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Archival Report

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

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## ABSTRACT

**BACKGROUND:** Exposure to early-life adversity is known to predict DNA methylation (DNAm) patterns that may be related to psychiatric risk. However, few studies have investigated whether adversity has time-dependent effects based on the age at exposure.

**METHODS:** Using a two-stage structured life course modeling approach, we tested the hypothesis that there are sensitive periods when adversity induces greater DNAm changes. We tested this hypothesis in relation to two alternatives: an accumulation hypothesis, in which the effect of adversity increases with the number of occasions exposed, regardless of timing; and a recency model, in which the effect of adversity is stronger for more proximal events. Data came from the Accessible Resource for Integrated Epigenomic Studies, a subsample of mother–child pairs from the Avon Longitudinal Study of Parents and Children ($n$ = 691–774).

**RESULTS:** After covariate adjustment and multiple testing correction, we identified 38 CpG sites that were differentially methylated at 7 years of age following exposure to adversity. Most loci ($n$ = 35) were predicted by the timing of adversity, namely exposures before 3 years of age. Neither the accumulation nor recency of the adversity explained considerable variability in DNAm. A standard epigenome-wide association study of lifetime exposure (vs. no exposure) failed to detect these associations.

**CONCLUSIONS:** The developmental timing of adversity explains more variability in DNAm than the accumulation or recency of exposure. Very early childhood appears to be a sensitive period when exposure to adversity predicts differential DNAm patterns. Classification of individuals as exposed versus unexposed to early-life adversity may dilute observed effects.

*Keywords:* Childhood adversity, Children, DNA methylation, Epigenetics, Longitudinal, Sensitive periods

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Exposure to childhood adversity, including poverty (1), abuse (2,3), family dysfunction (4,5), and other stressors (6,7), is a common and potent determinant of mental health across the lifespan, increasing risk of childhood- and adult-onset psychiatric disorders by at least twofold (8–10). Although the biological mechanisms explaining this relationship are poorly understood, accumulating evidence suggests that adversity may become programmed molecularly, leaving behind biological memories that persistently alter genome function and increase susceptibility to mental disorders. Indeed, dozens of candidate gene and epigenome-wide association studies (EWASs) in both animals and humans have shown that early-life adversity is associated with persistent alterations in the epigenome (11–15), including changes in DNA methylation (DNAm), which is the most studied epigenetic mechanism involving the addition of methyl groups to cytosines in the DNA sequence (16,17). These differential DNAm sites can alter gene expression, providing a mechanism by which gene by environment interactions affect biological responses (18).

Recent evidence, particularly from animal studies, suggests that epigenetic programming may be developmentally time sensitive and that there may be sensitive periods (19,20) when adversity exposure is more likely to induce DNAm changes. For instance, rodent experiments have demonstrated the existence of sensitive periods for different aspects of epigenetic regulation—from embryonic reprogramming to postnatal exposure—leading to differences in epigenetic outcomes and gene expression (21–25). Recent work in nonhuman primates also suggests that there are differential effects on DNAm based on whether adversity exposure, including maternal separation, occurred at birth or later in development (26). However, few human studies, whether candidate gene studies (16,27–29) or EWASs (30–32), have examined the time-dependent effects of psychosocial adversity on DNAm; nearly all human epigenetic studies have instead focused on the presence versus absence of exposure to early-life adversity. Thus, it is unknown whether there are age stages at which adversity differentially affects DNAm, children are therefore

SEE COMMENTARY ON PAGE 789

 
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more vulnerable, and prevention efforts could be most efficacious.

This study aimed to address this limitation by using data from a prospective birth cohort of children to test the hypothesis that there are sensitive periods associated with DNAm alterations following adversity exposure. To test this hypothesis, we used a two-stage structured life course modeling approach (SLCMA) (33,34) to examine the effect of repeated exposure to seven types of childhood adversities across three developmental periods (in very early childhood, before 3 years of age; early childhood, 3–5 years of age; and middle childhood, 6–7 years of age) on DNAm profiles at 7 years of age. Recognizing that alternative conceptual models have been proposed to explain the effects of adversity, we also used the SLCMA to determine whether the sensitive period model explained more variability in DNAm relative to two other theoretical models described in the life course epidemiology literature (35–37): 1) an accumulation model (38–40), in which the effect of adversity on DNAm increases with the number of occasions exposed, regardless of timing; and 2) a recency model (41), in which the effect of adversity on DNAm is stronger for more proximal events. Finally, to evaluate the potential advantage of the SLCMA relative to the standard EWAS approach, which would ignore the timing or frequency of adversity, we examined the number of epigenome-wide significant loci identified by each approach and evaluated their degree of overlap.

## METHODS AND MATERIALS

### Sample and Procedures

Data came from the Avon Longitudinal Study of Parents and Children (ALSPAC), a population-based birth cohort (42–44). The ALSPAC generated blood-based DNAm profiles at birth and 7 years of age as part of the Accessible Resource for Integrated Epigenomic Studies (ARIES), a subsample of 1018 mother–child pairs from the ALSPAC (45). The ARIES mother–child pairs were randomly selected out of those with complete data across at least five waves of data collection (Supplement 1).

### Measures

**Exposure to Adversity.** We examined the effect of seven adversities shown previously to associate with epigenetic marks (46–48): 1) caregiver physical or emotional abuse (49–52); 2) sexual or physical abuse (by anyone) (49–52); 3) maternal psychopathology (53,54); 4) one adult in the household (55); 5) family instability (29,56); 6) financial stress and/or poverty (57,58); and 7) neighborhood disadvantage and/or poverty (59). These adversities were chosen because they capture experiences that deviate from a child's expected social and physical environment (60). Each adversity was measured via maternal report on at least four occasions at or before 7 years of age either from a single item or from psychometrically validated standardized measures. Specific time periods of assessment varied across adversity type (Supplement 1). For each adversity type, we generated three sets of encoded variables (Supplement 1): 1) a set of variables indicating the presence of the adversity at a specific developmental stage versus absence of the adversity at that stage, to test the sensitive period hypothesis; 2) a single variable denoting the total number of time periods of exposure to a given adversity, to test the accumulation hypothesis; and 3) a single variable denoting the total number of developmental periods of exposure, with each exposure weighted by the age of the child during the measurement time period, to test the recency hypothesis; this variable upweighted more recent exposures, allowing us to determine whether more recent exposures were more impactful.

**DNA Methylation.** DNAm was measured at 485,000 CpG dinucleotide sites across the genome using the Illumina Infinium Human Methylation 450K BeadChip microarray (Illumina, San Diego, CA). DNA for this assay was obtained from cord blood at birth and peripheral blood leukocytes at 7 years of age. DNA was stored and extractions were completed at 5 to 8 years after collection of cord blood and within 3 weeks after collection of peripheral blood at age 7 (61). DNAm wet laboratory procedures, preprocessing analyses, and quality control were performed at the University of Bristol [Supplement 1 and Relton *et al.* (45)]. DNAm levels are expressed as a β value representing the proportion of cells methylated at each interrogated CpG site.

Prior to analysis, raw methylation β values, which are preferred over *M* values because of their interpretability (62), were normalized (63) to remove or minimize the effects of variation due to technical artifacts. To adjust for DNAm variation due to cell-type heterogeneity in peripheral and cord blood samples, we estimated cell counts from DNAm profiles (64) and regressed these estimates from the normalized β values. Additionally, to remove possible outliers, we winsorized the β values at each CpG site, setting the bottom 5% and top 95% of values to the 5th and 95th quantile, respectively (65).

**Covariates.** To adjust for baseline sociodemographic differences in the cohort, all analyses additionally controlled for the following variables, measured at birth (Supplement 1): child race and/or ethnicity; child birth weight; maternal age; number of previous pregnancies; sustained maternal smoking during pregnancy; and parent social class (66). Justification for the inclusion of parent social class as a covariate and alternative results from analyses that exclude social class as a covariate are presented in Supplement 1.

### Data Analysis

Our primary analyses involved comparing the three theoretical models using the SLCMA, which was originally developed by Mishra *et al.* (34) and later extended by Smith *et al.* (33,67) to analyze repeated exposure data across the life course (Supplement 1). The major advantage of the SLCMA is that it provides an unbiased way to compare multiple competing theoretical models simultaneously and identify the most parsimonious explanation for the observed outcome variation. The SLCMA uses least angle regression (LARS) (68) and an associated covariance test (69) to identify the single theoretical model (or potentially more than one model working in combination) that explains the most outcome variation ($R^2$). Compared with other methods for structured life course analysis, LARS has greater statistical power (33) and does not overinflate effect size estimates (68) or bias hypothesis tests (69). The SLCMA has been used in several life course epidemiology studies (70,71), including studies of other birth cohorts (72,73). The LARS procedure functions under the same assumptions as multiple linear regression.

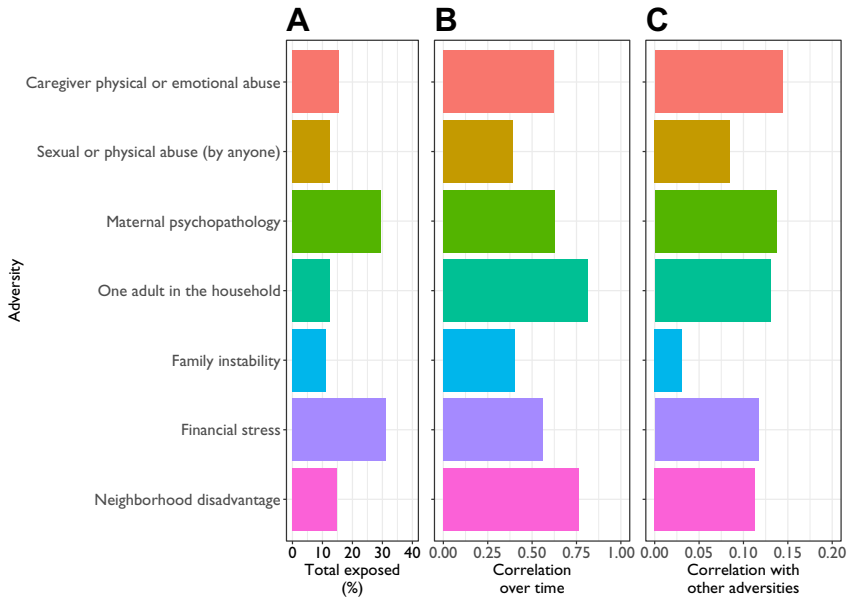


**Figure 1.** Exposure to adversity in the Accessible Resource for Integrated Epigenomic Studies data set. The figure displays the lifetime prevalence by 7 years of age of exposure to each adversity (labeled as "total exposed"), the average correlation between exposure to one type of adversity at one time point and exposure to that same adversity at a second time point (labeled as "correlation over time"), and the average correlation between exposure to one type of adversity and exposure to a second type of adversity (labeled as "correlation with other adversities"). **(A)** The lifetime prevalence of each adversity varied by type. The most commonly reported adversities were financial stress (31%) and maternal psychopathology (29%). The remaining adversities were less reported, but still common: caregiver physical or emotional abuse (15%), neighborhood disadvantage (15%), sexual or physical abuse (by anyone; 13%), one adult in the household (13%), and family instability (11%). **(B)** Among specific types of adversity, exposures tended to correlate over time, with neighboring time points being more related than distant time points. For instance, exposure to one adult in the household and neighborhood disadvantage were most strongly correlated over time ($r$ = .54–.93 and $r$ = .67–.89, respectively), whereas exposure to family instability ($r$ = .11–.74) and sexual or physical abuse ($r$ = .02–.69) were more weakly correlated across time. **(C)** The average correlation of having ever been exposed to the other adversities was modest across adversities, suggesting that we were capturing unique subtypes of adversity.

