Adversity exposure during sensitive periods predicts accelerated epigenetic aging in children

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Abstract

Objectives: Exposure to adversity has been linked to accelerated biological aging, which in turn has been shown to predict numerous physical and mental health problems. In recent years, measures of DNA methylation-based epigenetic age—known as "epigenetic clocks"—have been used to estimate accelerated epigenetic aging. Although a small number of studies have found an effect of adversity exposure on epigenetic age in children, none have investigated if there are "sensitive periods" when adversity is most impactful.

Methods: Using data from the Avon Longitudinal Study of Parents and Children (ALSPAC; n=973), we tested the prospective association between repeated measures of childhood exposure to seven types of adversity on epigenetic age assessed at age 7.5 using the Horvath and Hannum epigenetic clocks. With a Least Angle Regression variable selection procedure, we evaluated potential sensitive period effects.

Results: We found that exposure to abuse, financial hardship, or neighborhood disadvantage during sensitive periods in early and middle childhood best explained variability in the deviation of Hannum-based epigenetic age from chronological age, even after considering the role of adversity accumulation and recency. Secondary sex-stratified analyses identified particularly strong sensitive period effects. These effects were undetected in analyses comparing children "exposed" versus "unexposed" to adversity. We did not identify any associations between adversity and epigenetic age using the Horvath epigenetic clock.

Conclusions: Our results suggest that adversity may alter methylation processes in ways that either directly or indirectly perturb normal cellular aging and that these effects may be heightened during specific life stages.

Keywords: sensitive periods; epigenetic clock; aging; ALSPAC; adversity

Highlights

- Exposure to adversity was associated with accelerated epigenetic aging in childhood.
- Associations were observed when using the Hannum but not Horvath epigenetic clock.
- Effects were driven by exposure during early and middle childhood sensitive periods.
- Adversity differentially affected epigenetic age acceleration in boys and girls.

1. Introduction

Exposure to childhood adversity, such as abuse or poverty, represents one of the most potent risk factors for a range of negative health outcomes across the lifespan, with estimates linking such exposures to at least a two-fold increase in subsequent risk for mental disorders (Dunn et al., 2012; McLaughlin et al., 2010). Although these associations are well-established, the specific mechanisms through which adversity becomes biologically embedded remain poorly understood.

Accumulating evidence suggests adversity may become biologically embedded through accelerated aging of cells, tissues, and organs (Gassen et al., 2017; Zannas et al., 2015). Accelerated biological aging, in which biological age outpaces chronological age, is known to be a valid indicator of impaired functionality of both the cell and the biological system in which the cell interacts (Teschendorff et al., 2013).

Recently, DNA methylation (DNAm) patterns at specific CpG sites have been proposed as a promising measure of biological aging. These DNAm-based measures are referred to as "epigenetic clocks" due to their remarkably high correlation with chronological age (Hannum et al., 2013; Horvath, 2013). Two independent algorithms developed to generate these DNAm-based age estimates are the Horvath clock (Horvath, 2013) and the Hannum clock (Hannum et al., 2013). Both clocks can be used to capture accelerated epigenetic aging, which represents the discrepancy between the estimate of epigenetic age based on DNAm patterns and an individual's chronological age (Hannum et al., 2013; Horvath, 2013). In adults, accelerated epigenetic aging as measured by these epigenetic clocks has been correlated with numerous adverse health outcomes (Breitling et al., 2016; Dhingra et al., 2018), including increased mortality risk (Marioni et al., 2016). These epigenetic clocks have been shown to reliably correlate with chronological age in younger populations as well (Horvath et al., 2016; Simpkin et al., 2017); accelerated epigenetic aging in children and adolescents has been associated with both more advanced growth and development and increased youth mental health problems (Suarez et al., 2018a; Sumner et al., 2018).

