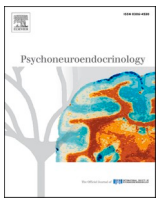
Psychoneuroendocrinology 144 (2022) 105854

Contents lists available at ScienceDirect

Psychoneuroendocrinology

journal homepage: www.elsevier.com/locate/psyneuen



# Examining the epigenetic mechanisms of childhood adversity and sensitive periods: A gene set-based approach

Yiwen Zhu [a,b,*], Alexandre A. Lussier [a,c,d], Andrew D.A.C. Smith [e], Andrew J. Simpkin [f], Matthew J. Suderman [g], Esther Walton [h], Caroline L. Relton [g,i], Erin C. Dunn [a,c,d,j,**,1]

[a] Center for Genomic Medicine, Massachusetts General Hospital, Boston, MA, United States
[b] Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA, United States
[c] Department of Psychiatry, Harvard Medical School, Boston, MA, United States
[d] Stanley Center for Psychiatric Research, The Broad Institute of Harvard and MIT, Cambridge, MA, United States
[e] Mathematics and Statistics Research Group, University of the West of England, Bristol, UK
[f] School of Mathematics, Statistics and Applied Mathematics, National University of Ireland, Galway, Ireland
[g] MRC Integrative Epidemiology Unit, Population Health Sciences, Bristol Medical School, University of Bristol, Bristol, UK
[h] Department of Psychology, University of Bath, Bath, UK
[i] Population Health Sciences, Bristol Medical School, University of Bristol, Bristol, UK
[j] Harvard Center on the Developing Child, Cambridge, MA, United States

ARTICLE INFO

*Keywords:*
Childhood adversity
Sensitive periods
ALSPAC
Plasticity
Epigenetics

ABSTRACT

*Background:* Sensitive periods are developmental stages of heightened plasticity when life experiences, including exposure to childhood adversity, have the potential to exert more lasting impacts. Epigenetic mechanisms, including DNA methylation (DNAm), may provide a pathway through which adversity induces long-term biological changes. DNAm shifts may be more likely to occur during sensitive periods, especially within genes that regulate the timing of sensitive periods. Here, we investigated the possibility that childhood adversity during specific life stages is associated with DNAm changes in genes known to regulate the timing and duration of sensitive periods.

*Methods:* Genome-wide DNAm profiles came from the Avon Longitudinal Study of Parents and Children (n = 785). We first used principal component analysis (PCA) to summarize DNAm variation across 530 CpG sites mapped to the promoters of 58 genes previously-identified as regulating sensitive periods. Gene-level DNAm summaries were calculated for genes regulating sensitive period *opening* ($n_{genes}$ = 15), *closing* ($n_{genes}$ = 36), and *expression* ($n_{genes}$ = 8). We then performed linear discriminant analysis (LDA) to test associations between seven types of parent-reported, time-varying measures of exposure to childhood adversity and DNAm principal components. To our knowledge, this is the first time LDA has been applied to analyze functionally grouped DNAm data to characterize associations between an environmental exposure and epigenetic differences.

*Results:* Suggestive evidence emerged for associations between sexual or physical abuse as well as financial hardship during middle childhood, and DNAm of genetic pathways regulating sensitive period opening and expression. However, no statistically significant associations were identified after multiple testing correction.

*Conclusions:* Our gene set-based method combining PCA and LDA complements epigenome-wide approaches. Although our results were largely null, these findings provide a proof-of-concept for studying time-varying exposures and gene- or pathway-level epigenetic modifications.

* Correspondence to: Department of Epidemiology, Harvard T.H. Chan School of Public Health, 677 Huntington Avenue, Boston, MA 02115, United States.
** Correspndence to: Psychiatric and Neurodevelopmental Genetics Unit, Center for Genomic Medicine, Massachusetts General Hospital, 185 Cambridge Street, Simches Research Building 6th Floor, Boston, MA 02114, United States.
*E-mail addresses:* yiwenzhu@mail.harvard.edu (Y. Zhu), edunn2@mgh.harvard.edu (E.C. Dunn).
[1] www.thedunnlab.com.

https://doi.org/10.1016/j.psyneuen.2022.105854
Received 3 September 2021; Received in revised form 24 June 2022; Accepted 24 June 2022
Available online 27 June 2022

## 1. Introduction

Childhood adversity is commonly defined as social, emotional, and physical experiences that deviate from expected environmental inputs during development, including the presence of aversive stimuli or absence of an adaptive learning environment (Gilbert et al., 2009; McLaughlin et al., 2017). Exposure to childhood adversity can impact development, leading to changes in myriad neurological, behavioral, and health outcomes (Berens et al., 2017). These consequences are thought to be more impactful when adversity occurs during *sensitive periods* in development, meaning periods of increased plasticity (encompassing both sensitivity and vulnerability) when individuals are more susceptible to life experiences, including exposure to childhood adversity (Knudsen, 2004). To that end, and consistent with sensitive period theories, recent studies are beginning to reveal that the effects of childhood adversity might be time-varying, with adversity exposures during early childhood possibly being more detrimental than exposures occurring earlier or later in development (Dunn et al., 2020).

Genetic processes play a key role in regulating the timing of sensitive periods. Both animal and human studies have identified more than 50 genes involved in shaping the *timing* of sensitive periods across brain development (Takesian and Hensch, 2013). Specifically, these genes are involved in the opening, closing, and maintenance (or expression) of sensitive periods. *Opening* genes regulate when sensitive periods *begin,* accelerating or delaying the onset of sensitive period plasticity by modifying parvalbumin cell maturation or excitatory-inhibitory circuitry balance (Anomal et al., 2013; Takesian and Hensch, 2013). *Closing* genes regulate when sensitive periods terminate; these genes play important roles in the formation of perineuronal nets, which emerge with sensitive period closure and limit plasticity (Lee et al., 2017). Finally, *expression* genes maintain the duration of sensitive periods, orchestrating structural changes that lead to circuit consolidation over time (Fagiolini et al., 2003).

The functions of these sensitive period genes have been predominantly identified in the primary sensory cortex using animal models (Hensch, 2004; Hooks and Chen, 2007; Sharma et al., 2015). However, evidence for similar plasticity regulating mechanisms in the prefrontal cortex and subcortical structures is emerging (Gogolla et al., 2009; Guirado et al., 2020). For example, we recently applied a gene set approach to examine how genetic pathways involved in sensitive period functioning are associated with risk for depression in humans (Zhu et al., 2022). Our results suggest that genetic variation in *opening* genes is implicated in depression risk in the general population. We also found that individuals with high genetic risk for depression conferred by *opening*-related genes and who were exposed to caregiver abuse during an empirically identified sensitive period (from ages 1–5) had the highest levels of depression in adolescence, compared to individuals who did not have sensitive-period related genetic susceptibility or exposure to childhood adversity. Together, these findings show gene--by-development interplay (dGxE) and highlight the role of sensitive period-regulating pathways in shaping psychiatric vulnerabilities during development.

Evidence is lacking on how adversity *timing* shapes epigenetic modifications of these sensitive period pathways in humans. One major type of epigenetic modification, DNA methylation (DNAm), has been increasingly studied as a potential biological signature of exposure to childhood adversity. Emerging evidence suggests DNAm patterns may be most strongly influenced by adversity that occurs during the first five years of life, compared to adversity at other ages or the accumulation of adversity across time (Dunn et al., 2019; Lussier et al., 2022). However, existing research on epigenetic profiles linked to childhood adversity is limited. Candidate gene studies have narrow scope, cannot generate novel hypotheses, and lack replicability and sometimes generalizability (Parade et al., 2021). Results from discovery-driven epigenome-wide analyses (EWAS) may have limited interpretability, as they do not specify or test a priori hypotheses.

Gene set-based analyses of DNAm data combine the advantages of discovery- and hypothesis-driven studies by investigating *biological pathways* rather than specific genes. As such, gene set-based approaches can complement EWAS by providing additional insights into mechanisms implicated in the pathophysiology of neuropsychiatric disorders (Do et al., 2015). For example, by considering the DNAm patterns of genes linked to sensitive periods, researchers can investigate the extent to which exposure to childhood adversity has time-dependent associations with epigenetic modifications that might disrupt processes involved in developmental plasticity.

In this study, we analyzed data from a large, population-based sample to identify if and when childhood adversity exerts stronger effects on DNAm during the first seven years of life. We analyzed DNAm for loci mapped to three gene sets shown to regulate sensitive periods. Gene sets were identified from a literature review of animal and human studies on known genetic components governing the *opening* ($n_{genes}$ = 15), *closing* ($n_{genes}$ = 36), and *expression* ($n_{genes}$ = 8) of sensitive periods for brain development (Fig. 1; Takesian and Hensch, 2013; Zhu et al., 2022). To our knowledge, our study is the first to implement linear discriminant analysis (LDA) with functionally grouped epigenetic data to examine associations between time-varying exposures and DNAm.

## 2. Materials and methods

### 2.1. Sample and procedures

Data came from a UK-based prospective birth cohort, called the Avon Longitudinal Study of Parents and Children (ALSPAC) (Boyd et al., 2013; Fraser et al., 2013). The ALSPAC is a longitudinal population-based cohort based in Avon, UK. Pregnant women residing in the area with estimated delivery dates between April 1, 1991, and December 31, 1992, were invited to participate in the study. The initial sample included 14,514 women who returned at least one questionnaire or attended one focus clinic by July 19, 1999. Out of the initial sample, a total of 14,676 fetuses and 13,988 children who were alive at 1 year of age were included. A second wave of participants were recruited when the oldest children in the cohort reached approximately 7 years of age, which consisted of eligible individuals who did not join the study initially, resulting in the enrollment of an additional 913 children. With the two waves combined, the ALSPAC sample consisted of 14,901 children alive at age 1 year (Boyd et al., 2013; Fraser et al., 2013).

Our analytic sample came from a subsample of ALSPAC, called the Accessible Resource for Integrated Epigenomics Studies (ARIES). The ARIES subsample includes 1018 mother-child pairs from whom blood-based DNAm data were collected. Participants in the ARIES subsample were randomly selected from ALSPAC participants with complete data across at least five timepoints of data collection (Relton et al., 2015). There were 785 singleton children with DNAm at age 7 in our analytic sample, who had complete data on all covariates and at least one type of childhood adversity across all time points.

Ethical approval for ALSPAC and ARIES was obtained from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committee. Consent for biological samples was collected in accordance with the Human Tissue Act (Human Tissue Act, 2004). Please note the study website contains details of all the data that is available through a fully searchable data dictionary and variable search tool (http://www.bristol.ac.uk/alspac/researchers/our-data/). Secondary analyses of ALSPAC data were approved with oversight by the Mass General Brigham Institutional Review Board (IRB) (Protocol 2017P001110).