In the first stage, we entered the set of encoded variables described previously into the LARS variable selection procedure (68). LARS identified the variable with the strongest association with the outcome, thus identifying whether the sensitive period, accumulation, or recency model was most supported by the data. Therefore, for each CpG site, one unique LARS model was selected for each of the seven types of adversity. For each selected model, we performed a covariance test of the null hypothesis that the variable selected is unassociated with the outcome. With respect to multiple testing, the covariance test $p$ values are adjusted for the number of variables included in the LARS procedure, controlling the type I error rate for each adversity type and CpG site. To adjust for confounding during the first stage, we regressed each encoded variable on the covariates and implemented LARS on the regression residuals (67).

In the second stage, the theoretical model shown in the first stage to best fit the observed data for a specific type of adversity was then carried forward to a multiple regression framework, where measures of effect were estimated. Positive effect estimates thus indicate elevated (hyper-) methylation, and negative effect estimates indicate decreased (hypo-) methylation. Only models with a covariance test $p$ value $< 1 \times 10^{-7}$, the standard Bonferroni correction threshold for epigenome-wide statistical significance, were included in the second stage. The same covariates were also included in the second stage. We compared the distribution of theoretical models across the Bonferroni-significant CpG sites with an omnibus $\chi^2$ test, which tested the null hypothesis that the theoretical models were likely to be represented among the significant results in proportion to the frequency in which they were tested.

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 (coded as ever vs. never exposed) and DNAm across all

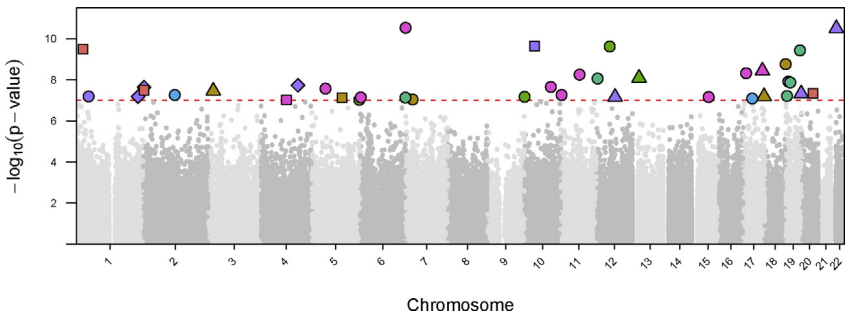


**Figure 2.** A Manhattan plot displays top CpG sites associated with exposure to adversity. In this Manhattan plot, the x-axis is the chromosomal position for each CpG site and the y-axis is the $-\log_{10} p$ value for the association between exposure to adversity and DNA methylation values at each CpG site. The dashed line shows the epigenome-wide significance level, with each CpG site above the line representing a statistically significant association ($p < 1 \times 10^{-7}$). The color of each CpG site refers to the type of adversity. The shape of each CpG site indicates the life course model tested. The sensitive period hypotheses were encoded as a circle for very early childhood, a triangle for early childhood, and a square for middle childhood. The recency and accumulation hypotheses were encoded as a diamond. As shown, CpG sites significantly affected by exposure adversity were distributed throughout the genome. There was no obvious genomic spatial pattern by adversity type or timing of exposure.

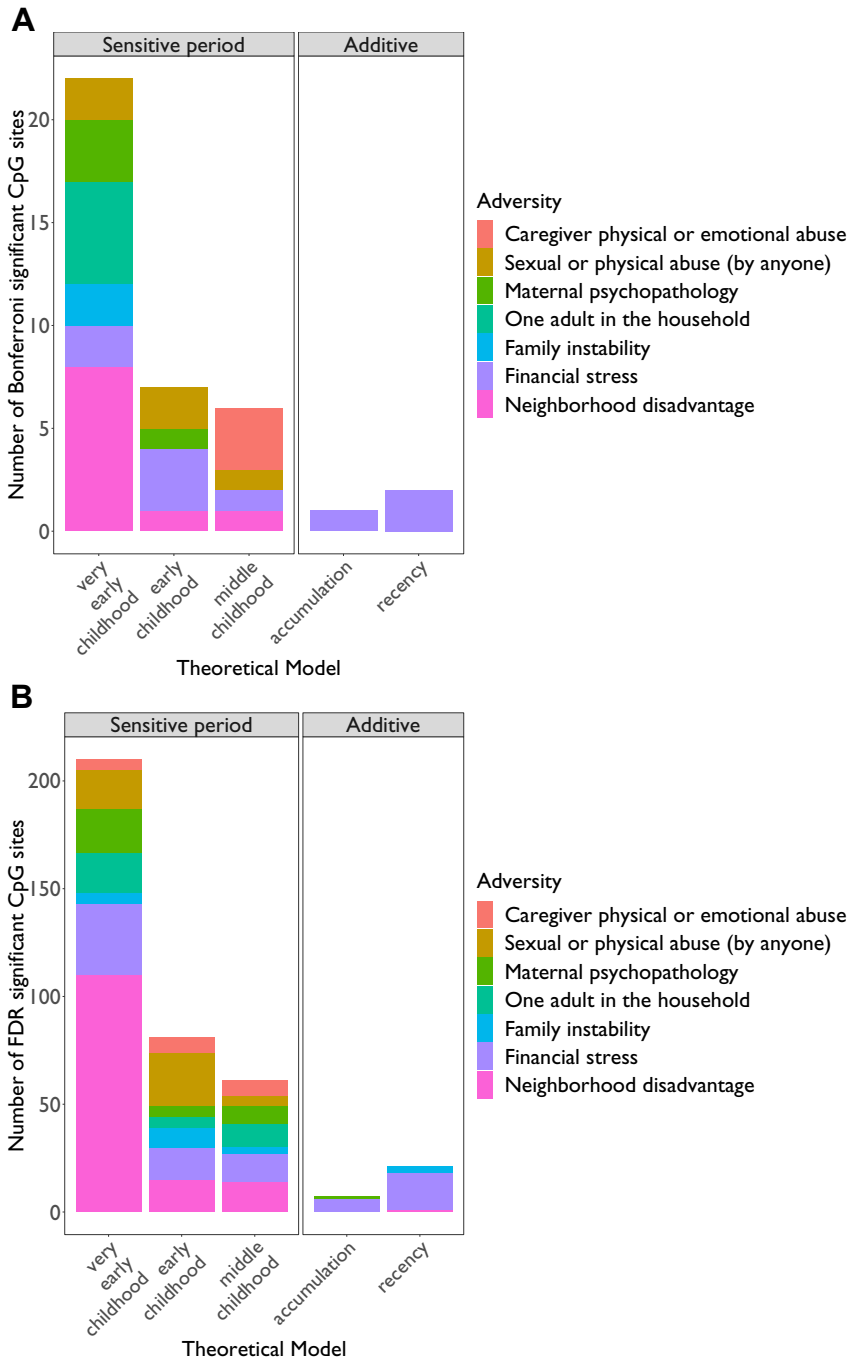


**Figure 3.** The figure illustrates the frequency at which each life course theoretical model was chosen for each type of adversity. Each plot displays the number of CpG sites for which adversity significantly predicted methylation, after controlling for covariates and correcting for multiple comparisons using **(A)** a Bonferroni threshold ($p < 1 \times 10^{-7}$, $n$ = 38 sites) and **(B)** a false discovery rate (FDR) correction $q < .05$ ($n$ = 380 sites). The distribution of theoretical models chosen first by the least angle regression procedure for top CpG sites was significantly different from what would be expected by chance, with exposure to adversity during sensitive periods, especially during very early childhood, more frequently predicting methylation.

CpG sites. The EWAS results were then compared with the SLCMA to determine whether the two approaches yielded similar or distinct conclusions regarding the number of significant loci detected.

We also performed sensitivity analyses to evaluate the fit of the LARS selection procedure, determine the degree of differential methylation present at birth, and control for genetic variation. We examined the biological significance of the findings by 1) examining the correlation in methylation between blood and brain tissue for the top CpG sites using an online database (74); 2) investigating enrichment of regulatory elements annotated to false discovery rate (FDR)–significant CpG sites; 3) performing a functional clustering analysis of all gene ontology terms for genes annotated to FDR-significant sites in DAVID 6.8 (75); and 4) assessing the selective constraint of these genes using the Exome Aggregation Consortium (76).

**Table 1. Results of the SLCMA in ARIES, With Annotation to the Closest Gene, for the Bonferroni–Significant CpG Sites ($p < 1 \times 10^{-7}$)**