A handful of recent studies have explored how exposure to adversity influences epigenetic aging in adulthood (Brody et al., 2016; Fiorito et al., 2017; Lawn et al., 2018; Simons et al., 2016; Wolf et al.,

2017; Zannas et al., 2015). These studies have shown that individuals who have perceived subjective high levels of stress across their lifetimes (Zannas et al., 2015), including exposure to sexual abuse (Lawn et al., 2018), a parent's mental illness (Brody et al., 2016; Davis et al., 2017), or chronic financial stress (Simons et al., 2016), have epigenetic ages that outpace their chronological age. One recent meta-analysis quantified this age acceleration, showing that any exposure to childhood trauma was associated with an epigenetic "outpace" of as much as 6 months (when epigenetic age was estimated with Hannum's, but not with Horvath's clock) (Wolf et al., 2017).

However, to our knowledge, only two studies—both of which are cross-sectional—have investigated these associations in children. In one study of youth ages 6-13 years, children who were at least one standard deviation epigenetically older than their peers were found to score twice as high on a measure of lifetime violence exposure (Jovanovic et al., 2017). A more recent study of youth ages 8-16 years reported that each childhood experience of threat (e.g., abuse, domestic violence) was associated with approximately one additional month of epigenetic age acceleration (Sumner et al., 2018).

Although evidence from these studies suggests a link between adversity exposure and accelerated aging, most of this work has primarily focused on one or two types of adversity, as opposed to a range of possible exposure types. As noted, previous studies investigating adversity-induced epigenetic aging in children have also all been limited to cross-sectional designs, rather than studies using prospective assessment of adversity exposure. Furthermore, to our knowledge, no studies have examined the importance of the timing of adversity exposure. Given the growing body of support for "sensitive periods" in development, during which time developing organs, tissues, and biological systems may be particularly susceptible to the effects of experience (Bornstein, 1989; Knudsen, 2004; Shonkoff et al., 2009), consideration of the timing of adversity across the life course is warranted. Indeed, a recent study found that the effects of childhood adversity on epigenetic patterns were largely driven by *when* the adversity occurred, with the period from birth to age 3 emerging as a sensitive period when exposure to adversity was associated with more epigenetic changes (Dunn et al., 2019). Importantly, a standard epigenome-wide association study of lifetime adversity exposure (versus no exposure) failed to detect

these associations (Dunn et al., 2019). Findings like these emphasize the need to investigate not only the biological consequences of adverse experiences, but also the possibility of time-dependent effects that may be obscured by simple exposed vs. unexposed models.

In the current study, we aimed to address these limitations and test the central hypothesis that postnatal adversity exposure does have an accelerating effect on epigenetic age in childhood, and that these effects may be strongest and most detectable during sensitive periods in development. Investigating sensitive periods may not only help to reveal otherwise undetectable time-dependent effects, but it may also help to identify "high risk/high reward" periods in development, when adversity exposure can be most potent but health-promoting interventions might be most impactful.

2. Methods

2.1. Study Overview

We tested three consecutive hypotheses. We first assessed the independent associations between a set of postnatal adversity exposures and accelerated epigenetic age at age 7.5, regardless of the timing of exposure. Second, given the previously described evidence from epigenetic studies that simple classification of individuals as exposed versus unexposed to adversity may dilute observed effects (Dunn et al., 2019), we then tested—for each adversity type—a *sensitive period model*, which posits that the developmental timing of exposure is most important in shaping accelerated aging (Bailey et al., 2001; Knudsen, 2004). Third, recognizing that there are other ways to conceptualize time-dependent effects, we then compared the sensitive period model to two alternative theoretical models derived from life course theory (Ben-Shlomo and Kuh, 2002; Kuh and Ben-Shlomo, 2004): an *accumulation model*, which posits that every additional year of exposure is associated with an increased risk for accelerated aging (Evans et al., 2013; Sameroff, 2000), and a *recency* model, which suggests that the effects of adversity can be time-limited, and thus accelerated epigenetic aging may be more strongly linked to proximal rather than distal events (Shanahan et al., 2011). Finally, we performed two secondary analyses focused on using a broader set of age ranges to define sensitive periods and understanding sex-specific effects.