### 2.2. Measures

#### 2.2.1. Exposure to childhood adversity

We analyzed seven commonly-occurring types of childhood adversity: (a) caregiver physical or emotional abuse; (b) sexual or physical abuse (by anyone); (c) maternal psychopathology; (d) one adult in the

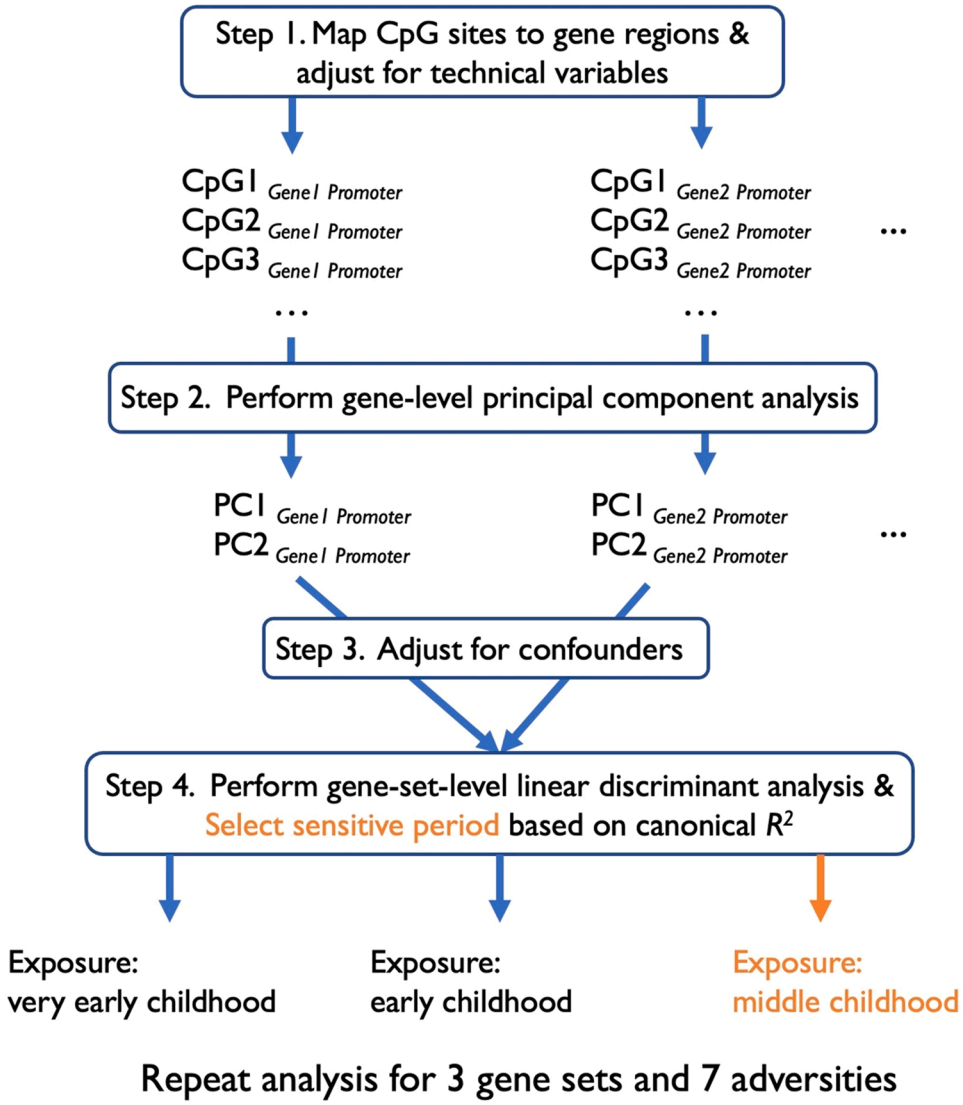



**Fig. 1.** Flowchart of analyses. After adjusting for technical variables (Step 1), variation among CpG sites annotated to the promoter of a given gene was summarized at the *gene* level into two principal component (PC) scores (Step 2). PCs were then adjusted for confounders (Step 3). Finally, at the *gene set* level, associations between time-varying exposures to childhood adversity and PCs of all genes within a gene set were assessed using a linear discriminant analysis (Step 4). The sensitive period most strongly associated with the PCs was selected based on canonical $R^2$, as highlighted in orange in this example.

household; (e) family instability; (f) financial hardship; and (g) neighborhood disadvantage. These adversities were selected because they were frequently examined in prior studies of early life stress on epigenetic profiles (Barker et al., 2018; Dunn et al., 2019; Krause et al., 2020). They also had at least four repeated assessments before age 7 in ALSPAC, enabling analysis of adversity timing. Maternal psychopathology was assessed using two psychometrically validated instruments (namely, the Crown-Crisp Experiential Index (Crown and Crisp, 1979) and the Edinburgh Postnatal Depression Scale (Cox et al., 1987)) and one item evaluating suicidality. The other six adversities analyzed were measured using maternal (and if available, also partner) prospective self-assessments. Tables 1–3 presents a summary of the respondent(s), instrument(s) or questionnaire items, exposure definition, and available time points for each adversity.

To identify potential sensitive period effects of childhood adversity on DNAm, we created three exposure variables per adversity, based on whether children were exposed to that type of adversity, during each of three developmental periods: very early childhood (0–2 years), early childhood (3–5 years), and middle childhood (6–7 years). The developmental periods were defined to be consistent with our prior research on epigenetic markers of childhood adversity (Dunn et al., 2019), and were based on subject matter knowledge only, independent of any results. For a given adversity, *any* reporting of exposure (within one or more developmental periods) led us to code a child as exposed during that developmental period(s); reporting of no exposure (at *all* assessment periods) led us to code a child as unexposed. Overall, 21 variables were created in total, for three developmental periods and seven types of adversity.

### 2.2.2. DNA methylation

DNAm profiles were obtained from blood samples collected at age 7 and assayed using the Illumina Infinium Human Methylation 450k BeadChip microarray, which measured DNAm at 485,577 CpG sites (Illumina, San Diego, CA). The 450K microarray captures DNAm variation at 99 % of RefSeq genes. To minimize batch effects, samples were semi-randomly assigned across time points to different plates (Relton et al., 2015). Beta values for DNAm were analyzed, which represent the proportion of cells methylated at each CpG. Data quality control was performed using the protocol described elsewhere (Min et al., 2018). The pipeline applied functional normalization and background correction using the *meffil* R package. Additionally, cross-hybridizing CpGs, polymorphic CpGs, and CpGs located in sex chromosomes were removed before analyses. To reduce the impact of outliers, beta values at each CpG site were winsorized by setting values outside the 5th and 95th percentiles to the 5th and 95th cutoffs, respectively.

We focused on DNAm in promoter regions, as promoters show more

**Table 1**
Summary of childhood adversity measures analyzed in the current study.

| Adversity | Respondent | Instrument or questionnaire items | Exposure definition | Time points of assessment |
|---|---|---|---|---|
| Caregiver physical or emotional abuse | Mother and partner | 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 | Children were coded as exposed if either the mother, the partner, or both, endorsed any of the items.<br>Children were coded as unexposed if any negative response was available and no positive response was provided. | 8 months,<br>1.75 years,<br>2.75 years,<br>4 years,<br>5 years, and<br>6 years |
| Sexual or physical abuse | Mother | An item asking whether or not the child had been exposed to either sexual or physical abuse from anyone | Children were coded as exposed if an affirmative response was provided to the item.<br>Children were coded as unexposed if any negative response was available and no positive response was provided. | 1.5 years,<br>2.5 years,<br>3.5 years,<br>4.75 years,<br>5.75 years, and<br>6.75 years |
| Maternal psychopathology | Mother | 1) the Crown-Crisp Experiential Index (CCEI), assessing anxiety and depression; 2) the Edinburgh Postnatal Depression Scale (EPDS); and 3) a question asking about suicide attempts in the past 1.5 years | Consistent with prior studies and established cutoffs, children were coded as exposed if one or more of the following criteria was met: 1) CCEI depression score $>$ 9; 2) CCEI anxiety score $>$ 10; 3) EPDS score $>$ 12; or 4) a suicide attempt since the time of the last interview.<br>Children were coded as unexposed if none of the criteria above were met and none of the scores were missing. | 8 months,<br>1.75 years,<br>2.75 years,<br>5 years, and<br>6 years |
| One adult in the household | Mother | An item asking about the number of adults (>18 years of age) living in the household | Children were coded as exposed if fewer than two adults were residing in the household.<br>Children were coded as unexposed if two or more adults resided in the household. | 8 months,<br>1.75 years,<br>2.75 years,<br>4 years, and<br>7 years |
| Family instability | Mother | 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. | Children were coded as exposed if at least two of these events occurred at a single time point.<br>Children were coded as unexposed if none of the events occurred and no questions were missing. | 1.5 years,<br>2.5 years,<br>3.5 years,<br>4.75 years,<br>5.75 years, and<br>6.75 years |
| Financial hardship | Mother | 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) | Children were coded as exposed if their mothers reported at least fair difficulty for three or more items at each time point.<br>Children were coded as unexposed if mothers provided responses to all five items and the above criterion was not met. | 8 months,<br>1.75 years,<br>2.75 years,<br>5 years, and<br>7 years |
| Neighborhood disadvantage | Mother | There were problems in the 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 | A sum score was derived, ranging from 0 to 16. Children with scores $\geq$8, generally corresponding to the 95th percentile, were classified as exposed.<br>Children were coded as unexposed if the sum scores were below 8 and no questions were missing. | 1.75 years,<br>2.75 years,<br>5 years, and<br>7 years |

**Table 2**
Distribution of covariates and childhood adversity in the ARIES analytic sample (n = 785), compared to the full ALSPAC sample (n = 14,901).

| | ALSPAC full sample (n = 14,901) | ARIES analytic sample (n = 785) | Comparison p-value |
|---|---|---|---|
| ***Covariates*** | | | |
| | *n ( %)* | *n ( %)* | |
| Race | | | |
| Non-White | 611 (5.1) | 24 (3.1) | 0.01 |
| White | 11,488 (94.9) | 761 (96.9) | |
| Sex | | | |
| Male | 7542 (51.3) | 405 (51.6) | 0.91 |
| Female | 7152 (48.7) | 380 (48.4) | |
| Age of mother at child's birth | | | |
| Ages 15–19 | 650 (4.6) | < 5 (0.5) | < 0.001 |
| Ages 20–35 | 12,363 (88.4) | 698 (88.9) | |
| Age 36+ | 968 (6.9) | 83 (10.6) | |
| Number of previous pregnancies | | | |
| 0 | 5800 (44.7) | 357 (45.5) | 0.02 |
| 1 | 4550 (35.0) | 295 (37.6) | |
| 2 | 1860 (14.3) | 104 (13.2) | |
| 3+ | 772 (5.9) | 29 (3.7) | |
| Sustained smoking during pregnancy | | | |
| No | 9565 (78.8) | 704 (89.7) | < 0.001 |
| Yes | 2577 (21.2) | 81 (10.3) | |
| Maternal education at baseline | | | |
| Below O-level | 3735 (30.0) | 101 (12.9) | < 0.001 |
| O-level | 4303 (34.6) | 268 (34.1) | |
| A-level | 2795 (22.5) | 244 (31.1) | |
| Degree or above | 1603 (12.9) | 172 (21.9) | |
| | *Mean (SD)* | *Mean (SD)* | |
| Birth weight, grams | 3391.65 (560.04) | 3488.56 (488.04) | < 0.001 |
| ***Exposure prevalence*** | | | |
| | *n ( %)* | n ( %) | |
| Physical or sexual abuse | 1564 (23.4) | 122 (18.2) | 0.001 |
| Financial hardship | 3273 (41.0) | 164 (24.1) | < 0.001 |
| One adult in the household | 2336 (32.1) | 108 (16.0) | < 0.001 |
| Family instability | 1760 (23.8) | 125 (18.0) | < 0.001 |
| Neighborhood disadvantage | 1815 (24.2) | 112 (16.0) | < 0.001 |
| Caregiver physical or emotional abuse | 1944 (28.6) | 147 (21.0) | < 0.001 |
| Maternal psychopathology | 5350 (60.3) | 297 (43.4) | < 0.001 |

*Note.* P-values were obtained from $\chi^2$ tests or independent t-tests comparing distributions of covariates and exposure variables in the analytic sample to distributions in the rest of the entire ALSPAC sample.

robust associations with transcription levels (Vialou et al., 2013). Promoters were defined as the genomic regions 1500 base pairs upstream of the transcription start site and from the 5′-untranslated region through the first exon (Lokk et al., 2014). A total of 530 CpG sites were annotated to the promoters of the 58 sensitive period regulating genes. The number of CpG sites annotated to each gene promoter varied from 1 (*PILRB*) to 31 (*PTPRS*) (Fig. 2). A full list of CpG sites examined (and their annotations) is available in Table S1.

Of note, although our list of sensitive period genes came from published studies on plasticity in the brain, DNAm was measured in blood by ALSPAC researchers. Correlation analyses between DNAm levels in blood and brain at these 530 CpG sites were modestly concordant (Supplemental Materials), similar to the distribution across the entire genome (Hannon et al., 2015; Braun et al., 2019).