| Adversity | CpG Site | First Hypothesis Chosen by LARS Procedure | DNAm in Unexposed Group, β[a] | DNAm in Exposed Group, β[a] | Increases in $R^2$[b] | *p* Value[c] | Effect Estimate, β[d] | SE[d] | Lower 95% CI[d] | Upper 95% CI[d] | Chr[e] | Coordinate, bp[e] | Nearest Gene[f] | Distance to Nearest Gene, bp[f] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Caregiver physical or emotional abuse (*n* = 719) | cg10713431 | Middle childhood (6 years of age) | .132 | .139 | .025 | $4.59 \times 10^{-8}$ | .008 | .0019 | .004 | .012 | 20 | 43933204 | *MATN4* | 0 |
| | cg12023170[g] | Middle childhood (6 years of age) | .074 | .086 | .038 | $3.17 \times 10^{-10}$[i] | .013 | .0022 | .008 | .017 | 1 | 23751761 | *TCEA3* | 499 |
| | cg19825600[g,h] | Middle childhood (6 years of age) | .458 | .384 | .027 | $3.23 \times 10^{-8}$ | −.072 | .0158 | −.103 | −.041 | 2 | 3704501 | *ALLC* | 1283 |
| Sexual or physical abuse (by anyone) (*n* = 703) | cg01370449 | Very early childhood (2.5 years of age) | .244 | .334 | .030 | $8.87 \times 10^{-8}$ | .083 | .0168 | .050 | .116 | 7 | 27183369 | *HOXA-AS3* | 0 |
| | cg06430102 | Very early childhood (2.5 years of age) | .926 | .862 | .037 | $1.69 \times 10^{-9}$[i] | −.058 | .0103 | −.078 | −.038 | 19 | 1151960 | *SBNO2* | 0 |
| | cg19170021 | Early childhood (4.75 years of age) | .734 | .827 | .028 | $6.41 \times 10^{-8}$ | .092 | .0209 | .051 | .134 | 17 | 79077169 | *BAIAP2* | 0 |
| | cg05072819[g] | Early childhood (5.75 years of age) | .040 | .053 | .030 | $3.49 \times 10^{-8}$ | .014 | .0027 | .009 | .019 | 3 | 20081367 | *KAT2B* | 155 |
| | cg05936516 | Middle childhood (6.75 years of age) | .128 | .153 | .031 | $7.47 \times 10^{-8}$ | .025 | .0048 | .016 | .035 | 5 | 114507066 | *TRIM36* | 0 |
| Maternal psychopathology (*n* = 691) | cg04583813 | Very early childhood (8 mo of age) | .900 | .878 | .031 | $6.57 \times 10^{-8}$ | −.023 | .0046 | −.032 | −.014 | 10 | 560323 | *DIP2C* | 0 |
| | cg08171937 | Very early childhood (2.75 years of age) | .016 | .017 | .034 | $2.33 \times 10^{-10}$[i] | .001 | .0003 | .001 | .002 | 12 | 49454761 | *RHEBL1* | 3705 |
| | cg10666628 | Very early childhood (2.75 years of age) | .020 | .021 | .029 | $9.29 \times 10^{-8}$ | .002 | .0004 | .001 | .003 | 5 | 179050666 | *HNRNPH1* | 0 |
| | cg17806989 | Early childhood (5 years of age) | .981 | .975 | .032 | $8.16 \times 10^{-9}$[i] | −.006 | .0012 | −.009 | −.004 | 13 | 25338287 | *RNF17* | 12 |
| One adult in the household (*n* = 710) | cg08337366[a] | Very early childhood (8 mo of age) | .934 | .906 | .029 | $6.07 \times 10^{-8}$ | −.032 | .0066 | −.045 | −.019 | 19 | 6371622 | *ALKBH7* | 820 |
| | cg10192047 | Very early childhood (8 mo of age) | .016 | .019 | .029 | $1.31 \times 10^{-8}$[i] | .003 | .0007 | .002 | .005 | 19 | 18722754 | *TMEM59L* | 926 |
| | cg26990406 | Very early childhood (8 mo of age) | .868 | .728 | .027 | $7.22 \times 10^{-8}$ | −.142 | .0308 | −.203 | −.082 | 7 | 178829 | *FAM20C* | 14138 |
| | cg24468070 | Very early childhood (1.75 years of age) | .038 | .058 | .034 | $3.63 \times 10^{-10}$[i] | .023 | .0044 | .014 | .031 | 19 | 54976501 | *CDC42EP5* | 0 |
| | cg03397307 | Very early childhood (2.75 years of age) | .025 | .030 | .030 | $8.46 \times 10^{-9}$[i] | .005 | .0010 | .003 | .007 | 12 | 3862423 | *CRACR2A* | 56 |
| Family instability (*n* = 703) | cg18311384 | Very early childhood (2.5 years of age) | .019 | .022 | .027 | $7.97 \times 10^{-8}$ | .002 | .0005 | .001 | .003 | 17 | 34842312 | *ZNHIT3* | 159 |
| | cg27637303 | Very early childhood (2.5 years of age) | .345 | .420 | .028 | $5.32 \times 10^{-8}$ | .078 | .0168 | .045 | .111 | 2 | 118942893 | *INSIG2* | 75295 |
| Financial stress (*n* = 774) | cg11631610 | Very early childhood (8 mo of age) | .949 | .923 | .027 | $1.20 \times 10^{-8}$[i] | −.027 | .0057 | −.038 | −.016 | 19 | 11322739 | *DOCK6* | 0 |
| | cg06783003 | Very early childhood (1.75 years of age) | .860 | .893 | .024 | $6.25 \times 10^{-8}$ | .037 | .0083 | .021 | .053 | 1 | 45116008 | *RNF220* | 0 |
| | cg01050704[g] | Early childhood (5 years of age) | .017 | .019 | .025 | $4.68 \times 10^{-8}$ | .002 | .0005 | .001 | .003 | 19 | 59084995 | *MZF1-AS1* | 0 |
| | cg02006977 | Early childhood (5 years of age) | .015 | .017 | .024 | $6.87 \times 10^{-8}$ | .002 | .0005 | .001 | .003 | 12 | 69139955 | *SLC35E3* | 0 |
| | cg21299458 | Early childhood (5 years of age) | .110 | .147 | .035 | $3.19 \times 10^{-11}$[i] | .038 | .0070 | .024 | .052 | 22 | 20779896 | *SCARF2* | 0 |
| | cg19219503 | Middle childhood (7 years of age) | .922 | .889 | .031 | $2.28 \times 10^{-10}$[i] | −.035 | .0071 | −.049 | −.021 | 10 | 37414802 | *ANKRD30A* | 0 |
| | cg11714846 | Accumulation | .923 | .915 | .023 | $6.64 \times 10^{-8}$ | −.005 | .0011 | −.007 | −.003 | 1 | 230419534 | *GALNT2* | 1658 |
| | cg21924472 | Recency | .756 | .770 | .027 | $1.87 \times 10^{-8}$ | .003 | .0006 | .002 | .004 | 4 | 139600734 | *LINC00499* | 255235 |
| | cg24996440 | Recency | .566 | .585 | .026 | $2.28 \times 10^{-8}$ | .004 | .0009 | .003 | .006 | 2 | 3583570 | *RNASEH1* | 9119 |
| Neighborhood disadvantage (*n* = 702) | cg00928478 | Very early childhood (1.75 years of age) | .020 | .018 | .027 | $2.19 \times 10^{-8}$ | −.002 | .0005 | −.003 | −.001 | 10 | 99078824 | *FRAT1* | 196 |
| | cg01954337 | Very early childhood (1.75 years of age) | .050 | .059 | .028 | $5.32 \times 10^{-8}$ | .008 | .0018 | .005 | .012 | 11 | 3819010 | *NUP98* | 0 |
| | cg04996689 | Very early childhood (1.75 years of age) | .029 | .035 | .028 | $2.63 \times 10^{-8}$ | .006 | .0011 | .003 | .008 | 5 | 52285560 | *ITGA2* | 0 |
| | cg12069925 | Very early childhood (1.75 years of age) | .042 | .048 | .030 | $4.72 \times 10^{-9}$[i] | .007 | .0014 | .004 | .009 | 17 | 11900858 | *ZNF18* | 72 |
| | cg14522055 | Very early childhood (1.75 years of age) | .030 | .035 | .028 | $6.77 \times 10^{-8}$ | .005 | .0011 | .003 | .007 | 15 | 64338757 | *DAPK2* | 235 |
| | cg19157140 | Very early childhood (1.75 years of age) | .014 | .016 | .037 | $2.87 \times 10^{-11}$[i] | .002 | .0005 | .001 | .003 | 7 | 766323 | *PRKAR1B* | 0 |

**Table 1. Continued**

| Adversity | CpG Site | First Hypothesis Chosen by LARS Procedure | DNAm in Unexposed Group, β[a] | DNAm in Exposed Group, β[a] | Increases in $R^2$[b] | *p* Value[c] | Effect Estimate, β[d] | SE[d] | Lower 95% CI[d] | Upper 95% CI[d] | Chr[e] | Coordinate, bp[e] | Nearest Gene[f] | Distance to Nearest Gene, bp[f] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | cg21740964 | Very early childhood (1.75 years of age) | .160 | .173 | .025 | $7.13 \times 10^{-8}$ | .014 | .0028 | .008 | .019 | 6 | 3849331 | *FAM50B* | 299 |
| | cg24826892[g] | Very early childhood (1.75 years of age) | .016 | .018 | .030 | $5.50 \times 10^{-9}$[i] | .003 | .0006 | .002 | .004 | 11 | 71159390 | *DHCR7* | 0 |
| | cg08546016 | Early childhood (5 years of age) | .050 | .056 | .029 | $3.63 \times 10^{-9}$[i] | .006 | .0012 | .004 | .009 | 17 | 72776238 | *TMEM104* | 0 |
| | cg12412390 | Middle childhood (7 years of age) | .038 | .046 | .030 | $9.59 \times 10^{-8}$ | .008 | .0016 | .005 | .011 | 4 | 96469286 | *UNC5C* | 0 |

ARIES, Accessible Resource for Integrated Epigenomics Studies; bp, base pair; Chr, chromosome; CI, confidence interval; DNAm, DNA methylation; LARS, least angle regression; SLCMA, structured life course modeling approach.

[a]In the DNAm column, values are unadjusted DNA methylation (β values) averaged within the group.

[b]In the Increases in $R^2$ column, values represent the increase in $R^2$ explained by the first hypothesis chosen after accounting for covariates.

[c]In the *p* column, each value gives the *p* value for the covariance test, which assesses the significance of the increase in $R^2$ explained.

[d]In the Effect Estimate, SE, Lower 95% CI, and Upper 95% CI columns, values are the parameter estimate, standard error, and lower and upper limits of the 95% confidence interval, respectively, of the regression coefficient of the first hypothesis chosen.

[e]The Chr and Coordinate columns, respectively, give the chromosome and position of the CpG site.

[f]The Nearest Gene and Distance to Nearest Gene columns give the gene symbol of and distance in bases to the nearest gene to the CpG site (as measured from the transcription start site), respectively.

[g]In list of potentially noisy probes compiled by Naeem *et al.* (86) (i.e., cross-reactive probes, probes with single nucleotide polymorphisms, insertions or deletions, and/or repeat regions, probes affected by unknown factors).

[h]In list of potentially noisy probes compiled by Chen *et al.* (87) (i.e., cross-reactive probes, probes with single nucleotide polymorphisms).

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

## RESULTS

### Sample Characteristics and Distribution of Exposure to Adversity

Demographic characteristics of the ARIES analytic sample are shown in Table S1 in Supplement 1 for the total sample and for children exposed to any adversity ($n = 650$, 67%, experienced at least one adversity at some point in their lifetime). Details on the prevalence and correlations of exposure across time are also reported in Figure 1 and Figure S1 and Table S2 in Supplement 1. Of note, differences in the prevalence of exposure across time are unlikely to affect model selection, as all variables are automatically standardized by the LARS procedure.

### Model Comparison and Effect Estimation

We identified 38 CpG sites ("top sites") that were differentially methylated at 7 years of age following exposure to adversity ($p < 1 \times 10^{-7}$) (Figure 2). Methylation at most sites ($n = 35$) was related to the developmental timing of exposure to adversity, especially adversity during very early childhood, meaning between birth and age 2 years (Figure 3A). In fact, exposure to adversity during very early childhood explained variability at more CpG sites (22 in total) than expected, while the accumulation and recency models were associated with fewer CpG sites than expected (one and two CpG sites, respectively; $\chi^2 = 7.40$, $p = .02$).

As shown in Table 1 and Figure 3A, neighborhood disadvantage was the type of adversity predicting the greatest number of genome-wide methylation differences (10 CpG sites), followed by financial stress (nine CpG sites), sexual or physical abuse (by anyone) (five CpG sites), and one adult in the household (five CpG sites). Maternal psychopathology, caregiver physical or emotional abuse, and family instability were associated with differences at four, three, and two CpG sites, respectively.

Across all 38 top sites, exposure to adversity was typically associated with hypermethylation (73.7% positive beta coefficients; $\chi^2 = 8.53$, $p = .004$) (Table 1). On average, exposure to adversity during a sensitive period was associated with a 2.5% difference in methylation level (β) after controlling for all covariates (range 0.1%–14.2%). For the two CpG sites associated with recency of exposure to financial stress, one additional adverse event was associated with a 0.3% to 0.4% increase in methylation per year of age at the event. For the single site associated with accumulation of exposure, one additional adverse event was associated with a 0.5% decrease in methylation. Of these 38 CpG sites, 14 remained statistically significant after we imposed a more stringent *p* value threshold that accounted for the testing of seven types of adversity ($p = [1 \times 10^{-7}]/7 = 1.43 \times 10^{-8}$) (Table 1).