2.2. Sample and Procedures

We analyzed data from the Avon Longitudinal Study of Parents and Children (ALSPAC), a large population-based birth cohort out of Avon, England of children followed from before birth through early adulthood (Boyd et al., 2013; Fraser et al., 2012; Golding et al., 2001). ALSPAC generated blood-based DNAm profiles at age 7.5 as part of the Accessible Resource for Integrated Epigenomics Studies (ARIES), which is a subsample of 1,018 mother-child pairs from ALSPAC who had complete data across at least five waves of data collection (Relton et al., 2015) (**Supplemental Materials).**

2.3. Measures

2.3.1. *Cellular Aging*

DNAm was determined at age 7.5 using procedures performed at the University of Bristol (**Supplemental Materials).** Using the level of methylation for each child in the sample, we generated two estimates of cell intrinsic epigenetic age based on the approaches of Horvath (Horvath, 2013) and Hannum (Hannum et al., 2013). For each clock, we estimated age acceleration using a regression procedure in which epigenetic age was the outcome and chronological age was the independent variable. In both the Horvath and Hannum epigenetic clocks, age acceleration or deceleration is represented by the residuals of the above described regression procedures (Wolf et al., 2016). Positive residuals indicate accelerated aging, in which the child's chronological age is lower than their estimated methylation age (hereafter referred to as accelerated aging).

2.3.2. *Exposure to Adversity*

We examined the effect of seven adversities on methylation age residuals: (a) caregiver physical or emotional abuse; (b) sexual or physical abuse (by anyone); (c) maternal psychopathology; (d) one adult in the household; (e) family instability; (f) financial hardship; and (g) neighborhood disadvantage/poverty. These adversity types were chosen based on previous research (Dunn et al., 2019;

Lawn et al., 2018) linking these exposures to epigenetic change (Brody et al., 2016; Lawn et al., 2018) or accelerated biological aging (Coimbra et al., 2017; Tyrka et al., 2010; Wojcicki et al., 2015). These adversity types were also chosen because they were each measured on at least four occasions at or before age 7 (see **Table 1**) from a single item or psychometrically validated standardized measures. To evaluate the effects of adversity exposure on epigenetic age without accounting for the timing of exposure, we created an "exposed" versus "unexposed" indicator for each adversity type, such that a child who was exposed to a particular adversity type at any time point was coded as "exposed" to that adversity. Second, for each type of adversity, we generated three sets of variables to test the three life course hypotheses: (a) for the *sensitive period hypothesis*, we created a set of variables indicating presence versus absence of the adversity at a specific developmental stage; specific time periods of assessment for each adversity are denoted in **Supplemental Table 2.** To test the (b) *accumulation hypothesis*, we generated a single variable denoting the total number of time periods of exposure to a given type of adversity. For the (c) *recency hypothesis*, we generated a single variable denoting the total number of developmental periods of exposure, with each exposure weighted by the age in months of the child during the measurement time period; this recency variable gave a larger weight to more recent exposures, thus, allowing us to determine whether more recent exposures were more impactful.

2.3.3. *Covariates*

We controlled for the following covariates, measured at child birth: child race/ethnicity; number of births in the pregnancy (pregnancy size); number of previous pregnancies; maternal marital status; highest level of maternal education; maternal age; maternal smoking during pregnancy; child birth weight; parental homeownership; and parent job status (**Supplemental Materials for rationale**).

2.4. Analyses

We began by running univariate and bivariate analyses to examine the distribution of covariates and exposures to adversity in the total analytic sample. To reduce potential bias and minimize loss of

power due to attrition, we performed multiple imputation on missing exposures and covariates (**Supplemental Materials**). Missing data for each adversity exposure and covariate are presented in **Supplemental Table 3**.