#### 2.2.3. Covariates

To control for potential confounding of the association between childhood adversity and DNAm by sociodemographic characteristics, we adjusted for the following covariates: child race/ethnicity (White; non-White); birth weight (continuous, in grams); maternal age (ages 15–19; ages 20–35; ages 35+); number of previous pregnancies (0;1;2; 3 +); sustained maternal smoking during pregnancy (yes; no); and maternal education (levels corresponding to schooling before age 16, until age 16, through ages 16–18, and university degree or above). Prior studies showed these variables could influence both exposure to childhood adversity and epigenetic modifications (Dunn et al., 2019; Marini et al., 2020).

### 2.3. Data analysis

#### 2.3.1. Primary analysis

Fig. 1 displays the analytic steps of our study. In step 1, technical variables were regressed on DNAm levels at each CpG site (estimated cell proportions and sample type, i.e., whole blood or white blood cells). In step 2, we used PCA as a data reduction strategy, as including all 530 CpGs was computationally challenging. PCA extracts linear combinations (i.e., principal components, or PCs) of DNAm values at the promoter of each CpG site within a certain gene. The goal was for these PCs to explain the maximal amount of variance across individual CpGs, and create parsimony in subsequent regression models. With one exception (*PILRB),* we retained two PCs per gene, which on average explained 75 % of variability in DNAm in each gene. For *PILRB,* only one CpG was available for analysis, thus only one PC (explaining 100 % of variability in DNAm) was retained. Using PCA, we reduced the original 530 individual CpGs to 16–77 PCs per analysis, which correspond to 8–39 genes in each gene set. In step 3, we adjusted for confounding by regressing the PCs on all pre-specified covariates listed under Section 2.2.3.

In step 4, we used linear discriminant analysis (LDA) to test associations between childhood adversity and the PCs in each gene set. LDA provides a measure of association between a binary exposure, in this case childhood adversity exposure status within one of the three developmental periods, and a multivariate outcome, here the DNAm PCs in a gene set. LDA is mathematically equivalent to canonical correlation analysis (Bach and Jordan, 2005), as both methods assess multivariate associations. The strength of association was calculated as the squared canonical correlation (canonical $R^2$), which is a multivariate equivalent of a Pearson's correlation coefficient. We calculated the canonical $R^2$ for each of the three developmental periods and selected the developmental period with the largest canonical $R^2$ for reporting. To account for the selection of the largest canonical $R^2$, we constructed an empirical null distribution for the largest $R^2$ through 5000 bootstrapping iterations and reported the bootstrap p-value.

Notably, the LDA models were adversity- and timing-specific. In other words, we tested a simple model, where only exposure to a specific type of adversity during the tested developmental period had an association with the outcome. Therefore, the comparison group was defined as children unexposed to the tested exposure during that period, because our hypothesis focused on the presence of effect at a given time point above and beyond exposures at other time periods. Construction of hypotheses and comparison groups in this manner is consistent with the structured life course modeling approach (SLCMA; pronounced slick-mah) (Smith et al., 2022). Step 4 was repeated for each adversity and gene set, summing to 21 tests of association. We therefore assessed p-values after Bonferroni corrections ($\alpha = 0.05/21 = 0.002$).

*Secondary analysis*

To further interpret our results, we pursued a secondary analysis using data available from the EWAS Catalog (http://www.ewascatalog.org). The EWAS catalog is a well-curated database containing published results from EWAS of DNAm and phenotypes spanning multiple domains, including biological functioning, disease risk, and social characteristics (Battram et al., 2021). We annotated the 530 probes analyzed here to any trait associations reported in the catalog. All associations from published analyses with a p-value$< 1 \times 10^{-4}$ were reported.

*Sensitivity analyses*

To evaluate the robustness and generalizability of our results, we pursued four sets of sensitivity analyses. First, DNAm patterns in different genomic regions (e.g., promoters vs. gene bodies) may be

**Table 3**
Associations between time-dependent exposure to childhood adversity and DNA methylation in promoter regions of three gene sets known to regulate sensitive period functioning in the brain.

| Childhood adversity | Sensitive period selected | Canonical $R^2$ | Wilks' lambda | Wilks' p-value | Bootstrap p-value |
|---|---|---|---|---|---|
| ***Opening genes*** | | | | | |
| Maternal psychopathology | Very early childhood (0–2 years) | 0.06 | 0.94 | 0.118 | 0.307 |
| Financial hardship | Very early childhood (0–2 years) | 0.05 | 0.95 | 0.427 | 0.766 |
| **Sexual or physical abuse** | **Middle childhood (6–7 years)** | **0.08** | **0.92** | **0.004** | **0.019** |
| One adult in the household | Middle childhood (6–7 years) | 0.04 | 0.96 | 0.633 | 0.904 |
| Family instability | Middle childhood (6–7 years) | 0.05 | 0.95 | 0.257 | 0.583 |
| Neighborhood disadvantage | Middle childhood (6–7 years) | 0.05 | 0.95 | 0.171 | 0.388 |
| Caregiver physical or emotional abuse | Middle childhood (6–7 years) | 0.04 | 0.96 | 0.549 | 0.872 |
| ***Closing genes*** | | | | | |
| Family instability | Early childhood (3–5 years) | 0.13 | 0.87 | 0.096 | 0.284 |
| Neighborhood disadvantage | Early childhood (3–5 years) | 0.09 | 0.91 | 0.817 | 0.977 |
| Caregiver physical or emotional abuse | Early childhood (3–5 years) | 0.12 | 0.88 | 0.155 | 0.387 |
| Sexual or physical abuse | Middle childhood (6–7 years) | 0.12 | 0.88 | 0.122 | 0.339 |
| Financial hardship | Middle childhood (6–7 years) | 0.12 | 0.88 | 0.063 | 0.401 |
| One adult in the household | Middle childhood (6–7 years) | 0.10 | 0.90 | 0.433 | 0.753 |
| Maternal psychopathology | Middle childhood (6–7 years) | 0.13 | 0.87 | 0.046 | 0.127 |
| ***Expression genes*** | | | | | |
| Sexual or physical abuse | Very early childhood (0–2 years) | 0.04 | 0.96 | 0.077 | 0.214 |
| Neighborhood disadvantage | Very early childhood (0–2 years) | 0.04 | 0.96 | 0.069 | 0.178 |
| Family instability | Early childhood (3–5 years) | 0.04 | 0.96 | 0.029 | 0.090 |
| Caregiver physical or emotional abuse | Early childhood (3–5 years) | 0.03 | 0.97 | 0.260 | 0.567 |
| **Financial hardship** | **Middle childhood (6–7 years)** | **0.05** | **0.95** | **0.008** | **0.028** |
| One adult in the household | Middle childhood (6–7 years) | 0.03 | 0.97 | 0.327 | 0.634 |
| Maternal psychopathology | Middle childhood (6–7 years) | 0.03 | 0.97 | 0.370 | 0.706 |

Bootstrap p-values accounted for the number of hypotheses tested per gene set and adversity (i.e., three sensitive period hypotheses). Associations with bootstrap p-values $< 0.05$ were in bold.

involved in different regulatory processes, sometimes in divergent directions (Jones, 2012). We therefore evaluated if our primary results, which were based on promoter regions, were similar to analyses examining variation in gene bodies of the three sensitive period gene pathways. The same analytic procedure was followed for these analyses: beta values at CpG sites in each gene body were summarized into two principal component scores per gene, and a LDA model was fit to each exposure time point.

Second, it is possible that any exposure to adversity before age 7 impacts DNAm, regardless of when the exposure occurred. Thus, we tested a basic model, comparing the presence (versus absence) of exposure to each type of adversity before age 7 on DNAm variation in promoters of sensitive period gene sets. Again, we pursued the same analytic approach, except that no selection of time period was examined, only the ever versus never exposure variable for each adversity type.

Third, there are ongoing debates regarding the benefits and drawbacks of lumping (versus splitting) adversity by type (Smith and Pollak, 2021). Some argue that different types of childhood adversity may impact epigenetic modifications through distinct pathways and thus should be examined separately (Dunn et al., 2019). Others suggest that early life stress, regardless of adversity type, could have similar effects on underlying biological processes (Heim and Binder, 2012); therefore, these effects can be captured under a broad classification of childhood adversity. To provide a contrast for our results, we investigated whether aggregating exposure status across the seven types of adversity, instead of examining each type separately as in the primary analysis, changed the results. During each developmental period (very early childhood, early childhood, and middle childhood), children exposed to any of the seven types of childhood adversity were coded as exposed, resulting in three models for each gene set (i.e., one for each sensitive period).

Fourth, as the gene set-based approach was meant to complement EWAS, meaning the agnostic search for signals across the genome, we were interested in determining if combining CpGs into genes and pathways yielded more information than CpG-level analyses. Therefore, we compared our findings to those obtained from a previous EWAS of the same dataset (Lussier et al., 2022). Specifically, Lussier et al. performed a SLCMA to compare multiple encoded exposures of childhood adversity and identify the life course hypothesis most strongly associated with DNAm at each locus across the epigenome using the ALSPAC cohort.

All analyses were performed using software package R. We made the scripts publicly available on GitHub (https://github.com/thedunnlab/geneset).

## 3. Results

### 3.1. Sample Characteristics

Compared to the entire ALSPAC, children in the ARIES analytic sample (n = 785) were more likely to be born to mothers who were older, had fewer previous pregnancies, were less likely to smoke during pregnancy, and were better educated at baseline. Children in the analytic sample were also less likely to experience childhood adversity (Table 1).

The prevalence of childhood adversity ranged from 2.2 % to 24 % across adversities and time periods (Fig. S1). Overall, 496 children (63.2 %) experienced at least one type of childhood adversity before age 7. Across time points, exposure to any type of childhood adversity was most prevalent during very early childhood (48.2 %), followed by early childhood (37.7 %) and middle childhood (29.0 %). Within a specific type of adversity, exposures were moderately to strongly correlated across time periods (Fig. S2). Across adversities, exposures were weakly to moderately correlated within a period (Fig. S3).

### 3.2. Principal component analysis, linear discriminant analysis, and model selection

#### 3.2.1. Primary results

For 53 of 58 genes, the first two PCs accounted for more than 50 % of the variation in promoter DNAm (Fig. 2). Adversity during middle childhood (6–7 years) was most frequently associated with DNAm in the promoters of genes involved in regulating sensitive period functioning (selected for 12 out of the 21 LDA models). Two associations between the timing of adversity and DNAm of gene sets were identified at a