After relaxing the multiple testing correction threshold to an FDR $q < .05$, there were 380 CpG sites affected by exposure to adversity (Figure 3B, and Table S3 in Supplement 2). As with the top 38 Bonferroni-significant sites, methylation at 352 of the 380 FDR-significant sites was best explained by sensitive period models (Figure 3B, and Table S3 in Supplement 2). Exposure in very early childhood explained methylation variation at more CpG sites than expected from the background for neighborhood

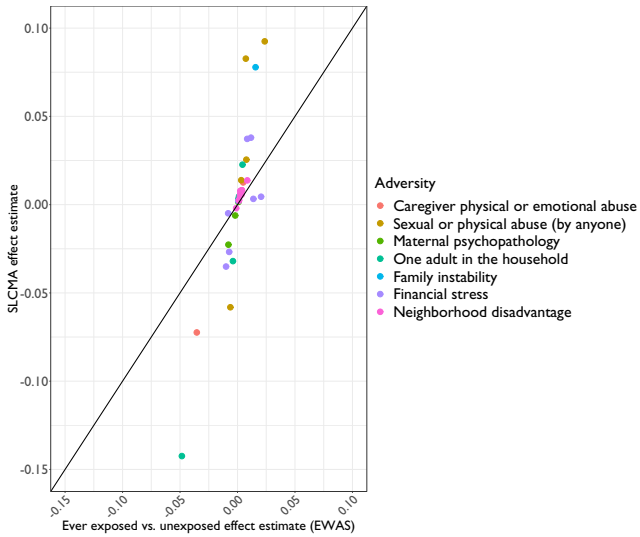


**Figure 4.** A scatterplot displays increased power in the structured life course modeling approach (SLCMA) shown by the comparison of beta estimates from the epigenome-wide association study (EWAS) vs. SLCMA approaches. In this scatterplot, the y-axis represents the β estimates associated with the 38 top CpG sites derived for the SLCMA; the x-axis represents the β estimates associated with the same 38 CpG sites obtained from EWASs. Different types of adversity are indicated by colors. The black straight line denotes the 1:1 correspondence between the two sets of β values. Substantial positive deviation from the line suggests increased power in the SLCMA. For most CpG sites, the magnitudes of effect were larger for the SLCMA compared with the EWAS results.

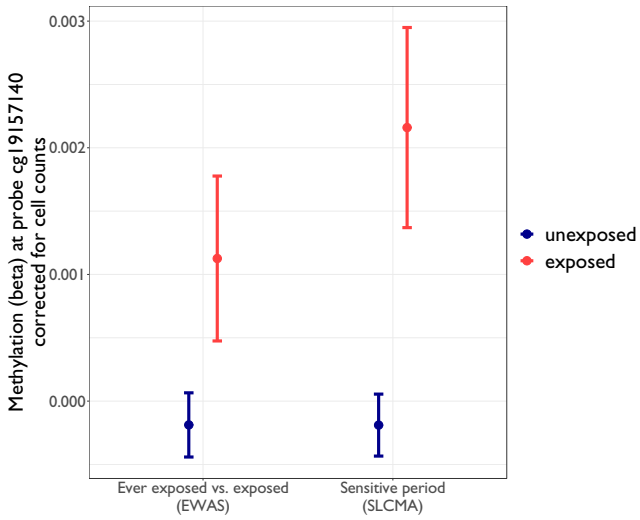


**Figure 5.** Comparison of epigenome-wide association study (EWAS) vs. structured life course modeling approach (SLCMA) estimates for the top CpG site identified in the SLCMA, cg19157140. The effect estimates and the confidence intervals obtained from the EWAS approach comparing ever exposed to never exposed to neighborhood disadvantage for cg19157140 are presented on the left. The stage 2 effect estimates and confidence intervals obtained from the SLCMA comparing being exposed to neighborhood disadvantage at 1.75 years of age with being unexposed at 1.75 years of age for the same CpG site are displayed on the right. The top CpG site in the SLCMA, which suggested a sensitive period at 1.75 years of age associated with the effects of neighborhood disadvantage, was nonsignificant after correction for multiple testing ($p$ = .0002) in the epigenome-wide association study of neighborhood disadvantage.

disadvantage (Figure S2 in Supplement 1). The effects of adversity type and timing on methylation were distributed throughout the genome (Figure S3 in Supplement 1).

## Exposed Versus Unexposed Analysis

Across the seven EWASs, which separately evaluated the effect of ever versus never exposed to each type of adversity on CpG site DNAm, only one statistically significant result emerged (Figure S4 in Supplement 1); this was for cg02431672, a locus located on chromosome 1 79 kb away from the gene *FAM183A*, and it was associated with exposure to abuse ($\beta = -.005$; $p = 1.77 \times 10^{-8}$).

Overall, there was very little overlap in identified CpG sites across the top SLCMA and EWAS results. Most of the top 38 sites had effect estimates that were larger in the SLCMA compared with the EWAS (Figure 4). There was also little overlap in findings across specific CpG sites. For example, the cg02431672 locus, which was the top hit in the EWAS of abuse, did not emerge as a top hit in the SLCMA of abuse, failing to appear in the list of FDR-significant loci ($p$ = .0138). Similarly, the top CpG site in the SLCMA (cg19157140), which suggested a sensitive period at 1.75 years of age associated with the effects of neighborhood disadvantage, was nonsignificant in the corresponding EWAS ($\beta$ = .001; $p$ = .0002) (Figure 5). These results suggest that the SLCMA allowed us to more effectively identify methylation differences among children with and without a history of exposure to adversity.

## Sensitivity Analyses

**Evaluation of the LARS Selection Procedure.** There was no evidence in support of compound theoretical models, whereby more than one theoretical model explained the most outcome variability. For each of the top 38 CpG sites, the marginal increase in variance of methylation explained by additional steps of the LARS procedure was not significant (each $p > .05$) (Figure S5 in Supplement 1), suggesting that methylation was best explained by a single theoretical model.

**Evaluation of Methylation at Birth for Top CpG Sites.** Adversity-associated methylation differences occurred during very early childhood for most top CpG sites. To assess whether the observed differences in DNAm existed at birth, we examined the effect of the selected exposure on DNAm in cord blood for the top 38 sites. We found that DNAm differences at birth were significant for only one of the 38 sites ($p < .05/38$, or .00132), suggesting that the differences in DNAm at 7 years of age mainly occurred after birth, as a result of exposure to postnatal stressors (Table S4 in Supplement 2). Similar results were obtained when examining the 380 FDR-significant loci, where significant differences at birth were detected at only six of the 380 probes (Table S4 in Supplement 2). An example of a site differentially methylated at birth and an example of a site nondifferentially methylated at birth are shown in Figure S6 in Supplement 1.

**Correction for Genetic Variation.** Genetic variation did not appear to influence observed DNAm differences at the top CpG sites. Using a database of methylation quantitative trait loci of the ARIES cohort (77), there were 658 single nucleotide polymorphisms associated with DNAm at 17 of the top 38 sites. After controlling for genetic variation at methylation quantitative trait loci linked to these 17 sites, the effect of exposure to adversity remained significant (each FDR $q < .05$; Table S5 in Supplement 1), suggesting that adversity could have caused these methylation differences distinct from genetic sequence variation.

### Exploring the Biological Significance of Findings

**Correlation Between Blood and Brain Tissue.** On average, methylation in blood at the top 38 sites was slightly positively correlated with methylation in four brain regions (prefrontal cortex: $r_{avg} = .10$, entorhinal cortex: $r_{avg} = .11$, superior temporal gyrus: $r_{avg} = .11$, cerebellum: $r_{avg} = .06$) (Table S6 in Supplement 1). CpG sites with methylation that is highly correlated between blood and brain tissue may be indicative of important interindividual covariation (i.e., due to adversity) or a strong genetic influence on methylation, while those that are uncorrelated may still be biomarkers of a response to adversity.

**Enrichment of Regulatory Elements.** As compared with all autosomal loci tested, FDR-significant loci were more likely to be located in gene promoters ($\chi^2 = 9.92$, $p = .002$) and less likely to be in gene enhancers ($\chi^2 = 3.86$, $p = .049$; Figure S7A in Supplement 1). Furthermore, the location of FDR-significant loci differed from all other loci tested relative to CpG islands ($\chi^2 = 42.92$, $p < .0001$) (Figure S7B in Supplement 1). With eFORGE 1.2 (78), we also tested whether FDR-significant loci colocalize with markers of transcriptional activity. FDR-significant loci were not enriched for DNase I hypersensitivity sites or histone marks in any tissue or cell type after correction for multiple comparisons (each $q > .05$). The strongest trend for enrichment was detected in the analysis of all histone marks in fetal thymus cells (uncorrected $p = .0007$). Annotations at each FDR-significant site are presented in Table S3 in Supplement 2.

**Biological Processes Potentially Affected by Adversity.** Genes near the FDR-significant sites ($n = 365$ genes) corresponded to 158 clusters of gene ontology biological process terms (75). The top 11 gene ontology term clusters, including positive regulation of developmental growth, axon development, and neuron apoptotic process, were more likely to be represented than by chance (average enrichment $p < .05$) (Figure S8 in Supplement 1).

Additionally, we uncovered evidence of functional constraint for these genes. Genes annotated to FDR-significant sites were more highly constrained, as measured by the probability of intolerance to loss-of-function variation from the Exome Aggregation Consortium (76), than the rest of the autosomal genes tested (permutation $p = .0001$) (Figure S9 in Supplement 1). This indicates a greater importance of these genes, on average, to survival and reproduction over human evolution.

## DISCUSSION

This prospective study used data from a large population-based sample of children to test three competing life course theoretical models describing the association between exposure to childhood adversity, measured repeatedly across the first 7 years of life, and DNAm at 7 years of age. By comparing these theoretical models to each other, we could evaluate which one explained the most variation in DNAm. To our knowledge, this is the first use of the SLCMA in an epigenome-wide context.

The main finding of this study is that the effect of adversity on DNAm depends primarily on the developmental timing of exposure. In our Bonferroni-corrected analysis, we identified 38 CpG sites that were differently methylated following exposure to adversity, with more than half of these loci showing associations based on adversity occurring during very early childhood, meaning before 3 years of age. Exposure in very early childhood was associated with DNAm differences for nearly all adversity types. In contrast, the effects of exposure in middle childhood were largely detected only for arguably the most severe forms of adversity exposure (e.g., sexual or physical abuse). These results are consistent with those of at least one human longitudinal study (16) and multiple animal studies (21,22,24,25) in emphasizing the existence of sensitive periods (19,20)—particularly occurring shortly after birth—when epigenetic programming is maximally dynamic in response to parental care disruptions and other environmental inputs. The lack of detectable sensitive periods in one recent study (32) may be due to focusing only on adversities occurring at or after 5 years of age. Interestingly, neither the accumulation nor recency of the adversity explained considerable variability in DNAm. The observed DNAm differences were absent at birth, identified for a range of adversities, and unrelated to genetic variation. The absence of support for an accumulation model is surprising, given previous research linking cumulative time spent in institutional care to DNAm status in stress-related genes (29).

Perhaps more importantly, our results suggest that broad classifications of individuals as exposed versus unexposed to "early life" adversity—although commonly used—may dilute observed effects and fail to detect DNAm differences among people exposed to adversity during specific life stages. These findings support the value of more detailed phenotyping, which is meaningful given the trend in psychiatric genetics toward minimizing phenotypic precision in the service of maximizing sample size. The lack of overlap in identified loci across the SLCMA and EWAS suggest that refinement of the environmental phenotype—by treating each time point of exposure as unique—may better capture underlying signal. Indeed, results of a power analysis suggest that the EWAS of exposed versus unexposed will be underpowered when the true underlying relationship between exposure and outcome depends on the timing or amount of exposure (Supplement 1). Thus, more precise phenotyping could preserve study power and provide more mechanistic insights to guide targeted interventions.