We first tested the association between lifetime adversity exposure and epigenetic age by testing a simple *ever versus never exposed* model for each adversity type. Expecting that this model could dilute any effects of adversity exposure on epigenetic aging, we then used a novel two-stage structured life course modeling approach (SLCMA) (Simpkin et al., 2015; Smith et al., 2016) to evaluate, separately for each adversity type, whether a sensitive period model might better explained the relationship between adversity exposure and epigenetic age. We also compared this sensitive period model to accumulation or recency of exposure models. Compared to other methods, such as standard multiple regression, the SLCMA provides an unbiased way to compare multiple competing theoretical models simultaneously and identify the most parsimonious explanation for variation in epigenetic age.

Details about the SLCMA modeling approach are outlined in the **Supplemental Materials**. Briefly, in the first stage of the SLCMA, we entered the set of variables described earlier into the Least Angle Regression (LARS) variable selection procedure (Efron et al., 2004). LARS identifies the single theoretical model (or potentially more than one models working in combination) that explains the most amount of outcome variation (in this case, epigenetic age acceleration). To identify these models, we used a covariance test (Lockhart et al., 2014) and examined elbow plots (**Supplemental Figure 1**). The covariance test provides a p-value for the selected variable, conditioned on the fact that LARS has selected the predictor with the largest correlation with the response. This approach resolves the common issue of "cherry-picking" when model fitting following selection. In the second stage, the life course theoretical models found in the first stage to best fit the observed data – that is, the model(s) appearing at the "elbow" of the plot (**Supplemental Figure 1**) and/or those with p-values <.05 in the covariance test (Lockhart et al., 2014) – were then carried forward to a multivariate regression framework to generate effect estimates for all selected hypotheses (**Supplemental Materials**). The goal of this second stage is to determine the contribution of a selected theoretical model after adjustment for covariates as well as other

selected theoretical models, in instances where more than one theoretical model is chosen in the first stage. Importantly, the SLCMA method takes multiple testing into account; 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 (**Supplemental Materials**). Thus, for each adversity, the testing of multiple competing lifecourse hypotheses *within* each SLCMA model is accounted for and the corresponding p-value is not inflated regardless of number of lifecourse hypotheses tested. Given the testing of multiple adversities across two epigenetic clocks, we additionally used a Bonferroni-adjusted significance threshold of p=.004 (0.05/7 adversities * 2 outcomes) to reduce the possibility of spurious results that may be incurred by multiple testing *across* 14 SLCMA models.

In addition, we also performed two sets of secondary analyses, which tested a broader definition of sensitive periods (**Supplemental Materials**) and the sex-specific effects of adversity on epigenetic age. These broader sensitive periods were defined as: *very early childhood* (ages 8 months – 2.75 years), *early childhood* (ages 3.5 – 5.75 years), and *middle childhood* (ages 6 – 7 years). These time windows were selected to facilitate interpretation of our findings in comparison to prior studies using similarly-defined developmental windows (Andersen et al., 2008; Dunn et al., 2018; Kaplow and Widom, 2007; Slopen et al., 2014).

3. Results

There were 973 children in the analytic sample (50.2% female, 97.2% white). Descriptive statistics on other covariates are presented in **Supplemental Table 4**.

3.1. Distribution of Exposure to Adversity and Age Acceleration

Table 1 shows the prevalence of childhood adversity overall and by each age period of assessment. The lifetime prevalence of adversity exposure ranged from 12.6% for physical abuse to 48.7% for family instability. Children exposed to any type of adversity were more likely than their

unexposed peers to be non-white and born to non-married mothers with low education, low social class, and with more than three previous pregnancies (**Supplemental Table 4**).

