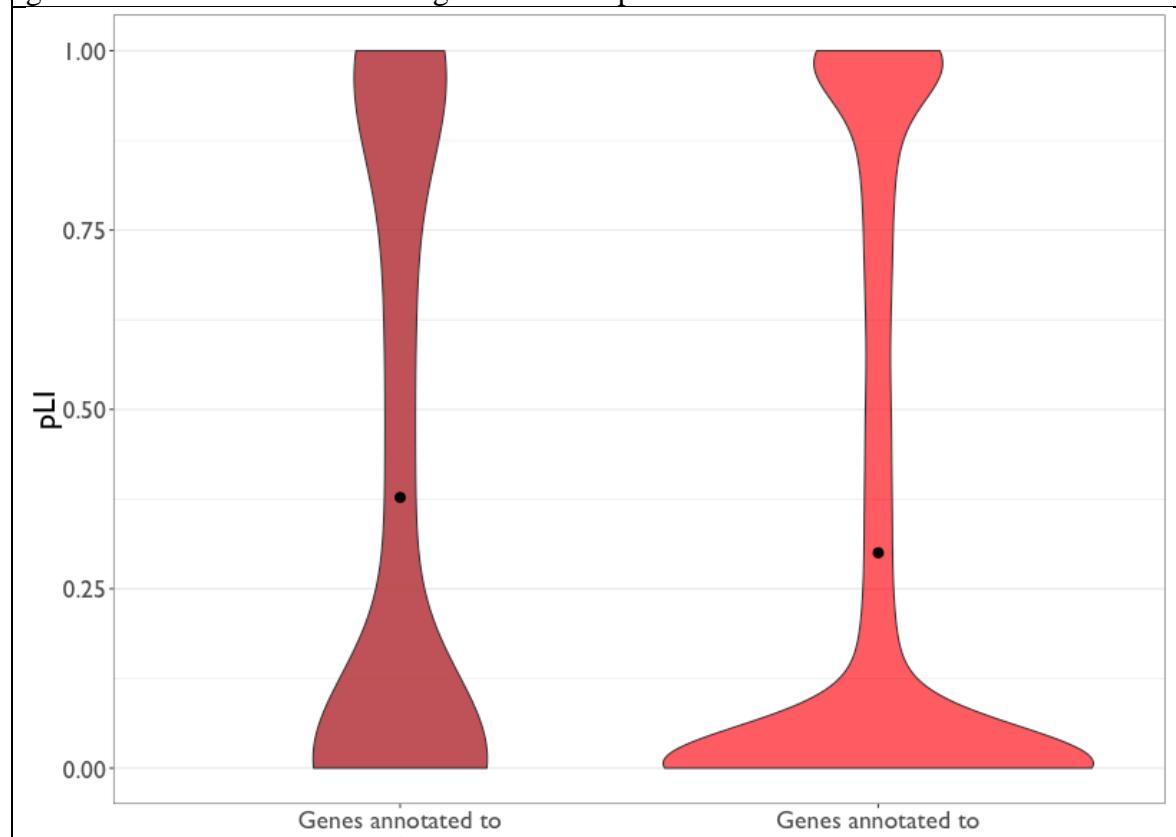


Figure S8. Enrichment of Gene Ontology (GO) term clusters for the 380 FDR-significant CpG sites