These findings also raise important questions regarding why exposure to adversity in the first 3 years of life may be particularly salient in influencing DNAm patterns. When adversity occurs early in life, it coincides with the initial and foundational sculpting

of brain architecture. Experiences of childhood adversity, which represent deviations from expected cognitive, social, and sensory inputs (60), may be more likely to be wired into neural circuitry during this especially vulnerable stage in brain development. Relatedly, DNAm patterns are known to be dynamic across the life course. It may be that very early exposure to adversity produces more stable DNAm changes that persist across the life course, in contrast with later exposure to adversity. With more longitudinal studies of DNAm, the field of psychiatric epigenetics will be better positioned to determine not only when are the most vulnerable life stages for DNAm changes to occur, but also the extent to which these adversity-induced DNAm patterns persist over time.

Although these findings emphasize the importance of exposure timing, greater insights are needed regarding the age stages when adversity may be most harmful, as mixed results have emerged among the small number of studies comparing the effects of early versus later adversity. Some retrospective studies have shown that adolescent DNAm patterns are more strongly associated with life stress during adolescence than earlier periods (27). However, other studies have found potentially persistent effects of childhood adversity into adolescence (31) and adulthood (11), even after accounting for subsequent stress exposure. A recent study also found that the effects of adversity timing may be gene specific (29). As epigenetic patterns appear to vary over the life course (26,79), longitudinal studies are needed to study the developmental trajectories of DNAm and evaluate the extent to which these adversity-induced DNAm differences persist or attenuate over time, and operate independently of or in interaction with subsequent experience to ultimately predict mental health outcomes. Ideally, these longitudinal studies would include repeated measures of prenatal and postnatal adversity exposure and investigate whether any adversity-associated DNAm signatures predict psychopathology. If our findings about the importance of sensitive periods do replicate, these results would emphasize the need to prioritize policies and interventions toward children exposed to adversity within the first 3 years of life, when the biological effects of adversity may be most profound.

Several limitations are noted. First, some adversity measures were drawn from single items. Parents may have also underreported exposure to stigmatizing experiences (80,81), especially if they were implicated in the exposure (82). However, the prevalence of several adversities, including those capturing possible experiences of abuse, were similar to and even greater than those reported from some nationally representative samples (9,83). Second, as with any longitudinal study, there was attrition over time, which could result in bias owing to loss of follow-up. However, ARIES children were sampled from among those with the most complete longitudinal data. Within the field of epigenetics, efforts are now underway to understand the consequences of attrition and how potential biases arising from attrition could be mitigated through multiple imputation or other strategies. Third, we were unable to examine the impact of experiencing multiple adversities simultaneously, because each adversity was measured at a slightly different time point. Fourth, the DNAm samples were obtained from peripheral tissue and not the brain; multiple data sets, however, are starting to identify limited though important shared DNAm patterns across central nervous system and peripheral tissue (84). Fifth, we were unable to directly examine whether DNAm at the identified loci influenced gene expression of the nearest genes. Future work using a sample with both methylation and expression data is needed to clarify the functional consequences of significant CpG sites. Finally, the $p$ values derived from the covariance tests could be potentially inflated, as the test relies on asymptotic theories and therefore does not theoretically guarantee the control of type I error rate in a finite sample (69). However, the covariance test might be a more sensitive method to detect signals compared with other postselection significance tests that make fewer assumptions (85). As the relative statistical power of the available tests remains unclear, simulation studies are underway to identify the best inference tools in different settings and the statistical power of the SLCMA with varying effect sizes.

In summary, this study lends further support to the evidence base showing that DNAm patterns are responsive to experience. However, these results reveal that DNAm patterns may be most influenced by exposures during sensitive periods in development. Efforts may therefore be needed to move beyond crude comparisons of those exposed versus unexposed to early-life adversity.

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

## *Supplement 1*

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 $\alpha = 100$ ($\beta$ = intensity of the Methylated allele (M) / 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, …, 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, ..., $J_i$, $J_i \geq 4$, 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:

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

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:

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

*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 ($p<1x10^{-7}$), 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 (MAF>0.01), Hardy-Weinberg equilibrium (HWE>$5x10^{-7}$), and imputation quality (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 $10^{th}$ and $90^{th}$ percentile and were left with 292,686 variable probes, resulting in a more liberal Bonferroni corrected p-value threshold of p<$1.71x10^{-7}$. 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

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

Regression of $Y$ on $X_s$ (i.e., fitting the correct sensitive period model) will give an average regression coefficient of $\beta_1$.

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

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

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 $2.5 \times 10^{-6}$. 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 $R^2$ typically associated with fitting an exposed vs. unexposed model instead of the correct sensitive period model.

Theorem:

Let $X_1, X_2, \ldots, X_J$ 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

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

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

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

Fitting the ever exposed vs. unexposed model,

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

On average,

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

Therefore on average,

$$\begin{aligned}\hat{\beta}_{any} &= \frac{\sum_i X_{any\,i}\,Y_i/n-(\sum_i X_{any\,i}/n)(\sum_i Y_i/n)}{\sum_i X_{any\,i}^2/n-\big(\sum_i X_{any\,i}\big)^2}\\ &= \frac{p_{any}\,\beta_0+p_s\beta_i-p_{any}(\beta_0+p_1\beta_1)}{p_{any}-p_{any}^2}\\ &= \frac{p_s}{p_{any}}\beta_1.\end{aligned}$$

The residuals resulting from this regression are given by

$$\begin{aligned}Y_i-\hat{Y}_i &= \beta_0+\beta_1X_{si}+\varepsilon_i-\beta_0-\frac{p_1}{p_{any}}\beta_1X_{any\,i}\\ &= \varepsilon_i+\beta_1\left(X_{si}-\frac{p_s}{p_{any}}X_{any\,i}\right).\end{aligned}$$

The sum of squares of residuals will average

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

Hence the average $R^2$ will be

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

The average standard error of the regression coefficient will be

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

Leading to the average standardized test statistic of

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

For comparison, the residuals resulting from regression of $Y$ on $X_s$ are $\varepsilon_i$, 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

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

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 ≤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 DNAm , 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 ($p < 1x10^{-7}$).

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 $\frac{(\theta_{\text{SES}} - \theta_{\text{no SES}})}{\theta_{\text{no SES}}}$, 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** | | | |
|---|---|---|---|---|---|---|
| | % | N | % | N | chi-squared | p-value |
| 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) | | | | | | |
|---|---|---|---|---|---|---|
| **Age** | **8 mo** | **1.75** | **2.75** | **4** | **5** | **6** |
| **8 mo** | 1 | --- | --- | --- | --- | --- |
| **1.75** | 0.82 | 1 | --- | --- | --- | --- |
| **2.75** | 0.69 | 0.77 | 1 | --- | --- | --- |
| **4** | 0.62 | 0.7 | 0.78 | 1 | --- | --- |
| **5** | 0.58 | 0.58 | 0.69 | 0.66 | 1 | --- |
| **6** | 0.45 | 0.46 | 0.5 | 0.56 | 0.67 | 1 |

| Maternal psychopathology (N=760) | | | | | |
|---|---|---|---|---|---|
| **Age** | **8 mo** | **1.75** | **2.75** | **5** | **6** |
| **8 mo** | 1 | --- | --- | --- | --- |
| **1.75** | 0.67 | 1 | --- | --- | --- |
| **2.75** | 0.56 | 0.67 | 1 | --- | --- |
| **5** | 0.61 | 0.6 | 0.65 | 1 | --- |
| **6** | 0.44 | 0.53 | 0.57 | 0.71 | 1 |

| Sexual or physical abuse (by anyone) (N=769) | | | | | | |
|---|---|---|---|---|---|---|
| **Age** | **1.5** | **2.5** | **3.5** | **4.75** | **5.75** | **6.75** |
| **1.5** | 1 | --- | --- | --- | --- | --- |
| **2.5** | 0.44 | 1 | --- | --- | --- | --- |
| **3.5** | 0.02 | 0.32 | 1 | --- | --- | --- |
| **4.75** | 0.33 | 0.44 | 0.69 | 1 | --- | --- |
| **5.75** | 0.4 | 0.51 | 0.61 | 0.49 | 1 | --- |
| **6.75** | 0.28 | 0.42 | 0.25 | 0.4 | 0.56 | 1 |

| Family instability (N=769) | | | | | | |
|---|---|---|---|---|---|---|
| **Age** | **1.5** | **2.5** | **3.5** | **4.75** | **5.75** | **6.75** |
| **1.5** | 1 | --- | --- | --- | --- | --- |
| **2.5** | 0.74 | 1 | --- | --- | --- | --- |
| **3.5** | 0.48 | 0.6 | 1 | --- | --- | --- |
| **4.75** | 0.27 | 0.41 | 0.28 | 1 | --- | --- |
| **5.75** | 0.24 | 0.21 | 0.34 | 0.52 | 1 | --- |
| **6.75** | 0.28 | 0.37 | 0.11 | 0.61 | 0.58 | 1 |

| One adult in the household (N=726) | | | | | |
|---|---|---|---|---|---|
| **Age** | **8 mo** | **1.75** | **2.75** | **4** | **7** |
| **8 mo** | 1 | --- | --- | --- | --- |
| **1.75** | 0.9 | 1 | --- | --- | --- |
| **2.75** | 0.78 | 0.93 | 1 | --- | --- |
| **4** | 0.64 | 0.82 | 0.91 | 1 | --- |
| **7** | 0.54 | 0.75 | 0.81 | 0.79 | 1 |

| Financial stress (N=846) | | | | | |
|---|---|---|---|---|---|
| **Age** | **8 mo** | **1.75** | **2.75** | **5** | **7** |
| **8 mo** | 1 | --- | --- | --- | --- |
| **1.75** | 0.71 | 1 | --- | --- | --- |
| **2.75** | 0.59 | 0.67 | 1 | --- | --- |
| **5** | 0.53 | 0.54 | 0.59 | 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 DNAm after controlling for known mQTLs. After controlling for genotypes at mQTL SNPs, the direction of the effect of exposure to adversity on DNAm 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<1x10-7), 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) (N=703) | 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 | | 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] | | 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 | Maternal psychopathology (N=691) | 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 | ALKBH7 | 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[†] | | 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 †] | | middle childhood (age 7) | 0.883 | 0.858 | 0.029 | 5.35E-08 | -0.025 | 0.0053 | -0.036 | -0.015 | 10 | 80981129 | ZMIZ1 | 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 $p < 1.43x10^{-8}$ , a more stringent p-value threshold that accounted for the testing of seven types of adversity ($1x10^{-7}$ / 7=$1.43x10^{-8}$).