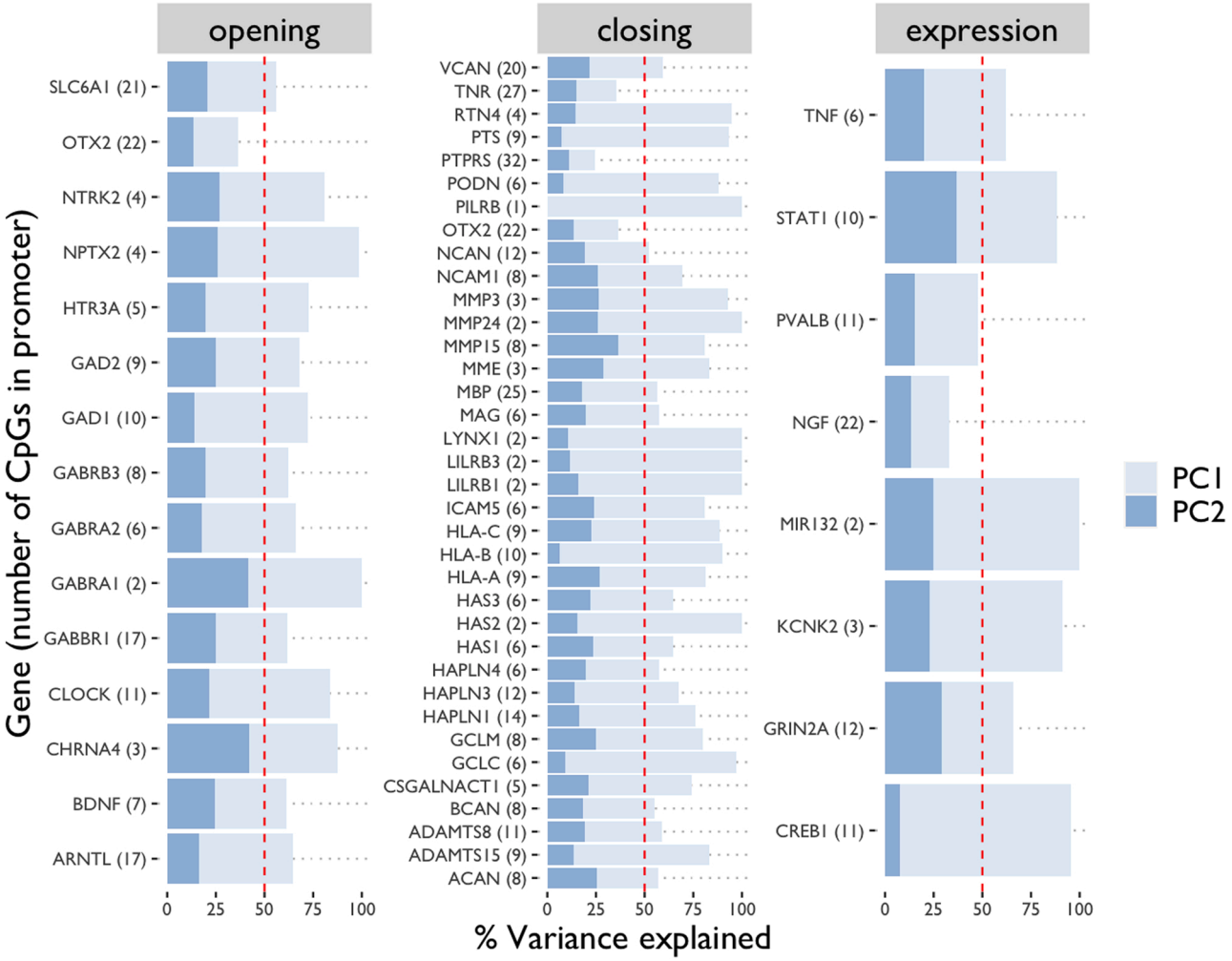



**Fig. 2.** Variances of CpGs located in promoters of three sets of sensitive period genes, as explained by the first two principal components (PCs). The number of CpGs annotated to the promoter of each gene is noted in parentheses. The red dashed line indicates 50 % of the variance. For 53 of 58 genes, variance explained by the first two PCs exceeded 50.

bootstrap p-value < *0.05*, which was corrected for the three sensitive period hypotheses tested per gene set and adversity type. Specifically, exposure to sexual or physical abuse in middle childhood was associated with differences in DNAm among *opening* genes (p = 0.019). Exposure to financial hardship in middle childhood was associated with DNAm differences in *expression* genes (p = 0.028). After a Bonferroni correction for multiple testing, no association remained significant.

### 3.2.2. Secondary analysis results

CpGs annotated to promoters of the sensitive period genes in our study were linked to other traits in previous EWAS. Queries of the 530 CpG sites using results from the EWAS Catalog yielded 2000 entries across 503 CpGs and 58 genes (Table S1). The number of studies reported for each CpG ranged from 14 (cg16762684, annotated to the closing gene, *MBP*, encoding myelin basic protein) to 1, capturing 79 unique traits. Out of the 503 CpGs, the majority (89 %; n = 446) reported associations with biological age, an expected finding given that these gene sets are implicated in developmental plasticity and sensitive period timing. The second most common trait was tissue type (n = 393), followed by sex (n = 78), gestational age (n = 73), and rheumatoid arthritis (n = 61). Notably, seven CpGs also appeared in study results of schizophrenia.

### 3.2.3. Sensitivity analysis results

Results from sensitivity analyses were as follows. First, DNAm annotated to gene bodies did not yield even a nominal signal after bootstrapping (Table S2). Second, analysis of lifetime exposure to adversity suggested sexual or physical abuse was nominally associated with DNAm in the *opening* gene promoters (p = 0.041), but this association was not statistically significant after multiple test correction (Table S3). The canonical $R^2$ associated with sexual or physical abuse was also attenuated from 0.08 to 0.06, and the corresponding Wilks' p-value increased from 0.004 to 0.041, suggesting any signal was weakened by combining exposures across time points. Third, the composite measure of any type of childhood adversity before age 7 was also unassociated with DNAm PCs (Table S4). Fourth, there was little overlap in our gene set-based analyses and the CpG-level associations shown by Lussier et al. (2022). No association emerged at FDR< 0.05 among all 530 CpGs (Table S5).

## 4. Discussion

In the current study, we implemented a novel analytic approach combining PCA and LDA to interrogate the joint contribution of time-varying exposures to childhood adversity and epigenetic modifications of pathways implicated in sensitive period functioning. As is often the case for an early work, particularly in the 'omics setting, our analyses did not detect any strong signals between adversity and DNAm in these gene sets. Nevertheless, our analytic approach provides a proof-of-concept for a gene set-based approach to examine time-dependent exposures and

pathway-specific DNAm. Specifically, our approach and results contribute to the existing literature in several meaningful ways.

First, our analyses demonstrate how PCA and LDA can be implemented to examine a pathway-specific hypothesis about time-varying environmental experiences and downstream differences in epigenetic profiles. The combination of PCA and LDA allowed us to achieve the goals of data reduction and hypothesis testing effectively, which has also been demonstrated in a study exploring biotypes of post-traumatic stress disorder (Yang et al., 2021). Compared to EWAS, candidate gene, or other research designs in the literature of social and psychiatric epigenetics, our approach has several unique strengths. For example, our framework extends existing pathway-based analyses, including enrichment or network analyses (Brown et al., 2020; Grillault Laroche et al., 2020), by examining gene sets pertaining to specific biological hypotheses and sourced from literature reviews or subject matter knowledge. Our approach also leverages perspectives from the SLCMA to account for time-dependent effects of correlated exposures via model selection and bootstrapping (Smith et al., 2022).

Second, although no conclusive evidence emerged from our results, the suggestive associations shed light on the value of considering developmental timing and sensitive periods at both the genetic and phenotypic level. After correcting for the number of sensitive period hypotheses tested via bootstrapping, exposure to adversity during middle childhood (ages 6–7), namely physical or sexual abuse and financial hardship, was potentially linked to differences in DNAm patterns within the promoters of genes involved in the onset or duration of sensitive periods. Middle childhood is a critical stage of development: key structures of neural plasticity, such as perineuronal nets, reach maturation around age 8 in brain regions implicated in psychopathology, including the medial prefrontal cortex and hippocampus (Mauney et al., 2013; Rogers et al., 2018). Adverse environmental stimuli during this period of rapid growth and maturation could lead to disruptions of experience-expectant mechanisms. Epigenetic modifications might also influence the activity of genes involved in neuronal plasticity, further disrupting molecular and behavioral responses. However, we emphasize our analyses were exploratory in nature and no association persisted after accounting for the number of adversities and gene sets examined. Repeated investigation of these findings in larger, more diverse samples, as well as studies focusing on the cascading effects of adversity later in life, are needed to further disentangle the role of epigenetic mechanisms in the biological embedding childhood adversity during sensitive periods.

Further, considering the time-sensitive nature of exposures allowed us to detect a few, albeit weak, signals compared to a traditional classification of ever versus never exposed. Although the decrease in effect estimate for physical or sexual abuse was not striking, as modest relationships between exposure to adversity and DNAm overall were expected, it nevertheless suggests meaningful gains from a developmentally informed approach. Our sensitivity analysis looking at the combined effects of all types of adversity also revealed no association across time periods. Early life stress in different forms may produce unique biological signatures and alter functioning differently even within the domain of sensitive period regulation. Therefore, as discussed in prior reviews and empirical articles, biological signatures resulting from childhood adversity could potentially be revealed through alternate conceptualizations of adversity, such as "splitting" rather than "lumping" adversity measures (Sumner et al., 2019; Colich et al., 2020; Smith and Pollak, 2021).

Lastly, our analyses serve as an important proof-of-concept for showing the feasibility of applying a developmentally informed gene set-based approach. We tested hypotheses about whether and how childhood adversity may influence epigenetic changes of pathways involved in genetic orchestration of developmental plasticity. The lack of strong support for our hypothesis requires additional investigation. It is unclear if the absence of findings could be attributed to inadequate sample sizes to identify modest effects, or true absence of association for our hypothesis. Although very few existing studies on DNAm collect longitudinal data on childhood adversity with the level of granularity that our analysis included, replication of these possibly null results in another independent sample is needed. If the effects of childhood adversity on DNAm at sensitive period-related genes were too small to be detected or diluted by measurement error, larger data collection effort with prospective reporting of adversity may need to be prioritized.

Future research should also test alternative hypotheses. It may be that genetic factors regulating sensitive periods and childhood adversity jointly influence developmental outcomes through other mechanisms than DNAm in promoter regions. As shown in a previous study, genetic risk conferred by common variants in specific sensitive period pathways modified the association between child abuse and depressive symptoms in adolescence (Zhu et al., 2022). Moreover, genetic or epigenetic variation in sensitive period-regulating components could modulate the timing and extent to which other key pathways were modified epigenetically, instead of directly affecting the sensitive period gene sets per se. Beyond hypotheses about developmental plasticity, our gene set-based approach can also be extended to test hypotheses about alternate neurobiological or physiological pathways underlying the effect of adversity, such inflammation or functionalities of the hypothalamic-pituitary-adrenal axis (Cecil et al., 2020).

Our analytic process was straightforward to implement, and the scripts we provide can be adapted to assess other pathways of interest. Preliminary data on the statistical power of the method are also provided. These data may offer guidance for future applications, especially with more refined gene sets or modest sample sizes (Fig. S4). We also encourage simulation-based power calculations to be performed for other pathways of interest in specific study contexts.

This study has several limitations. First, the sensitive period gene sets analyzed here may not be exhaustive. The current analysis primarily focuses on the genetic basis of neural plasticity regulated by GABAergic processes, whereas the time-varying effects of early life adversity may operate through other biological pathways. Thus, we may have potentially omitted possible genes implicated in sensitive period processes, which relate adversity to DNAm. As the field continues to identify molecular regulators of sensitive periods, our analyses can be expanded to include additional genes or pathways. Second, the definitions of adversity exposure and corresponding comparison groups did not encapsulate the full range of plausible mechanisms. Because we were interested in prospectively reported adversity in childhood and DNAm measured at age 7, we did not examine exposures to adversity after 7 years, due to considerations about temporality (i.e., exposure assessment should proceed DNAm sample collection). Thus, our analyses omitted exposures occurring in adolescence, a potential sensitive period linked to changes in brain functions and behavioral outcomes (Fuhrmann et al., 2015). Follow-up analyses investigating adversity exposure across development and later DNAm time points can more thoroughly examine the importance of exposure timing. Additionally, we examined each sensitive period exposure separately, following the parsimonious framework of the SLCMA. Our approach represents an initial step towards a more comprehensive understanding of adversity, sensitive periods, and epigenetic modifications in development. In future studies, potential interactions between time points and adversity types should be more carefully assessed. Third, our analyses may have been underpowered, due to the low prevalence of adversity during certain developmental periods, modest expected effect sizes, and the fact that statistical power of LDA decreases with the number of features included (Helmer et al., 2021). Although our sample size per feature was in line with prior recommendations for sufficient power and estimation stability (Barcikowski and Stevens, 1975; Helmer et al., 2021), we were inherently less powered to detect associations with larger gene sets, such as the *closing* set, due to the higher number of PCs analyzed. Thus, using our approach with larger datasets or conducting meta-analyses are critical next steps.

Finally, several factors could impact the generalizability of our

results. For instance, we analyzed DNAm data from blood samples, despite our gene sets capturing developmental plasticity in the brain. While brain and blood DNAm levels are generally only modestly correlated (Braun et al., 2019), DNAm variations in peripheral tissues may still serve as important biomarkers for brain-based disorders. In our analyses, almost half (28 out of the 58) of the genes included at least one CpG with strong blood-brain correlations ($\rho \geq 0.6$; Fig. S5). Thus, analyses of blood DNAm can allow identification of potential targets for follow-up experiments, where brain tissues might be accessible. Furthermore, ALSPAC participants are predominantly of European ancestry and the subsample with epigenetic data had lower levels of adversity compared to the larger cohort. It remains an important objective of future work to examine the generalizability of the current findings in children from minority groups, who often disproportionally experience childhood adversity (Slopen et al., 2016).