As shown in **Supplemental Table 4**, girls were, on average, epigenetically older than boys. Also, children born to married mothers with higher education and lower social class had lower age residuals (according to Hannum's epigenetic clock) compared to children whose mothers fell into other corresponding categories. No differences were observed for the remaining covariates (all p-values >.10) (**Supplemental Table 4**). **Supplemental Table 5** shows tetrachoric correlations between developmental time periods of exposure for each adversity. Exposures were moderately correlated across time, with neighboring time points generally being more highly correlated than distal time points (**Supplemental Table 5**). Different types of adversities showed low to moderate correlations (tetrachoric correlation coefficient *rho* ranged from 0.05 to 0.45; see S**upplemental Figure 2)**.

3.2. Association between Exposure to Adversity and Age Acceleration

We began with simple ever versus never exposed models for each adversity type. Based on these models, financial hardship was the only adversity associated with age acceleration (**Supplemental Tables 6 and 7**).

We then generated models that estimated the effects of the timing of exposure. **Table 2** displays, separately for each adversity type and epigenetic clock, the theoretical model selected by the LARS that best explained variability in age acceleration. As shown, evidence for three associations emerged for Hannum's epigenetic clock, all of which emphasized age acceleration following adversity exposure and the importance of sensitive periods. First, we found evidence that exposure to sexual or physical abuse at 3.5 years was associated with older epigenetic age (effect β=.07 years; 95% CI=.00-.14, p=.001, R^2=.01). Similarly, exposure to financial hardship at 7 years (effect β=.11, CI=.08-.14, p=.001, R^2=.05), and neighborhood disadvantage at 7 years (effect β=.12 years, CI=.01-.22, p=.001, R^2=.01) were associated with an acceleration in epigenetic aging. The magnitude of these beta estimates translates to an age acceleration of about one month among children exposed to adversity. None of the other life course

theoretical models were selected as explaining the variability in age acceleration for these three or any other adversity types. Using Horvath's epigenetic clock, none of the life course models were associated with epigenetic age acceleration for any of the adversities studied (**Table 2**). Of note, these effects survived correction for multiple testing both within the SLCMA and across the two clocks and adversities tested.

Comparable results were obtained when the sensitive periods were collapsed into three broader categories. In these secondary analyses, having only one adult in the household during early childhood (effect β=.06 years, CI=.02-.09, p=.002) and being exposed to maternal psychopathology in middle childhood (effect β=.03 years, CI=.06-.02, p=.023) were also associated with a modest acceleration in epigenetic age (**Supplemental Table 8**).

Sex-stratified analyses (**Table 3**) showed that for girls, having only one adult in the household (effect β=.10, CI=.002-.19, p=.030), or being exposed to maternal psychopathology (effect β=.06, CI=.02-.10, p=.0003), financial hardship (effect β=.008, CI=.004-.011, p<.0001), physical or emotional abuse (effect β=.08, CI=.006-.16, p=.027), or sexual abuse (effect β=.17, CI=.07-.27, p=.0004) was associated with increased epigenetic age. For example, by age 7.5, girls who were exposed to abuse at age 3.5 were biologically older than their unexposed peers by almost 2 months. In boys, exposure to financial hardship (effect β=.12, CI=.08-.16, p<.0001) and neighborhood disadvantage (effect β=.10, CI=.002-.20, p=.0005) were associated with increased epigenetic age. Each of these associations showed sensitive period specificity.

4. Discussion

This study tested the hypothesis that adversity exposure during sensitive periods in development is associated with accelerated epigenetic aging in childhood as measured by two epigenetic clocks, and that these associations can be better detected using methods that account for exposure timing, rather than simple comparisons of exposed versus unexposed individuals. To allow for the possibility of other timing effects, we also compared sensitive period models to alternative theoretical life course models of

exposure. To our knowledge, this study represents the first to prospectively investigate whether the effects of adversity on epigenetic aging are observable in children and the extent to which these relationships varies as a function of the timing and type of exposure.

We found that exposure to sexual or physical abuse in early childhood and exposure to financial hardship or neighborhood disadvantage in middle childhood were all associated with epigenetic age acceleration by about one month. We acknowledge that the incremental variance explained was limited, but this estimate of effect is consistent with previous literature (Horvath and Raj, 2018). It is also worth noting that the R^2 values reported do not represent the percentage of variation in age acceleration explained by a particular adversity exposure, but rather the percentage of variation in age acceleration that is explained by a *given lifecourse theoretical model* of exposure, *after* accounting for any variance explained by covariates.