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

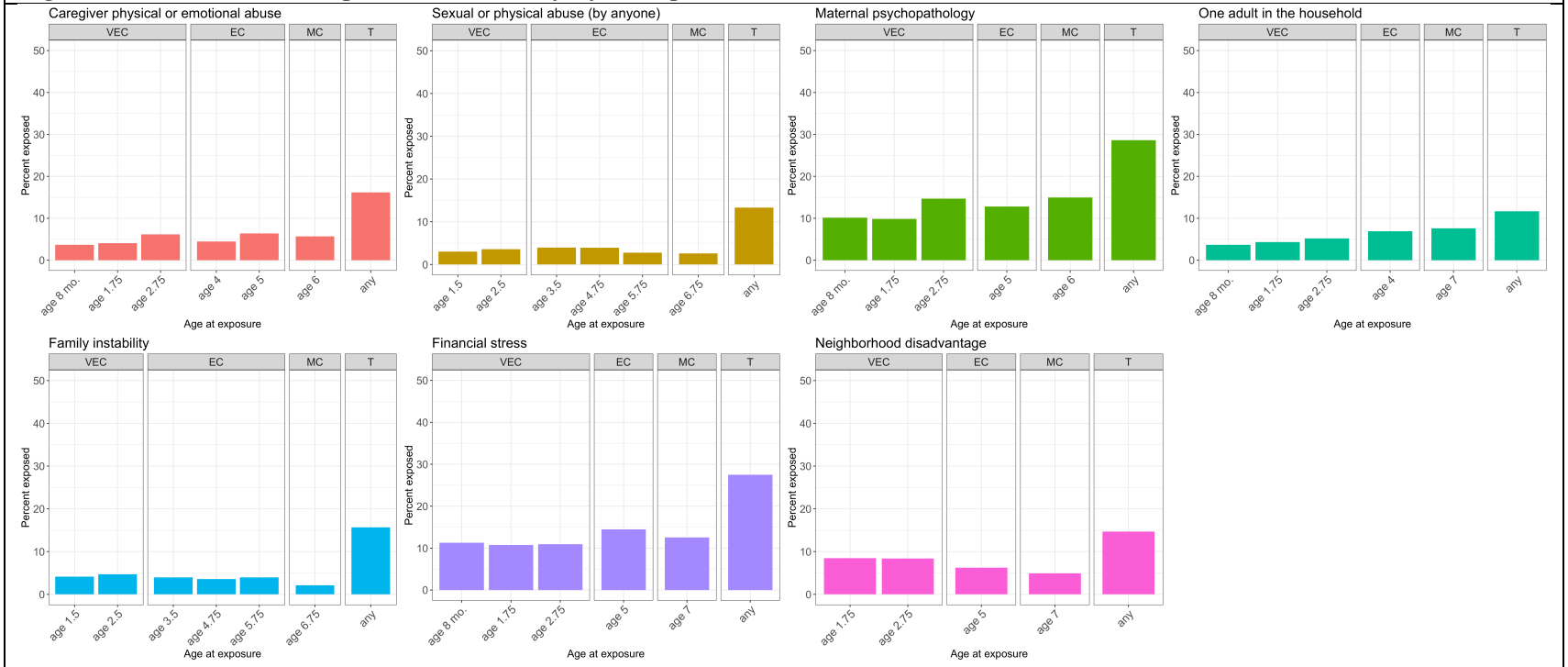
Table S8. Description of theoretical models used in the analysis, using exposure to abuse as an example

| 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.

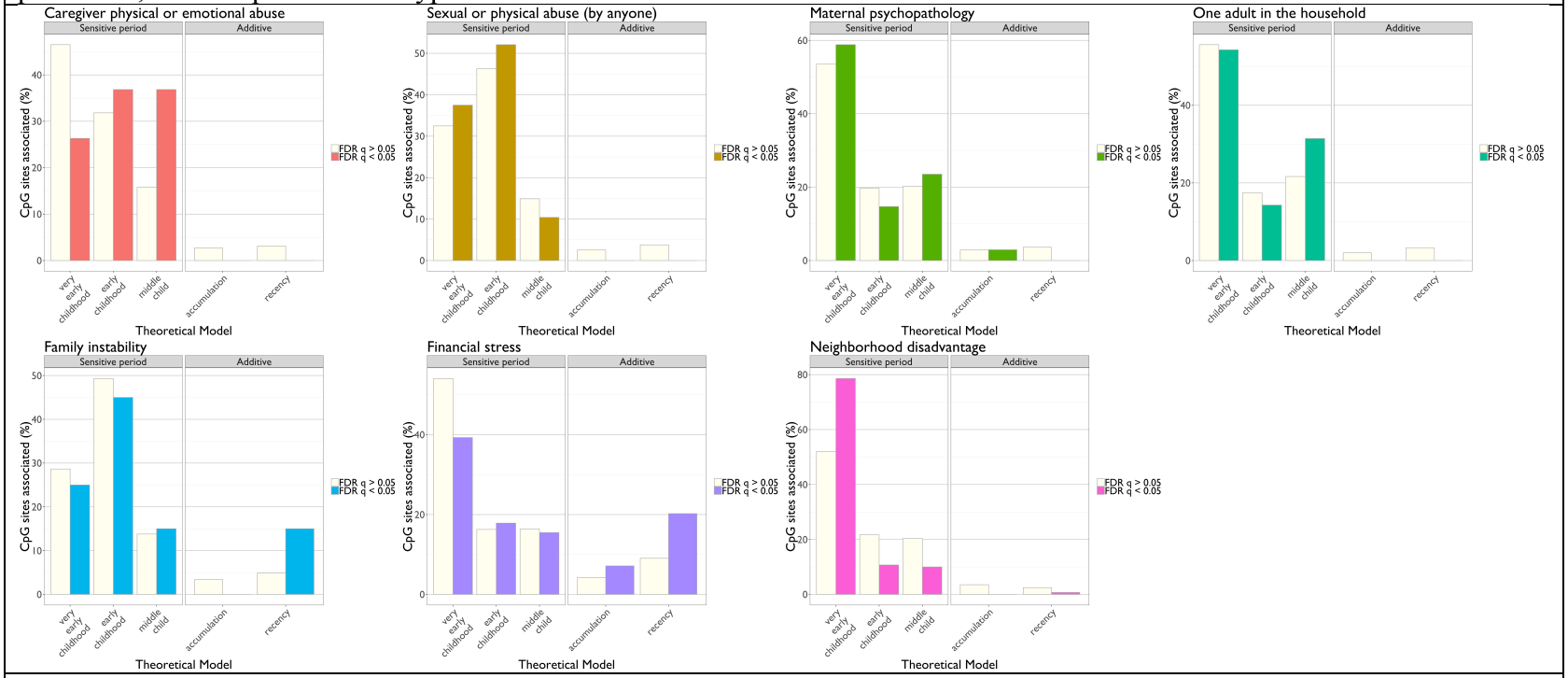
The *recency* hypothesis, in turn, assumes a linear increase in the effect of exposure over time, and weights more recent exposures more heavily than more distal ones (18). Unlike the last sensitive periods model, which captures only exposure to a given adversity within that specific time period, the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

Figure S1. Prevalence of exposure to adversity by time period



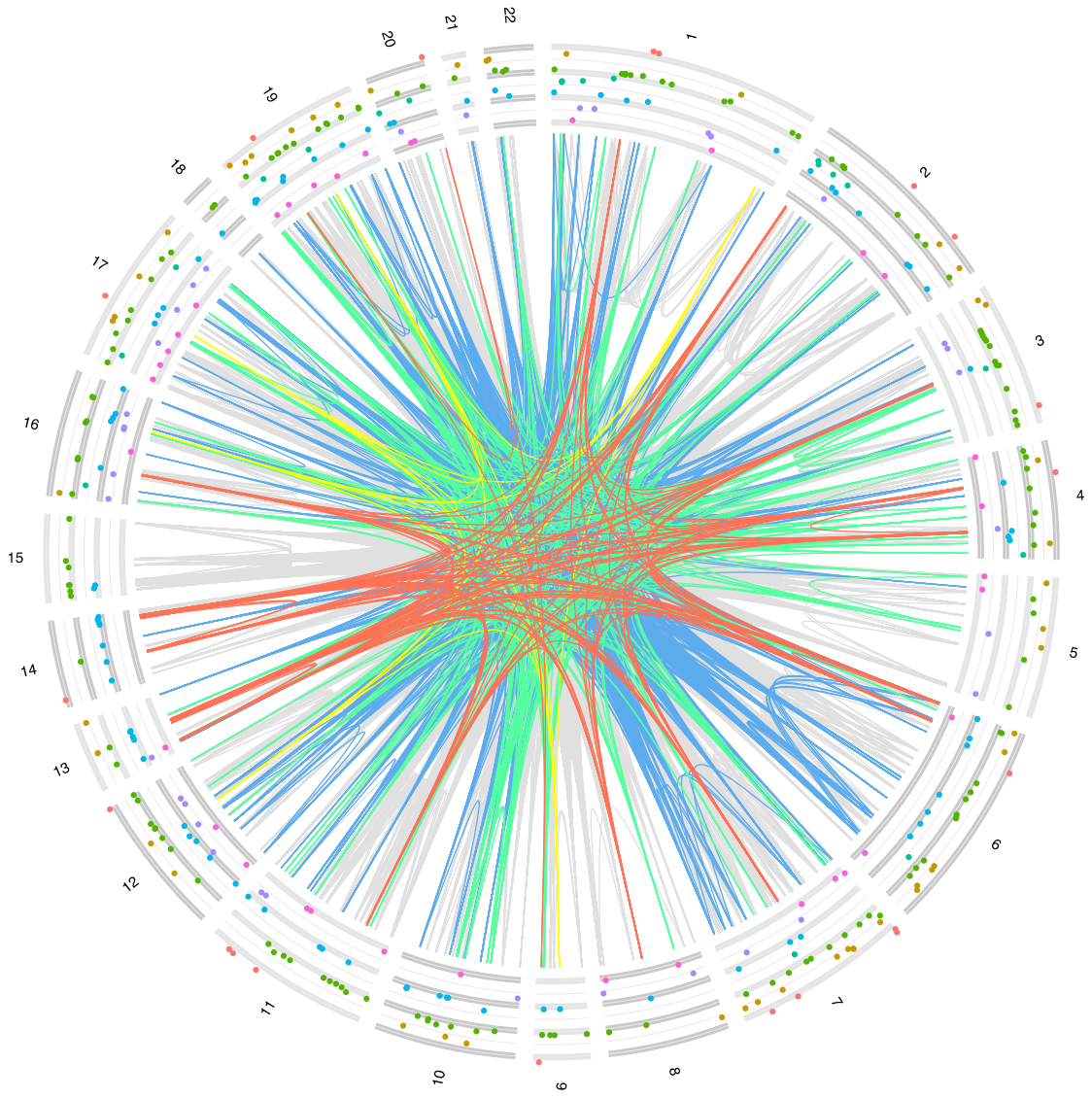

Time periods are very early childhood (I, before 3), early childhood (PS, ages 3-5), middle childhood (MC, ages 6-7), and total exposed at any time (T). As shown, age at exposure to adversity varied by the type of adversity. Family instability and neighborhood disadvantage were more common in very early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



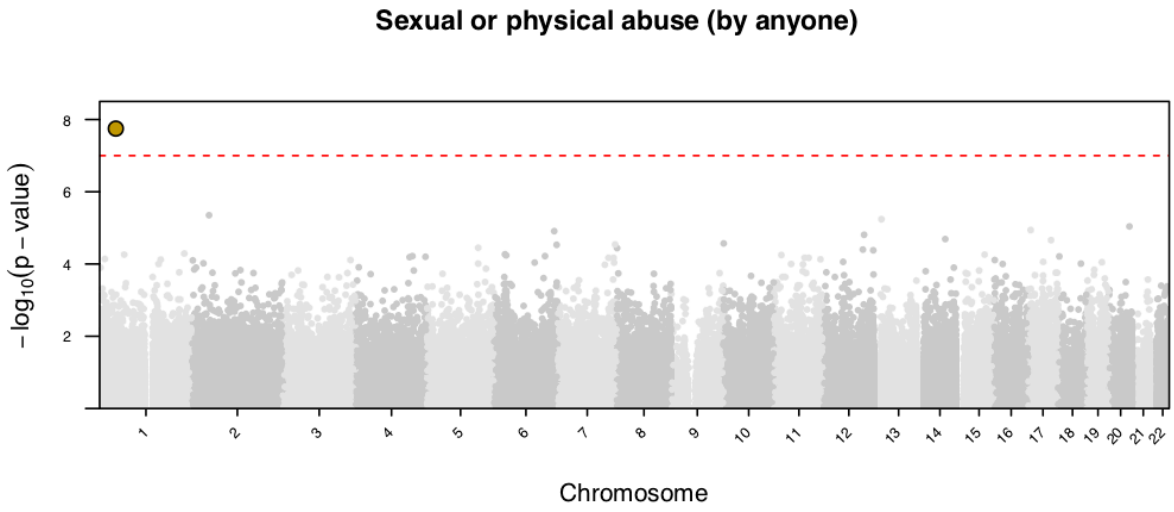

These figures display the percent of CpG sites for which methylation was best predicted by each of the theoretical models, after controlling for covariates. The distribution of hypotheses for FDR-significant CpG sites (FDR q< 0.05) was significantly different than that for the remaining CpG sites tested (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