In conclusion, our study outlines a gene set-based approach to studying time-varying exposures and pathway-level epigenetic modifications during development. This analytic approach offers a useful complement to current epigenome-wide analyses and could ultimately improve the interpretability of molecular findings in neuropsychiatric research.

## Role of funding source

Research reported in this publication was supported by the National Institute of Mental Health of the National Institutes of Health [Award Number R01MH113930; PI: ECD]. The NIMH had no further role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

The UK Medical Research Council and Wellcome (Grant ref: 217065/Z/19/Z) and the University of Bristol provide core support for ALSPAC. A comprehensive list of grants funding is available on the ALSPAC website (http://www.bristol.ac.uk/alspac/external/documents/grant-acknowledgements.pdf). This research was specifically funded by grants from the BBSRC (BBI025751/1; BB/I025263/1), IEU (MC_UU_12013/1; MC_UU_12013/2; MC_UU_12013/8, MRC_UU_00011/5), National Institute of Child and Human Development (R01HD068437), NIH (5RO1AI121226-02), and CONTAMED EU (212502). This publication is the work of the authors, and all authors will serve as guarantors for the contents of this paper.

Dr. Walton is funded by CLOSER, whose mission is to maximize the use, value and impact of longitudinal studies (www.closer.ac.uk). CLOSER was funded by the Economic and Social Research Council (ESRC) and the Medical Research Council (MRC) between 2012 and 2021 (grant reference: ES/K000357/1). The funders took no role in the design, execution, analysis or interpretation of the data or in the writing up of the findings. Dr. Walton is also supported by the European Union's Horizon 2020 research and innovation programme (Grant no. 848158).

## Author contributions

YZ, AAL, ADACS, and ECD designed the study. ECD acquired the data. YZ performed the data analyses. All authors provided feedback throughout the process of data analysis and interpreted the results. YZ, AAL, and ECD drafted the manuscript. All authors provided critical feedback on the manuscript and approved the final version before submission.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgments

We are extremely grateful to all the families who took part in this study, the midwives for their help in recruiting them, and the whole ALSPAC team, which includes interviewers, computer and laboratory technicians, clerical workers, research scientists, volunteers, managers, receptionists and nurses.

## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.psyneuen.2022.105854.

## References

Anomal, R., Villers-Sidani, E., de, Merzenich, M.M., Panizzutti, R., 2013. Manipulation of BDNF signaling modifies the experience-dependent plasticity induced by pure tone exposure during the critical period in the primary auditory cortex. PLoS One 8, e64208. https://doi.org/10.1371/journal.pone.0064208.

Bach, F.R., Jordan, M.I., 2005. A probabilistic interpretation of canonical correlation. Analysis 11.

Barcikowski, R.S., Stevens, J.P., 1975. A Monte Carlo Study of the stability of canonical correlations, canonical weights and canonical variate-variable correlations. Multivar. Behav. Res. 10, 353–364. https://doi.org/10.1207/s15327906mbr1003_8.

Barker, E.D., Walton, E., Cecil, C.A.M., 2018. Annual research review: DNA methylation as a mediator in the association between risk exposure and child and adolescent psychopathology. J. Child Psychol. Psychiatry 59, 303–322. https://doi.org/10.1111/jcpp.12782.

Battram, T., Yousefi, P., Crawford, G., Prince, C., Babei, M.S., Sharp, G., Hatcher, C., Vega-Salas, M.J., Khodabakhsh, S., Whitehurst, O., Langdon, R., Mahoney, L., Elliott, H.R., Mancano, G., Lee, M., Watkins, S.H., Lay, A.C., Hemani, G., Gaunt, T.R., Relton, C.L., Staley, J.R., Suderman, M., 2021. EWAS Cat.: A Database Epigenome-wide Assoc. Stud. https://doi.org/10.31219/osf.io/837wn.

Berens, A.E., Jensen, S.K.G., Nelson, C.A., 2017. Biological embedding of childhood adversity: from physiological mechanisms to clinical implications. BMC Med. 15, 135. https://doi.org/10.1186/s12916-017-0895-4.

Boyd, A., Golding, J., Macleod, J., Lawlor, D.A., Fraser, A., Henderson, J., Molloy, L., Ness, A., Ring, S., Davey Smith, G., 2013. Cohort Profile: the 'children of the 90s'—the index offspring of the avon longitudinal study of parents and children. Int. J. Epidemiol. 42, 111–127. https://doi.org/10.1093/ije/dys064.

Braun, P.R., Han, S., Hing, B., Nagahama, Y., Gaul, L.N., Heinzman, J.T., Grossbach, A.J., Close, L., Dlouhy, B.J., Howard, M.A., Kawasaki, H., Potash, J.B., Shinozaki, G., 2019. Genome-wide DNA methylation comparison between live human brain and peripheral tissues within individuals. Transl. Psychiatry 9, 47. https://doi.org/10.1038/s41398-019-0376-y.

Brown, K.M., Diez-Roux, A.V., Smith, J.A., Needham, B.L., Mukherjee, B., Ware, E.B., Liu, Y., Cole, S.W., Seeman, T.E., Kardia, S.L.R., 2020. Social regulation of inflammation related gene expression in the multi-ethnic study of atherosclerosis. Psychoneuroendocrinology 117, 104654. https://doi.org/10.1016/j.psyneuen.2020.104654.

Cecil, C.A.M., Zhang, Y., Nolte, T., 2020. Childhood maltreatment and DNA methylation: a systematic review. Neurosci. Biobehav. Rev. 112, 392–409. https://doi.org/10.1016/j.neubiorev.2020.02.019.

Colich, N.L., Rosen, M.L., Williams, E.S., McLaughlin, K.A., 2020. Biological aging in childhood and adolescence following experiences of threat and deprivation: a systematic review and meta-analysis. Psychol. Bull. 146, 721–764. https://doi.org/10.1037/bul0000270.

Cox, J.L., Holden, J.M., Sagovsky, R., 1987. Detection of postnatal depression: development of the 10-item edinburgh postnatal depression scale. Br. J. Psychiatry 150, 782–786. https://doi.org/10.1192/bjp.150.6.782.

Crown, S., Crisp, A.H., 1979. Manual of the crown-crisp experimental index. Hodder and Stoughton.

Do, K.Q., Cuenod, M., Hensch, T.K., 2015. Targeting oxidative stress and aberrant critical period plasticity in the developmental trajectory to schizophrenia. Schizophr. Bull. 41, 835–846. https://doi.org/10.1093/schbul/sbv065.

Dunn, E.C., Nishimi, K., Neumann, A., Renaud, A., Cecil, C.A.M., Susser, E.S., Tiemeier, H., 2020. Time-dependent effects of exposure to physical and sexual violence on psychopathology symptoms in late childhood: in search of sensitive periods in development. J. Am. Acad. Child Adolesc. Psychiatry 59 (283–295), e4. https://doi.org/10.1016/j.jaac.2019.02.022.

Dunn, E.C., Soare, T.W., Zhu, Y., Simpkin, A.J., Suderman, M.J., Klengel, T., Smith, A.D. A.C., Ressler, K.J., Relton, C.L., 2019. Sensitive periods for the effect of childhood adversity on DNA methylation: results from a prospective, longitudinal study. Biol. Psychiatry Longitud. Perspect. Stress Depress 85, 838–849. https://doi.org/10.1016/j.biopsych.2018.12.023.

Fagiolini, M., Katagiri, H., Miyamoto, H., Mori, H., Grant, S.G.N., Mishina, M., Hensch, T. K., 2003. Separable features of visual cortical plasticity revealed by N-methyl-d-aspartate receptor 2A signaling. PNAS 100, 2854–2859. https://doi.org/10.1073/pnas.0536089100.

Fraser, A., Macdonald-Wallis, C., Tilling, K., Boyd, A., Golding, J., Davey Smith, G., Henderson, J., Macleod, J., Molloy, L., Ness, A., Ring, S., Nelson, S.M., Lawlor, D.A.,