Our findings are also consistent with previous work linking adversities, such as abuse (Lawn et al., 2018), financial stress (Simons et al., 2016), and parental psychopathology (Brody et al., 2016; Lawn et al., 2018), with accelerated epigenetic aging in adulthood. Although the literature to date on the association between social environmental exposures and epigenetic aging in children is limited, the observed associations here between abuse experiences and accelerated epigenetic aging align with recent studies on the epigenetic consequences of violence exposure (Jovanovic et al., 2017; Sumner et al., 2018).

Our results extend previous findings by exploring the effects of the timing of prospectively-assessed exposure. We found evidence for sensitive periods during early and middle childhood, when the association between adversity exposure and epigenetic aging appears to be particularly strong. This finding aligns with human (Essex et al., 2013; McGowan et al., 2009) studies showing the importance of sensitive periods in epigenetic programming. It seems therefore plausible that the epigenetic age of cells is influenced by environmental inputs in a similar time-susceptibility manner. The current findings further emphasize the importance of attending to possible time-dependent effects when studying the effects of adversity on cellular aging, including DNAm and other cellular-based measures of accelerated aging. Our results suggest that an approach that does not account for the specific life stages when adversity occurs

may fail to detect effects of adversity on epigenetic age acceleration, and crude classifications of children as exposed vs. unexposed to "early life" adversity may mask observed differences among those exposed to adversity.

The sex-stratified analyses revealed that adversity could differentially affect epigenetic age acceleration in boys and girls. Some of these associations were particularly notable; for example, by age 7.5, girls who were exposed to abuse at age 3.5 were biologically older than their unexposed peers by almost 2 months. These findings suggest that the associations found in our main analyses may have been largely driven by the strength of the effect in girls. Our sex-stratified results are also consistent with previous findings indicating sex-specific effects in the patterning of epigenetic marks following prenatal (Suarez et al., 2018b) and childhood adversity (Essex et al., 2013; Massart et al., 2016), and underscore the value of sex-stratification in future analyses.

Disentangling the multiple possible mediational pathways driving these associations is challenging given the complex environmental contributions that comprise early life stress (Tyrka et al., 2013). One possible pathway may be through disrupted immune functioning, which has been implicated in a range of mental disorders (Misiak et al., 2019). Human post-traumatic stress disorder studies have found that adversity exposure may activate hypothalamic–pituitary–adrenal axis and disrupt neural-immune signaling (Agorastos et al., 2019). In keeping with this theory, a recent meta-analysis using data from more than 2,000 individuals from the Psychiatric Genomics Consortium PTSD Epigenetics Workgroup concluded that traumatic stress was associated with accelerated epigenetic aging in adulthood, and that cells integral to immune system maintenance and responsivity might play an important role in pacing the epigenetic clock (Wolf et al., 2017). Of note, this association was observed for childhood but not lifetime trauma exposure, suggesting the unique impact of adversity exposures occurring earlier in development.

In the current study, we did not find an association between exposure to the studied adversities and Horvath's epigenetic clock. Although a recent cross-sectional study by Sumner et al. found an association between threat-related experiences and Horvath-based estimates of accelerated epigenetic aging (Sumner et al., 2018), there are multiple possible explanations for this discrepancy. In comparison to the Sumner et

al. paper, our study population comprised a different racial and ethnic make-up and used different covariates. Moreover, our study also used distinct adversity types (such as physical abuse), rather than collapsing across types to create summed adversity exposure scores. Consistent with our results, other studies using both the Horvath and Hannum clocks have found that associations may exist for one clock, but not for another (Wolf et al., 2016; Wolf et al., 2017). The Horvath and Hannum models differ in the tissue and age of subjects used to develop them, and the sets of CpG sites used are largely different as well. An increasing body of literature suggests that the two clocks may in fact be suited to capture different aspects of biology, with the two clocks showing only modest correlation across disease phenotypes (Lu et al., 2018). Together, these factors may account for the observed difference in results between the two epigenetic clocks in our study.