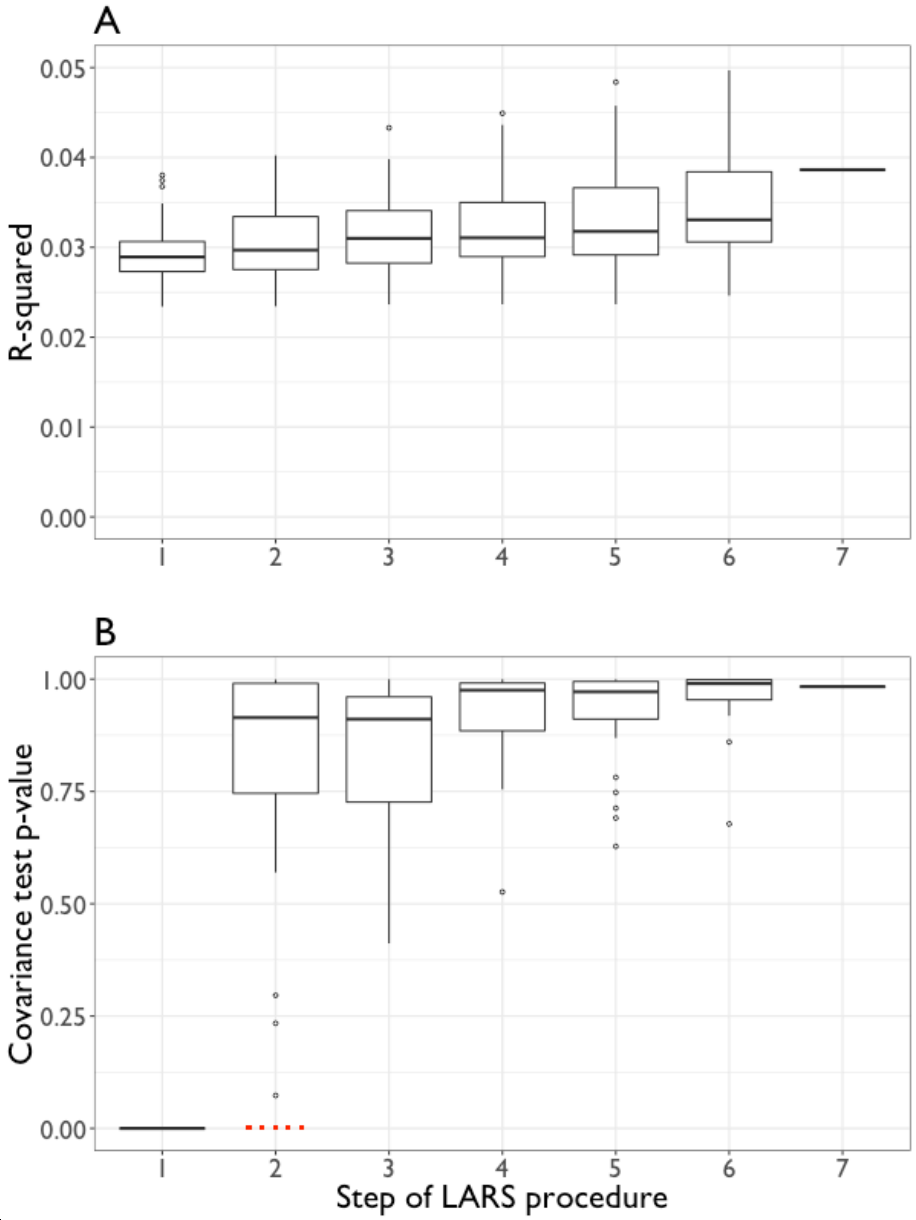

The effects of adversity on methylation were distributed throughout the genome. Outer rings: points represent genomic locations of all FDR-significant CpG sites, colored by adversity type (as above). Inner links: lines connect loci associated with the same adversity type and theoretical model, colored 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



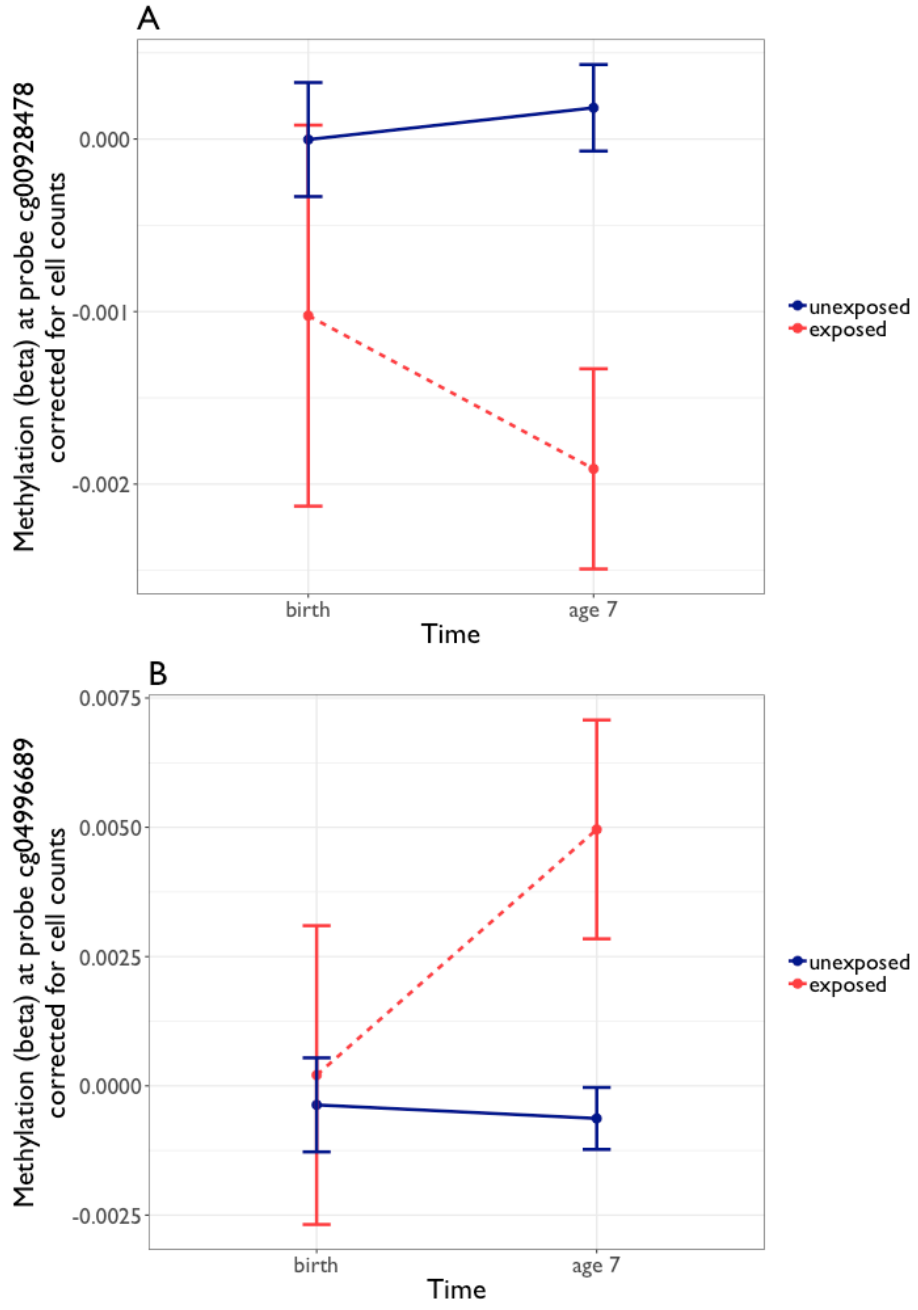

In this Manhattan plot, the x-axis is the chromosomal position for each CpG site and the y-axis is the -log10 p-value for the association between exposure to adversity and DNAm values at each CpG site. The dashed line shows the epigenome-wide significance level, with each CpG site above the line representing a statistically significant association ($p<1x10^{-7}$). 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



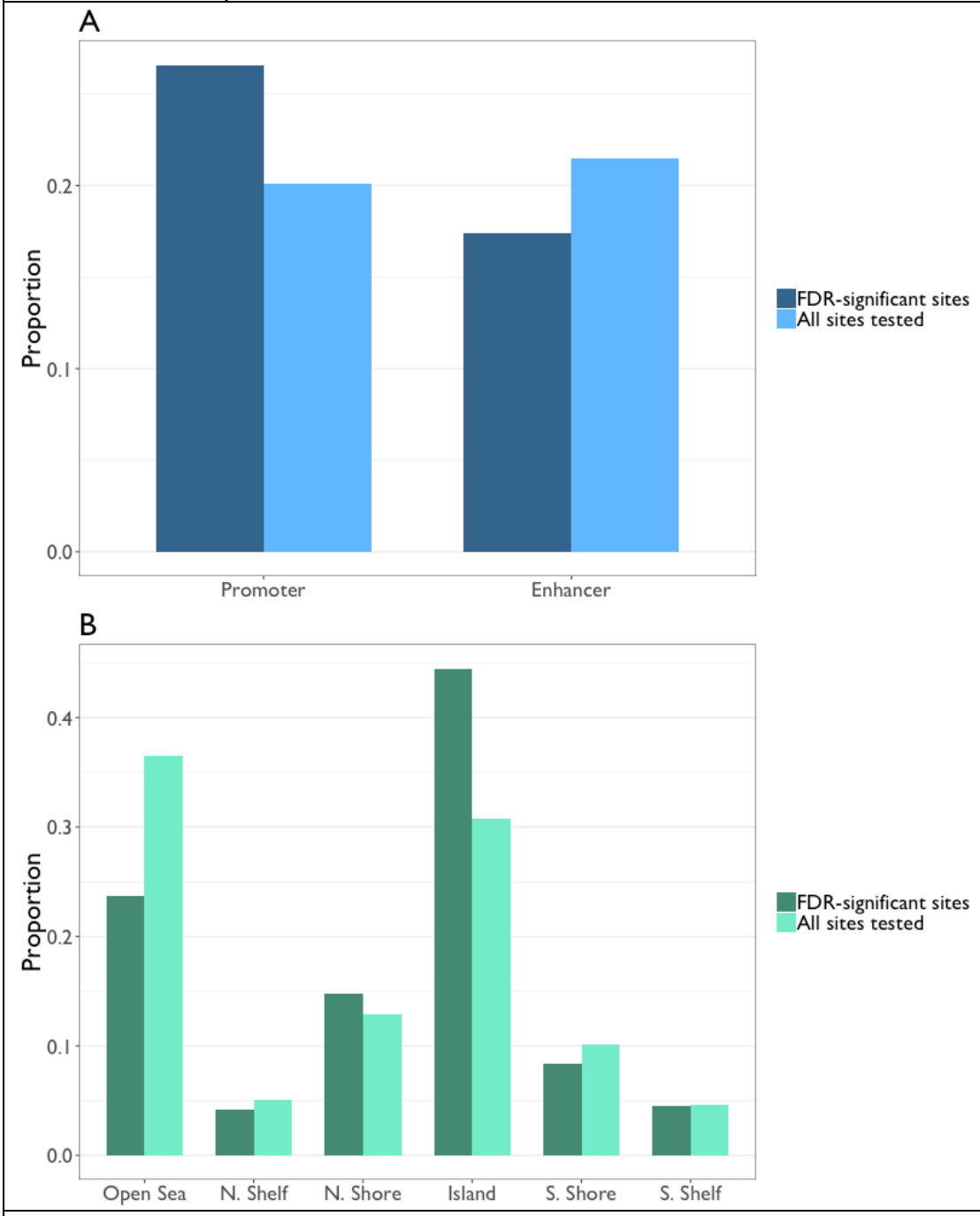

The CpG sites associated with adversity were detected by examining the first step of the LARS variable selection procedure. The first step of the LARS identified the *single* theoretical model that explained the most variation in DNAm at a given CpG site. However, it is possible that additional theoretical 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