2013. Cohort profile: the avon longitudinal study of parents and children: ALSPAC mothers cohort. Int. J. Epidemiol. 42, 97–110. https://doi.org/10.1093/ije/dys066.
Fuhrmann, D., Knoll, L.J., Blakemore, S.-J., 2015. Adolescence as a sensitive period of brain development. Trends Cogn. Sci. 19, 558–566. https://doi.org/10.1016/j.tics.2015.07.008.
Gilbert, R., Widom, C.S., Browne, K., Fergusson, D., Webb, E., Janson, S., 2009. Burden and consequences of child maltreatment in high-income countries. Lancet 373, 68–81. https://doi.org/10.1016/S0140-6736(08)61706-7.
Gogolla, N., Caroni, P., Lüthi, A., Herry, C., 2009. Perineuronal nets protect fear memories from erasure. Science 325, 1258–1261. https://doi.org/10.1126/science.1174146.
Grillault Laroche, D., Curis, E., Bellivier, F., Nepost, C., Courtin, C., Etain, B., Marie-Claire, C., 2020. Childhood maltreatment and HPA axis gene expression in bipolar disorders: a gene network analysis. Psychoneuroendocrinology 120, 104753. https://doi.org/10.1016/j.psyneuen.2020.104753.
Guirado, R., Perez-Rando, M., Ferragud, A., Gutierrez-Castellanos, N., Umemori, J., Carceller, H., Nacher, J., Castillo-Gómez, E., 2020. A critical period for prefrontal network configurations underlying psychiatric disorders and addiction. Front. Behav. Neurosci. 14. https://doi.org/10.3389/fnbeh.2020.00051.
Hannon, E., Lunnon, K., Schalkwyk, L., Mill, J., 2015. Interindividual methylomic variation across blood, cortex, and cerebellum: implications for epigenetic studies of neurological and neuropsychiatric phenotypes. Epigenetics 10, 1024–1032. https://doi.org/10.1080/15592294.2015.1100786.
Heim, C., Binder, E.B., 2012. Current research trends in early life stress and depression: review of human studies on sensitive periods, gene–environment interactions, and epigenetics. Exp. Neurol. Spec. Issue.: Stress Neurol. Dis. 233, 102–111. https://doi.org/10.1016/j.expneurol.2011.10.032.
Helmer, M., Warrington, S., Mohammadi-Nejad, A.-R., Ji, J.L., Howell, A., Rosand, B., Anticevic, A., Sotiropoulos, S.N., Murray, J.D., 2021. On stability of canonical correlation analysis and partial least squares with application to brain-behavior associations. bioRxiv. https://doi.org/10.1101/2020.08.25.265546.
Hensch, T.K., 2004. Critical period regulation. Annu. Rev. Neurosci. 27, 549–579. https://doi.org/10.1146/annurev.neuro.27.070203.144327.
Hooks, B.M., Chen, C., 2007. Critical periods in the visual system: changing views for a model of experience-dependent plasticity. Neuron 56, 312–326. https://doi.org/10.1016/j.neuron.2007.10.003.
Human Tissue Act, 2004. [WWW Document], 2004. URL ⟨http://www.legislation.gov.uk/ukpga/2004/30/pdfs/ukpga_20040030_en.pdf⟩ (Accessed 30 June 2019).
Jones, P.A., 2012. Functions of DNA methylation: islands, start sites, gene bodies and beyond. Nat. Rev. Genet. 13, 484–492. https://doi.org/10.1038/nrg3230.
Knudsen, E.I., 2004. Sensitive periods in the development of the brain and behavior. J. Cogn. Neurosci. 16, 1412–1425. https://doi.org/10.1162/0898929042304796.
Krause, B.J., Artigas, R., Sciolla, A.F., Hamilton, J., 2020. Epigenetic mechanisms activated by childhood adversity. Epigenomics. https://doi.org/10.2217/epi-2020-0042.
Lee, H.H.C., Bernard, C., Ye, Z., Acampora, D., Simeone, A., Prochiantz, A., Di Nardo, A. A., Hensch, T.K., 2017. Genetic Otx2 mis-localization delays critical period plasticity across brain regions. Mol. Psychiatry 22, 680–688. https://doi.org/10.1038/mp.2017.1.
Lokk, K., Modhukur, V., Rajashekar, B., Märtens, K., Mägi, R., Kolde, R., Koltšina, M., Nilsson, T.K., Vilo, J., Salumets, A., Tõnisson, N., 2014. DNA methylome profiling of human tissues identifies global and tissue-specific methylation patterns. Genome Biol. 15, r54. https://doi.org/10.1186/gb-2014-15-4-r54.
Lussier, A.A., Zhu, Y., Smith, B.J., Simpkin, A.J., Smith, A.D.A.C., Suderman, M.J., Walton, E., Ressler, K.J., Dunn, E.C., 2022. Updates to data versions and analytic methods influence the reproducibility of results from epigenome-wide association studies. Epigenetics 0, 1–16. https://doi.org/10.1080/15592294.2022.2028072.
Marini, S., Davis, K.A., Soare, T.W., Zhu, Y., Suderman, M.J., Simpkin, A.J., Smith, A.D. A.C., Wolf, E.J., Relton, C.L., Dunn, E.C., 2020. Adversity exposure during sensitive periods predicts accelerated epigenetic aging in children. Psychoneuroendocrinology 113, 104484. https://doi.org/10.1016/j.psyneuen.2019.104484.
Mauney, S.A., Athanas, K.M., Pantazopoulos, H., Shaskan, N., Passeri, E., Berretta, S., Woo, T.-U.W., 2013. Developmental pattern of perineuronal nets in the human prefrontal cortex and their deficit in schizophrenia. Biol. Psychiatry Schizophr.: Biomark. Risk Illn. Prog. 74, 427–435. https://doi.org/10.1016/j.biopsych.2013.05.007.
McLaughlin, K.A., Sheridan, M.A., Nelson, C.A., 2017. Neglect as a violation of species-expectant experience: neurodevelopmental consequences. Biol. Psychiatry 82, 462–471. https://doi.org/10.1016/j.biopsych.2017.02.1096.
Min, J.L., Hemani, G., Davey Smith, G., Relton, C., Suderman, M., 2018. Meffil: efficient normalization and analysis of very large DNA methylation datasets. Bioinformatics 34, 3983–3989. https://doi.org/10.1093/bioinformatics/bty476.
Parade, S.H., Huffhines, L., Daniels, T.E., Stroud, L.R., Nugent, N.R., Tyrka, A.R., 2021. A systematic review of childhood maltreatment and DNA methylation: candidate gene and epigenome-wide approaches. Transl. Psychiatry 11, 1–33. https://doi.org/10.1038/s41398-021-01207-y.
Relton, C.L., Gaunt, T., McArdle, W., Ho, K., Duggirala, A., Shihab, H., Woodward, G., Lyttleton, O., Evans, D.M., Reik, W., Paul, Y.-L., Ficz, G., Ozanne, S.E., Wipat, A., Flanagan, K., Lister, A., Heijmans, B.T., Ring, S.M., Davey Smith, G., 2015. Data resource profile: accessible resource for integrated epigenomic studies (ARIES). Int. J. Epidemiol. 44, 1181–1190. https://doi.org/10.1093/ije/dyv072.
Rogers, S.L., Rankin-Gee, E., Risbud, R.M., Porter, B.E., Marsh, E.D., 2018. Normal development of the perineuronal net in humans; in patients with and without epilepsy. Neuroscience 384, 350–360. https://doi.org/10.1016/j.neuroscience.2018.05.039.
Sharma, A., Campbell, J., Cardon, G., 2015. Developmental and cross-modal plasticity in deafness: Evidence from the P1 and N1 event related potentials in cochlear implanted children. Int. J. Psychophysiol. 95, 135–144. https://doi.org/10.1016/j.ijpsycho.2014.04.007.
Slopen, N., Shonkoff, J.P., Albert, M.A., Yoshikawa, H., Jacobs, A., Stoltz, R., Williams, D.R., 2016. Racial disparities in child adversity in the U.S.: interactions with family immigration history and income. Am. J. Prev. Med. 50, 47–56. https://doi.org/10.1016/j.amepre.2015.06.013.
Smith, B.J., Smith, A.D.A.C., Dunn, E.C., 2022. Statistical modeling of sensitive period effects using the structured life course modeling approach (SLCMA). Curr. Top. Behav. Neurosci. https://doi.org/10.1007/7854_2021_280.
Smith, K.E., Pollak, S.D., 2021. Rethinking concepts and categories for understanding the neurodevelopmental effects of childhood adversity. Perspect. Psychol. Sci. 16, 67–93. https://doi.org/10.1177/1745691620920725.
Sumner, J.A., Colich, N.L., Uddin, M., Armstrong, D., McLaughlin, K.A., 2019. Early experiences of threat, but not deprivation, are associated with accelerated biological aging in children and adolescents. Biol. Psychiatry 85, 268–278. https://doi.org/10.1016/j.biopsych.2018.09.008.
Takesian, A.E., Hensch, T.K., 2013. Balancing plasticity/stability across brain development. In: Progress in Brain Research. Elsevier, pp. 3–34. https://doi.org/10.1016/B978-0-444-63327-9.00001-1.
Vialou, V., Feng, J., Robison, A.J., Nestler, E.J., 2013. Epigenetic mechanisms of depression and antidepressants action. Annu. Rev. Pharmacol. Toxicol. 53, 59–87. https://doi.org/10.1146/annurev-pharmtox-010611-134540.
Yang, R., Gautam, A., Getnet, D., Daigle, B.J., Miller, S., Misganaw, B., Dean, K.R., Kumar, R., Muhie, S., Wang, K., Lee, I., Abu-Amara, D., Flory, J.D., Hood, L., Wolkowitz, O.M., Mellon, S.H., Doyle, F.J., Yehuda, R., Marmar, C.R., Ressler, K.J., Hammamieh, R., Jett, M., 2021. Epigenetic biotypes of post-traumatic stress disorder in war-zone exposed veteran and active duty males. Mol. Psychiatry 26, 4300–4314. https://doi.org/10.1038/s41380-020-00966-2.
Zhu, Y., Wang, M.-J., Crawford, K.M., Ramírez-Tapia, J.C., Lussier, A.A., Davis, K.A., de Leeuw, C., Takesian, A.E., Hensch, T.K., Smoller, J.W., Dunn, E.C., 2022. Sensitive period-regulating genetic pathways and exposure to adversity shape risk for depression. Neuropsychopharmacol 47, 497–506. https://doi.org/10.1038/s41386-021-01172-6.

# Supplemental Materials

## Blood-brain correlations

We performed correlation analyses between DNAm levels in blood and brain at the 530 CpG sites included in the current study, annotated to the promoter regions of the sensitive period-regulating genes. The strength of blood-brain correlations was comparable across the three gene sets, with the average correlation being slightly higher in the *expression* gene set. Further, results from IMAGE-CpG (http://han-lab.org/methylation/default/imageCpG), a web-based application for interrogations of DNAm levels in the human brain and peripheral tissues (Braun et al., 2019), suggested 64% of the analyzed CpGs had positive blood-brain correlations, with 7.8% showing strong correlations (Spearman's $\rho \geq 0.6$), spanning 28 out of the 58 genes examined the current study (**Figure S5**).

## Power analysis

To understand the statistical power of these analyses, we performed *post hoc* simulation-based power calculations of the linear discriminant analysis (LDA). In brief, because LDA can be considered equivalent to canonical correlation analysis (CCA; Bach and Jordan, n.d.), we used a recently-developed software tool (created after our analysis began), *gemmr*, to generate multivariate correlated data for CCA (Helmer et al., 2021). Prior studies suggest statistical power and stability of CCA-type analyses depend heavily on the number of samples per feature, with recommended numbers ranging between 10 and 70 (Barcikowski and Stevens, 1975; Helmer et al., 2021). In our analyses, the number of samples per feature was approximately 10 for the *closing* gene set, 25 for the *opening* gene set, and 44 for the *expression* gene set.

We performed simulations separately for each gene set. Data were generated with the same number of observations as our analytic sample (n=785) and the number of features was set to correspond to the actual number in each gene set. We varied the canonical correlation from 0.1 to 0.5, corresponding to a wide range of effect sizes. The prevalence of exposure was set to range from 5% to 25%, consistent with the observed time-varying prevalence across adversity in our study.

As shown in **Figure S4**, the estimated statistical power decreased when the gene set size increased and when the exposure prevalence decreased. Estimated power was the lowest for the *closing* gene set analysis, given the highest number of genes in that pathway. For an analysis to achieve at least 80% power to detect an effect comparable to the magnitude observed in our analyses (a canonical $R^2$ around 0.09), the exposure prevalence would need to be 10% or above for the *opening* gene set, 20% or above for the *closing* gene set, and at least between 5% and 10% for the *expression* gene set. For an exposure present in only 5% of the sample, the canonical $R^2$ would need to be approximately 0.15 or above for the analysis to be sufficiently powered.

**References**

Bach, F.R., Jordan, M.I., n.d. A Probabilistic Interpretation of Canonical Correlation Analysis 11.

Barcikowski, R.S., Stevens, J.P., 1975. A Monte Carlo Study of the Stability of Canonical Correlations, Canonical Weights and Canonical Variate-Variable Correlations. Multivariate Behavioral Research 10, 353–364. https://doi.org/10.1207/s15327906mbr1003_8

Braun, P.R., Han, S., Hing, B., Nagahama, Y., Gaul, L.N., Heinzman, J.T., Grossbach, A.J., Close, L., Dlouhy, B.J., Howard, M.A., Kawasaki, H., Potash, J.B., Shinozaki, G., 2019. Genome-wide DNA methylation comparison between live human brain and peripheral tissues within individuals. Translational Psychiatry 9, 47. https://doi.org/10.1038/s41398-019-0376-y

Helmer, M., Warrington, S., Mohammadi-Nejad, A.-R., Ji, J.L., Howell, A., Rosand, B., Anticevic, A., Sotiropoulos, S.N., Murray, J.D., 2021. On stability of Canonical Correlation Analysis and Partial Least Squares with application to brain-behavior associations. bioRxiv. https://doi.org/10.1101/2020.08.25.265546

## Supplementary Tables

Table S1. Full list of CpG sites included in the current study (n=530) and their identified EWAS catalog entries.