4.1. Strengths and Limitations

There are several strengths of the current study. We performed a more inclusive and detailed assessment of adversity types; most research in the field to date has focused on single types of adversity exposure, such as parental depression or low socioeconomic status only. Moreover, we also incorporated different life course theoretical models of adversity exposure, thereby allowing us to investigate which temporal features of exposure are most strongly associated with epigenetic aging. Finally, most studies to date have focused on older samples, often with a median chronological age above 45 years (Simpkin et al., 2016), whereas the current study focused on epigenetic aging in children.

Our study had limitations. First, our findings are based on DNA extracted from blood, which may be limiting as patterns of epigenetic change following social environmental stress exposure have been found to be tissue-specific, such that the same individual may have different Horvath's epigenetic clock estimates for different tissues (Levine et al., 2016). Therefore, we cannot exclude the possibility that childhood adversities affect cell methylation in a tissue-specific pattern and that peripheral blood-based measures of DNAm may not capture methylation changes of all tissues that occur following adversity. As others have noted (Tyrka et al., 2016), however, although leukocytes cannot be assumed to provide a clear

window into brain-based processes, they may be particularly interesting, given their susceptibility to widespread effects, such as glucocorticoid and immune signaling. The challenge of tissue and cell-type specificity is unfortunately a limitation of all epigenome-brain research in living human subjects. Second, given the scope and scale of ALSPAC (which contains more than 75,000 variables), we needed to select and operationalize discrete adversity types for analysis. To do this in a principled manner, we looked to the previous literature both in ALSPAC (Dunn et al., 2019; Lawn et al., 2018) and in other cohorts (Brody et al., 2016; Coimbra et al., 2017; Tyrka et al., 2010; Wojcicki et al., 2015) to guide our selection of the seven adversity types used in our analysis, focusing on standard scales and single item measures that were asked in consistent ways across the duration of the study. However, we were unable to include in our analysis other likely distressing adverse experiences, such as death of a parent, due to low prevalence of exposure in the current sample. Future research should investigate epigenetic aging following other serious adverse experiences in higher risk samples where this and other exposures would be more commonly recorded or consistently queried. Third, although the focus of the current study was limited to postnatal adversity exposures, future research should consider incorporating prenatal measures as well, particularly given evidence of the association between prenatal exposure to stressors like maternal psychopathology and epigenetic age at birth (Suarez et al., 2018b). Fourth, given the structure of the data and the lack of complete overlap in adversity assessment across time, we were unable to examine the adversities all together in the primary analyses. Although the correlations between adversities were low to moderate, it is nevertheless possible that attending to only one adversity type at a time could lead to overestimates of the effect of a given exposure. However, the results of a sensitivity analysis that examined mutually adjusted effects suggested that while the strength of associations was slightly attenuated, the overall patterns of associations remained similar (**Supplemental Materials**). One potential strength of examining each type of adversity individually is that we were able to identify meaningful differences in the associations between distinct adversity types and accelerated aging, which could yield different approaches for intervention. One challenge for future analyses will be to develop new ways to examine multiple adversities simultaneously without simply summing across number of exposures

(McLaughlin and Sheridan, 2016). Fifth, although we used multiple imputation in an effort to reduce potential bias and minimize loss of power, we cannot rule out the possibility that missing or incomplete outcome data due to attrition may have influenced our findings. Sixth, because the oldest sensitive period coincides with the most recent exposure occasion for all children, it may be difficult to discern between the oldest sensitive period and recent exposure. Seventh, we focused exclusively on adversity exposures and did not consider potentially positive environmental influences. Future research should consider both adverse and protective exposures. Finally, although we selected covariates (such as maternal smoking and child birthweight) that are routinely adjusted for in analyses of epigenetic aging (Simpkin et al., 2017, 2016), some of these covariates may be associated with downstream factors that could fall along the pathway between adversity and childhood epigenetic aging and therefore inadvertently capture effects of potential mediators. Thus, the described effects may be attenuated.