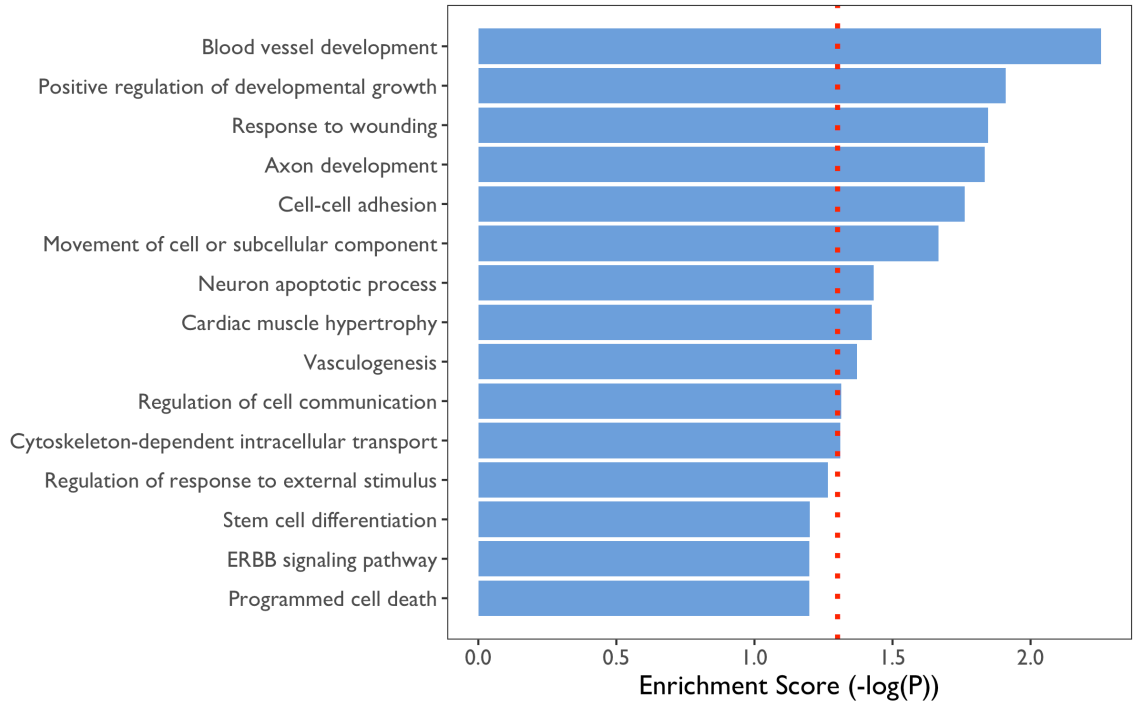

Because some adversities could have been present prenatally and could affect methylation *in utero*, we assessed methylation at birth in umbilical cord blood at the top CpG sites. At each top CpG site, we tested the predictive value of the theoretical model chosen at age 7 on methylation at birth with 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



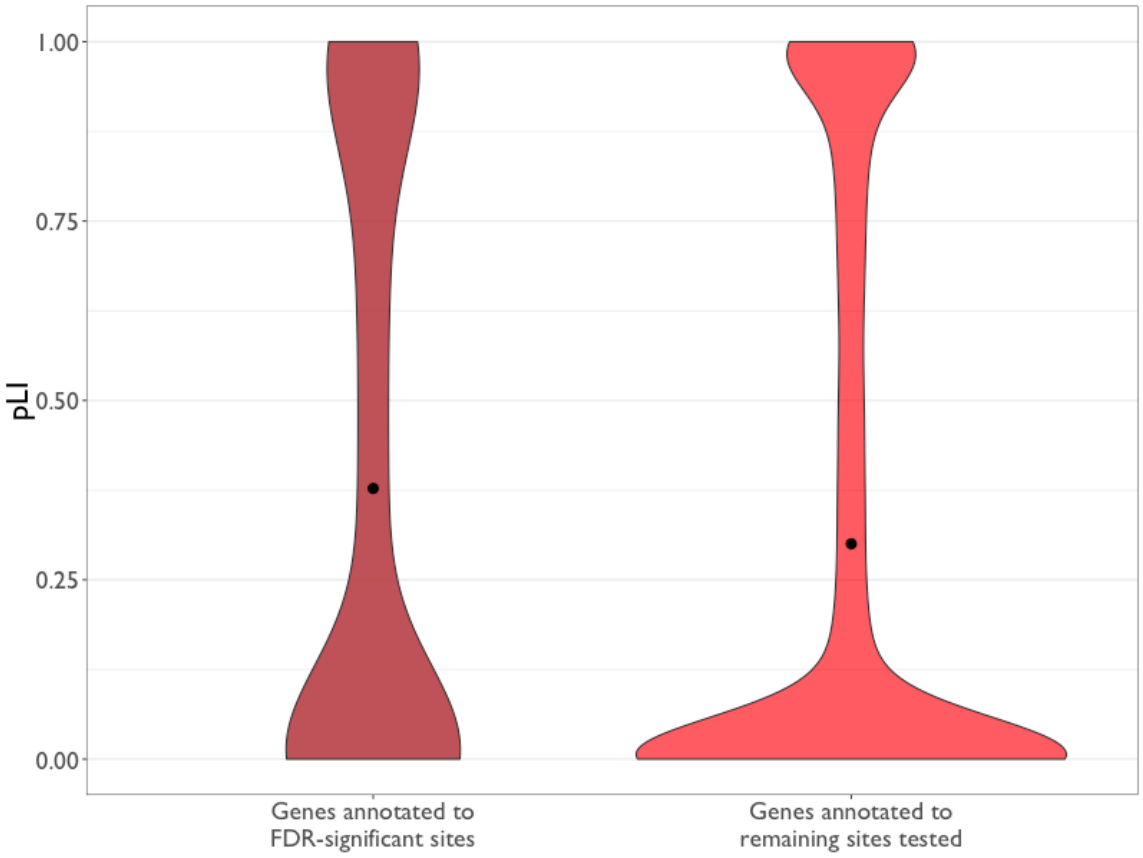

These plots display the proportion of FDR significant CpG sites (n=380) vs. all other CpG sites tested annotated to specific genomic regions. As shown, the 380 FDR-significant CpG sites **(a)** were enriched for promoter regions and depleted for enhancer regions and **(b)** differed by location relative to CpG islands.

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



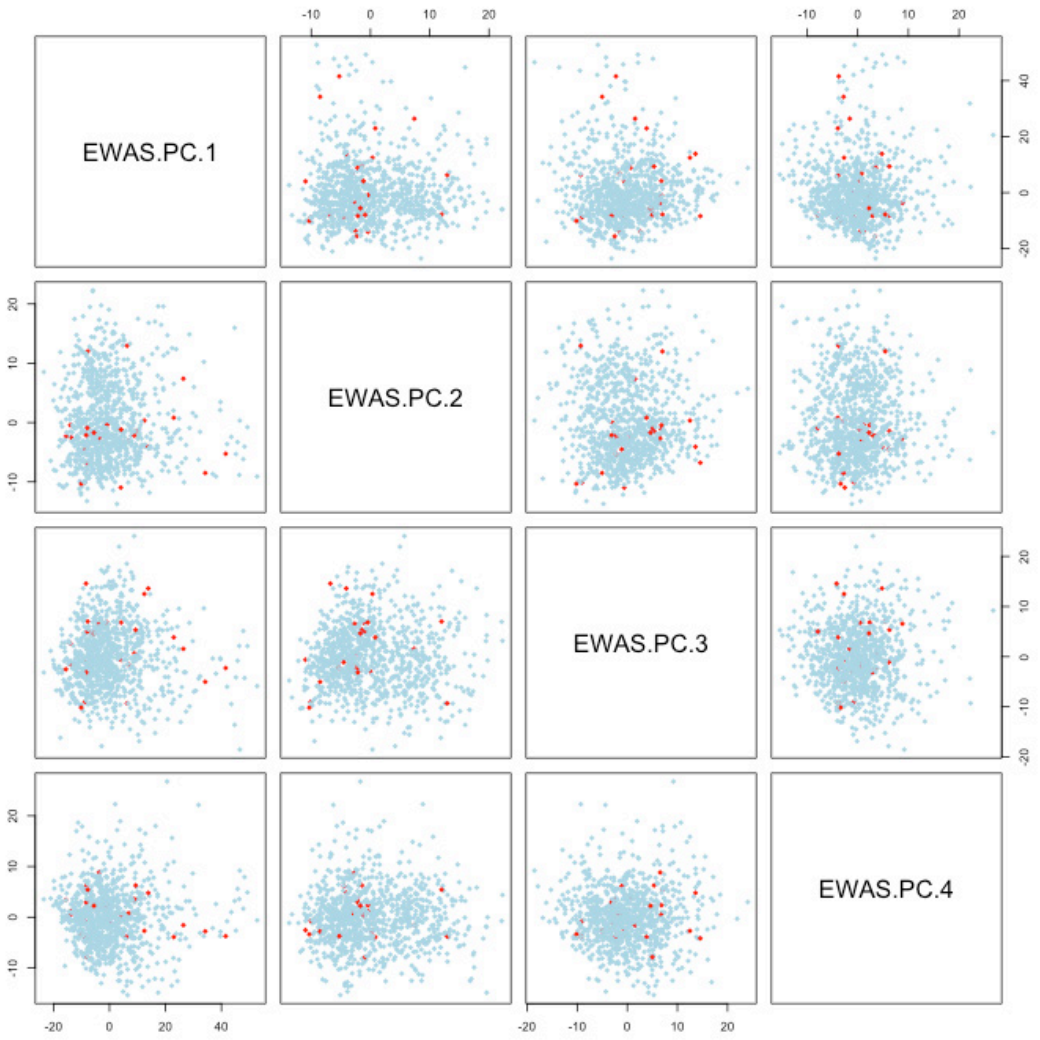

The 380 FDR-significant CpG sites were annotated to 365 genes. The plot displays enrichment scores (-log(P)) taken from 15 (out of 158 clusters) of GO biological process terms that corresponded to these 365 genes. As shown, 11 GO term clusters were enriched at a nominally significant level (red dashed 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.

## Supplemental References

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