*The table has been included as an excel spreadsheet. Please see the file attached.*

Table S2. Associations between time-dependent exposure to childhood adversity and DNA methylation in gene body regions of three gene sets known to regulate sensitive period functioning in the brain.

| Childhood adversity | Sensitive period selected | Canonical $R^2$ | Wilks' lambda | Wilks' p-value | Bootstrap p-value |
|---|---|---|---|---|---|
| ***Opening genes*** | | | | | |
| Family instability | Very early childhood (0-2 years) | 0.04 | 0.96 | 0.206 | 0.508 |
| Caregiver physical or emotional abuse | Early childhood (3-5 years) | 0.05 | 0.95 | 0.045 | 0.127 |
| Maternal psychopathology | Early childhood (3-5 years) | 0.04 | 0.96 | 0.141 | 0.340 |
| Sexual or physical abuse | Middle childhood (6-7 years) | 0.05 | 0.95 | 0.075 | 0.216 |
| Financial hardship | Middle childhood (6-7 years) | 0.04 | 0.96 | 0.293 | 0.608 |
| One adult in the household | Middle childhood (6-7 years) | 0.03 | 0.97 | 0.735 | 0.960 |
| Neighborhood disadvantage | Middle childhood (6-7 years) | 0.05 | 0.95 | 0.105 | 0.257 |
| ***Closing genes*** | | | | | |
| Sexual or physical abuse | Very early childhood (0-2 years) | 0.10 | 0.90 | 0.211 | 0.522 |
| Neighborhood disadvantage | Very early childhood (0-2 years) | 0.09 | 0.91 | 0.456 | 0.781 |
| Caregiver physical or emotional abuse | Early childhood (3-5 years) | 0.10 | 0.90 | 0.303 | 0.629 |
| Financial hardship | Middle childhood (6-7 years) | 0.11 | 0.89 | 0.190 | 0.449 |
| One adult in the household | Middle childhood (6-7 years) | 0.10 | 0.90 | 0.345 | 0.658 |
| Family instability | Middle childhood (6-7 years) | 0.11 | 0.89 | 0.164 | 0.430 |
| Maternal psychopathology | Middle childhood (6-7 years) | 0.11 | 0.89 | 0.143 | 0.347 |
| ***Expression genes*** | | | | | |
| One adult in the household | Very early childhood (0-2 years) | 0.03 | 0.97 | 0.277 | 0.559 |
| Neighborhood disadvantage | Very early childhood (0-2 years) | 0.03 | 0.97 | 0.312 | 0.625 |
| Maternal psychopathology | Early childhood (3-5 years) | 0.03 | 0.97 | 0.249 | 0.535 |
| Sexual or physical abuse | Middle childhood (6-7 years) | 0.03 | 0.97 | 0.127 | 0.331 |
| Financial hardship | Middle childhood (6-7 years) | 0.03 | 0.97 | 0.397 | 0.747 |
| Family instability | Middle childhood (6-7 years) | 0.03 | 0.97 | 0.104 | 0.286 |
| Caregiver physical or emotional abuse | Middle childhood (6-7 years) | 0.04 | 0.96 | 0.032 | 0.098 |

Bootstrap p-values accounted for the number of hypotheses tested per gene set and adversity (i.e., three sensitive periods). No association corresponded to a bootstrap p-value under 0.05.

Table S3. Associations between any exposure to each type of childhood adversity before age 7 and DNA methylation in promoter regions of three gene sets regulating sensitive period functioning.

| Childhood adversity | Canonical $R^2$ | Wilks' lambda | Wilks' p-value |
|---|---|---|---|
| ***Opening genes*** | | | |
| **Sexual or physical abuse** | **0.06** | **0.94** | **0.041** |
| Financial hardship | 0.04 | 0.96 | 0.658 |
| One adult in the household | 0.03 | 0.97 | 0.882 |
| Family instability | 0.03 | 0.97 | 0.881 |
| Neighborhood disadvantage | 0.04 | 0.96 | 0.532 |
| Caregiver physical or emotional abuse | 0.03 | 0.97 | 0.755 |
| Maternal psychopathology | 0.05 | 0.95 | 0.286 |
| ***Closing genes*** | | | |
| Sexual or physical abuse | 0.11 | 0.89 | 0.212 |
| Financial hardship | 0.08 | 0.92 | 0.867 |
| One adult in the household | 0.09 | 0.91 | 0.549 |
| Family instability | 0.11 | 0.89 | 0.209 |
| Neighborhood disadvantage | 0.07 | 0.93 | 0.879 |
| Caregiver physical or emotional abuse | 0.08 | 0.92 | 0.771 |
| Maternal psychopathology | 0.10 | 0.90 | 0.202 |
| ***Expression genes*** | | | |
| Sexual or physical abuse | 0.01 | 0.99 | 0.851 |
| Financial hardship | 0.03 | 0.97 | 0.243 |
| One adult in the household | 0.02 | 0.98 | 0.707 |
| Family instability | 0.03 | 0.97 | 0.258 |
| Neighborhood disadvantage | 0.02 | 0.98 | 0.554 |
| Caregiver physical or emotional abuse | 0.01 | 0.99 | 0.886 |
| Maternal psychopathology | 0.02 | 0.98 | 0.784 |

Because only one hypothesis was tested for each type of adversity, no bootstrapping was performed. Bolded value indicated associations with $p<0.05$.

Table S4. Associations between presence versus absence of exposure to any of the seven types of adversity during each time period and DNA methylation in promoter regions of three gene sets regulating sensitive period functioning.

| Gene set | Sensitive period selected | Canonical $R^2$ | Wilks' lambda | Wilks' p-value |
|---|---|---|---|---|
| Opening | Middle childhood (6-7 years) | 0.06 | 0.94 | 0.229 |
| Closing | Middle childhood (6-7 years) | 0.12 | 0.88 | 0.294 |
| Expression | Very early childhood (0-2 years) | 0.03 | 0.97 | 0.173 |

At each time period, an individual was coded as exposed if they were exposed to any type of childhood adversity.

Table S5. CpG-level results of loci with FDR q-value < 0.2 located in promoters of sensitive period genes, using the structured life course modeling approach (SLCMA) performed by Lussier et al. (2020).

| Gene set | Gene name | CpG | SLCMA p-value | Adversity | Selected hypothesis | FDR q-value[a] | $R^2$ |
|---|---|---|---|---|---|---|---|
| expression | KCNK2 | cg11346522 | 1.78E-04 | Neighborhood disadvantage | Very early childhood (1.75 years) | 0.094 | 0.022 |
| closing | MMP3 | cg16466334 | 4.03E-04 | Family instability | Early childhood (4.75 years) | 0.148 | 0.020 |
| expression | GRIN2A | cg06922606 | 6.24E-04 | Family instability | Middle childhood (6.75 years) | 0.148 | 0.018 |
| closing | MBP | cg26457248 | 8.38E-04 | Family instability | Early childhood (4.75 years) | 0.148 | 0.018 |

[a] FDR corrections were applied to correct for looking up results at 530 CpG sites. Lussier et al. assessed associations between time-varying exposures to the same seven types of childhood adversity as the current study and genome-wide DNA methylation (DNAm) at 440,257 CpG sites using the SLCMA, which simultaneously compares competing hypotheses for the mechanisms of exposures.

## Supplementary Figures

Figure S1. Prevalence of exposure to childhood adversity at each developmental time period before age 7 in the analytic sample (n=785).

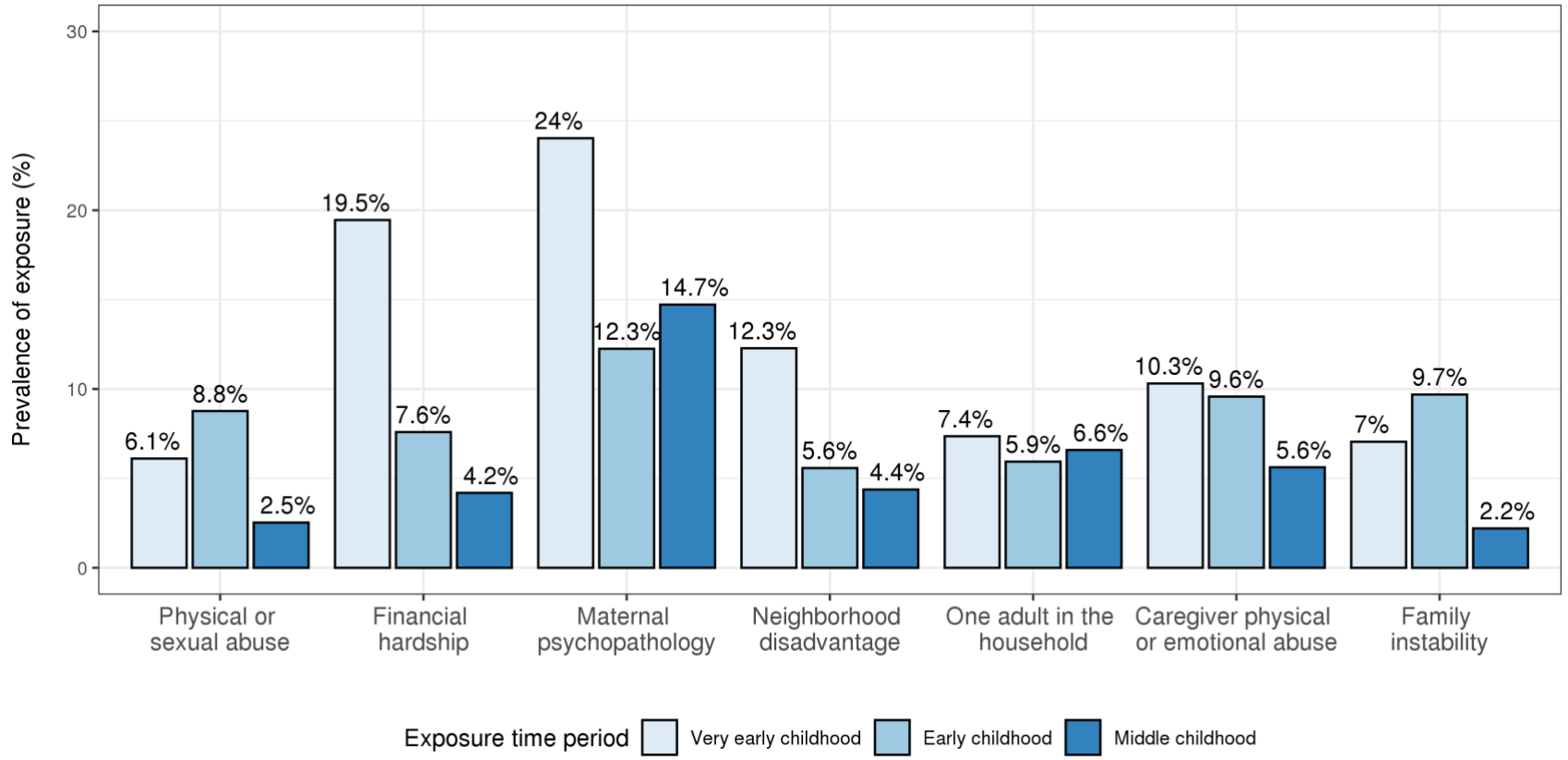


Figure S2. Tetrachoric correlations between exposures at different time periods within each type of childhood adversity before age 7 in the analytic sample (n=785). Time periods considered: very early childhood (0-2 years); early childhood (3-5 years); middle childhood (6-7 years).