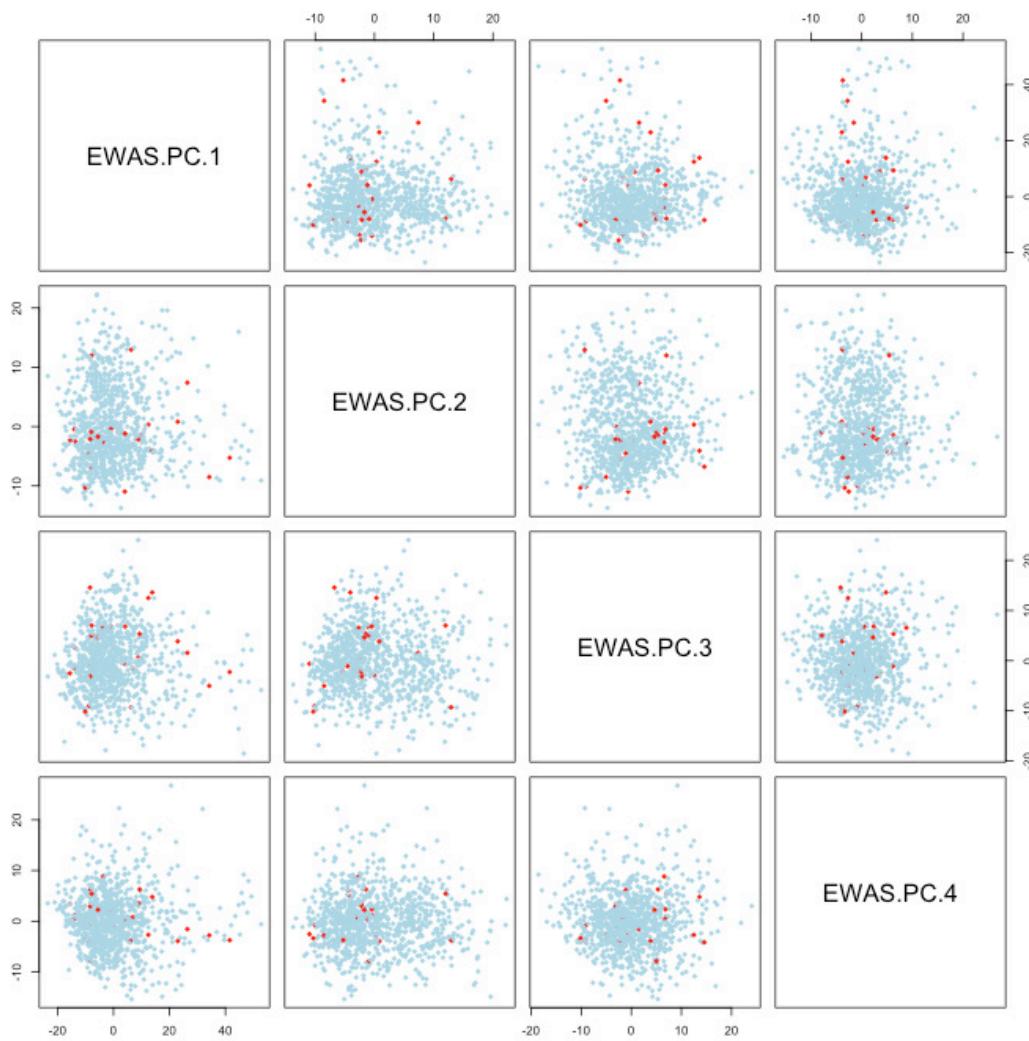
The 380 FDR-significant CpG sites were annotated to 365 genes. The plot displays enrichment scores (-log(P)) taken from 15 (out of 158 clusters) of GO biological process terms that corresponded to these 365 genes. As shown, 11 GO term clusters were enriched at a nominally significant level (red dashed line=1.3, the negative logarithm of $p=0.05$). These results suggest that the top 11 GO term clusters, including positive regulation of developmental growth, axon development, and neuron apoptotic process, were more likely to be represented among genes annotated to FDR-significant CpG sites than chance (average enrichment $p<0.05$).

Figure S9. Genes annotated to the FDR-significant sites were more highly constrained than genes annotated to the remaining autosomal CpG sites tested



Violin (rotated kernel density) plots of constraint scores (pLI) for genes annotated to FDR-significant sites and the remaining CpG sites tested. pLI = probability of a gene being intolerant to Loss-of-Function variation. Black points represent mean pLI values per gene set. Genes annotated to FDR-significant sites were more highly constrained than the rest of the autosomal genes tested (permutation $p=0.0001$), indicating a greater importance of these genes, on average, to survival and reproduction over human evolution.

Figure S10. Principal components of ancestry information inferred based on epigenome-wide DNA methylation data



Scatter plots showing patterns of ancestry inferred using an epigenome-wide DNAm data based principal component analysis (4). The method has been shown to reliably capture population structure even in the absence of genetic data. The same quality control procedure was performed following the guidelines provided by Rahmani et al. (4) and 473,864 CpGs were used in the principal component analysis, adjusting for sex and cell counts. Red dots indicate children who were self-reported to be non-white. As shown in these plots, we found no apparent outlier or pattern of population stratification; the principal components of self-reported white and nonwhite children seemed to be well blended.

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