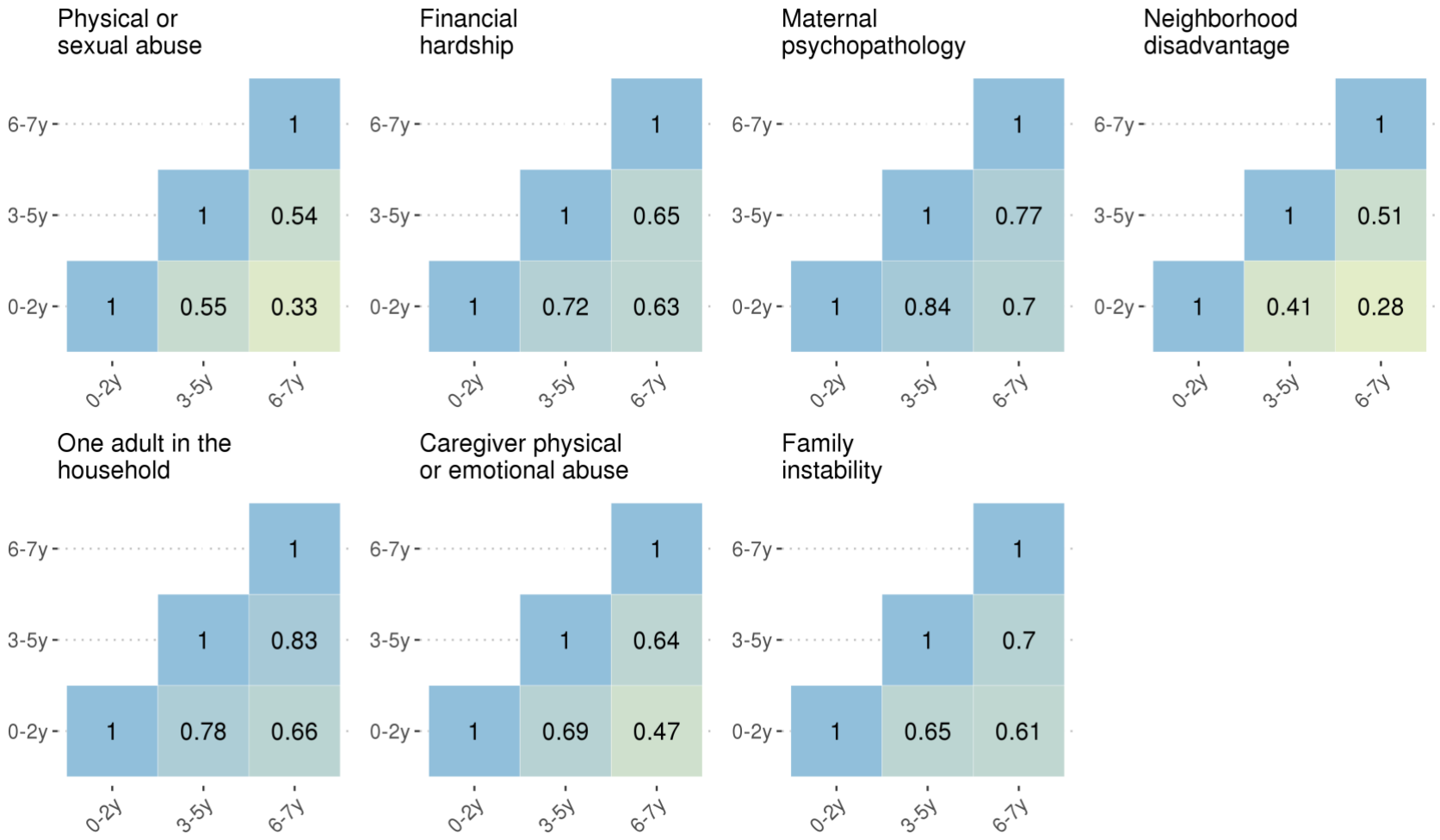


Figure S3. Tetrachoric correlations between exposures between different types of childhood adversity before age 7 within each time period in the analytic sample (n=785). Time periods considered: very early childhood (0-2 years); early childhood (3-5 years); middle childhood (6-7 years).

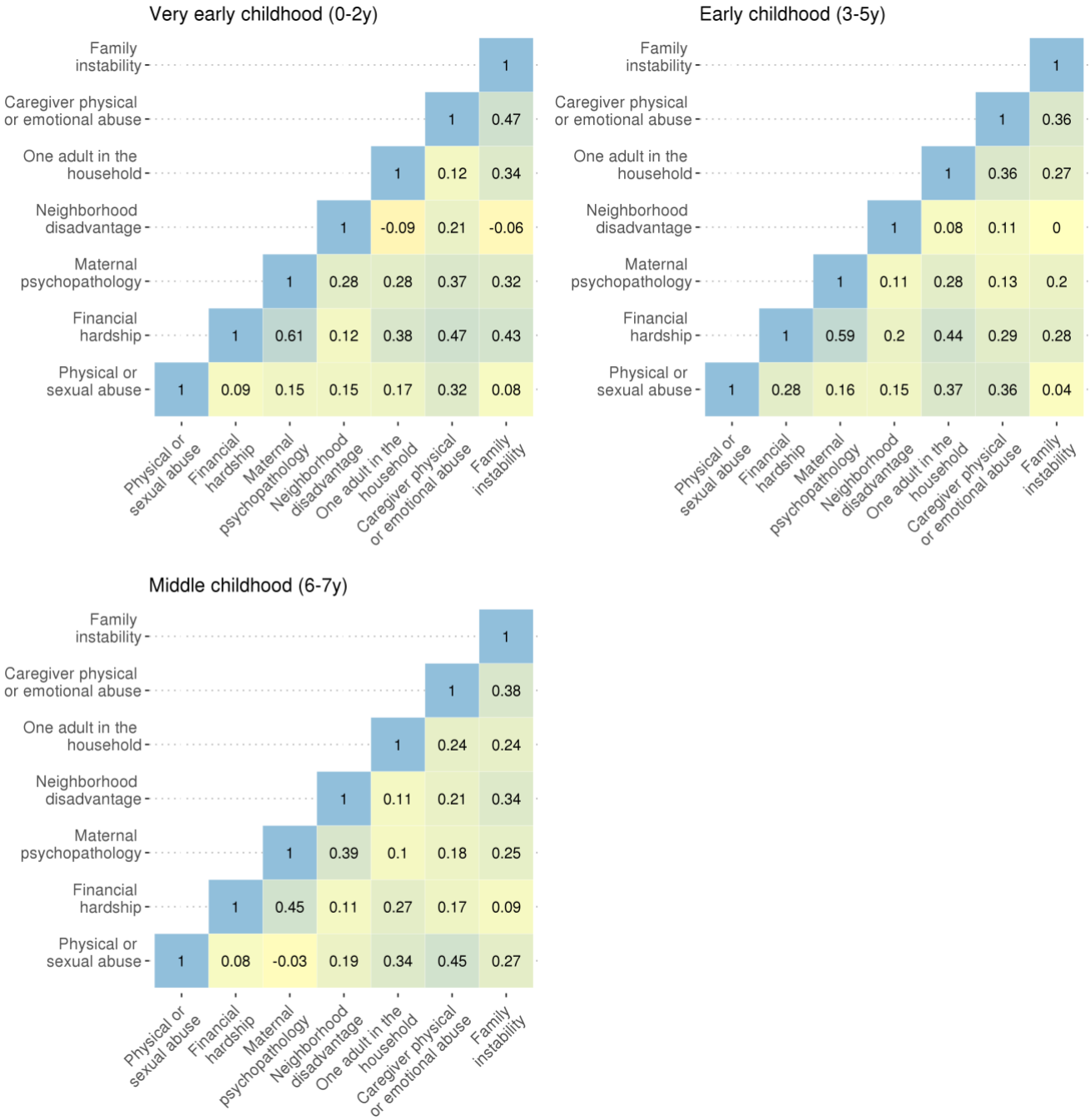


Figure S4. Simulation-based power analysis. Purple horizontal dotted line indicates 80% statistical power. Brown vertical dotted indicates a correlation of 0.3, or a canonical $R^2$ of 0.09, which was comparable to effects observed in our analyses.

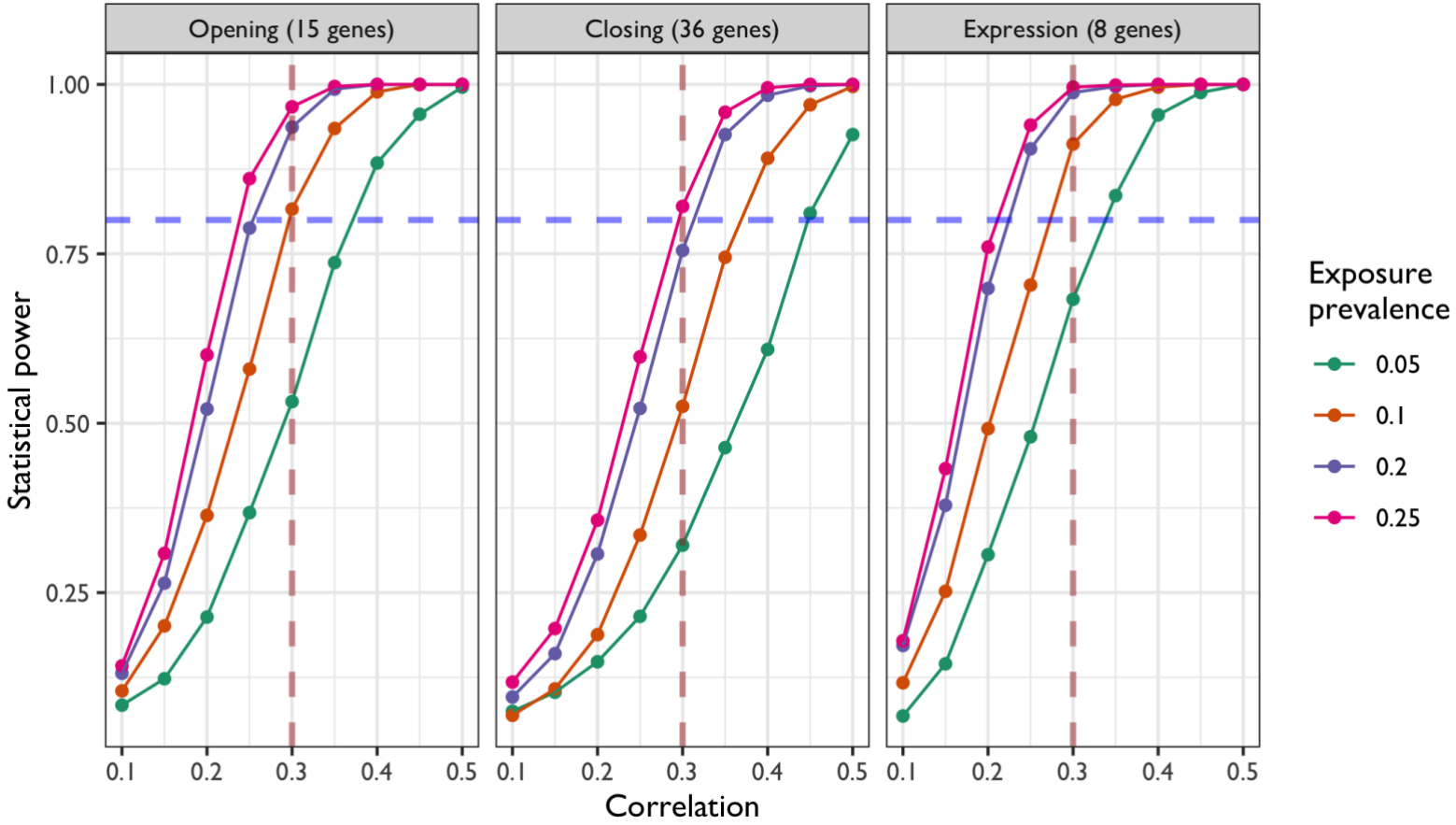
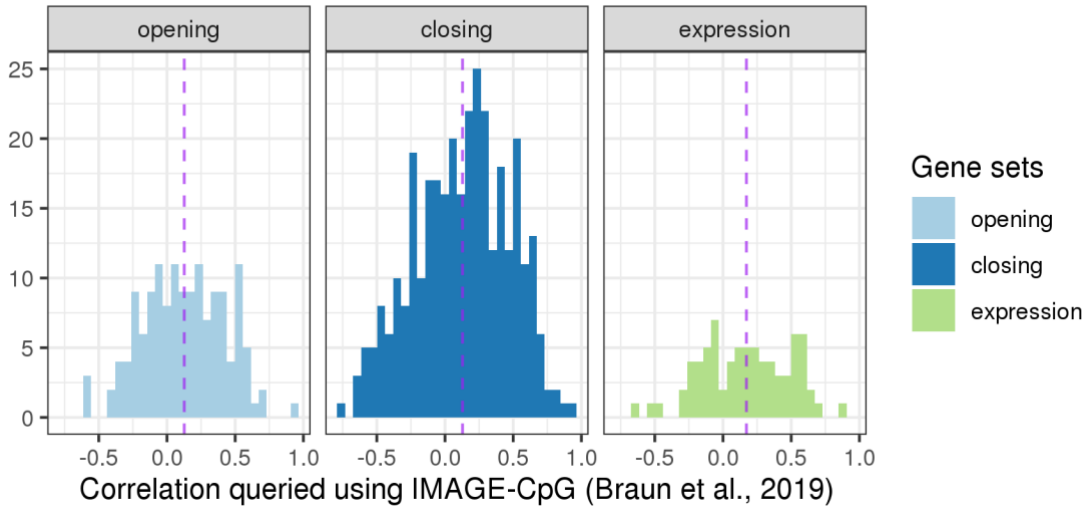


Figure S5. Correlation coefficient distributions for DNA methylation between brain and blood tissues for CpGs in promoters of sensitive period genes (data from Braun et al., 2019).




*Note.* A total of 527 CpGs are shown in the plot, annotated to 58 unique genes. The average correlation coefficients corresponding to CpGs in each gene set are: $r$=0.13 for opening genes; $r$=0.13 for closing genes; $r$=0.17 for expression genes, as indicated by the purple dashed lines.