Conclusions

In conclusion, we found that adversity experiences assessed in very early, early, and middle childhood were differentially associated with accelerated epigenetic aging at age 7.5. These findings suggest that accelerated epigenetic aging may function as one of the mechanisms through which childhood adversity becomes biologically embedded, and that adversity exposures during sensitive periods in childhood may have a particularly strong accelerating effect on epigenetic age. Future research leveraging repeated methylation measurements will be necessary to identify the varied trajectories of this acceleration across development, in the hopes of further teasing apart potential sensitive period effects from non-linearity in the ticking rate of the epigenetic clock (Horvath and Raj, 2018). Additional research is also needed to further test the effect of accelerated cellular aging on subsequent risk for depression and other neuropsychiatric disorders. Nevertheless, understanding the biological sequelae of childhood adversity—and how those sequelae differ depending on sensitive periods in exposure—represents the first step towards the development of targeted strategies designed to disrupt the processes linking adversity to psychiatric diseases as early in the life course as possible.

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Authors' Contributions

SM, ECD, TWS, ADACS and EJW designed the study. TWS, MJS, ADACS and CRL produced the data. Statistical analyses were performed by SM, TWS, YZ and AJS. SM, KAD and ECD wrote the manuscript. All authors revised the manuscript critically and approved it for submission.

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Table 1. Exposure to childhood adversity in the total analytic sample and by the age at exposure (n=973)

	Caregiver physical or emotional abuse		Sexual or physical abuse (by anyone)		Family instability		Maternal psychopathology		Financial hardship		One adult in the household		Neighborhood disadvantage	
	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)
Unexposed	822	84.5	850	87.4	499	51.3	686	70.5	669	68.8	833	85.6	829	85.2
Exposed	151	15.5	123	12.6	474	48.7	287	29.5	304	31.2	140	14.4	144	14.8
Age at Exposure														
Very early childhood														
8 months	34	3.7	---	---	---	---	95	10.2	104	11.3	243	3.7	---	---
1.5/1.75 yrs	38	4.2	28	3	170	18.2	89	9.8	98	10.7	288	4.3	76	8.4
2/2.75 yrs	56	6.3	32	3.6	182	20.3	130	14.8	97	10.9	350	5.2	74	8.4
Early childhood														
3.5 yrs	---	---	36	4.0	186	20.5	114	12.9	140	14.5	---	---	---	---
4/4.75 yrs	41	4.6	35	3.9	118	13.2	---	---	---	---	410	6.9	---	---
5/5.75 yrs	57	6.4	24	2.7	114	13.0	---	---	---	---	---	---	55	6.2
Middle childhood														
6/6.75 yrs	50	5.7	23	2.6	69	7.8	130	14.9	---	---	---	---	---	---
7 yrs	---	---	---	---	---	---	---	---	121	12.5	504	7.6	43	4.9

Table 2. Results of LARS models showing the life course theoretical model that best explained the relationship between adversity and age acceleration (n=973)

Adversity	Hannum's clock			Horvath's clock		
	Model selected	p-value	Improvement R^2	Model selected	p-value	Improvement R^2
Caregiver physical or emotional abuse	sensitive period (5 years)	.11	0.004	sensitive period (5 years)	.11	<0.001
Sexual or physical abuse	sensitive period (3.5 years)	**.0013**	**0.009**	sensitive period (4.75 years)	.99	<0.001
Maternal psychopathology	sensitive period (6 years)	.07	0.004	sensitive period (2.75 years)	.89	<0.001
One adult in the household	sensitive period (4 years)	.09	0.003	sensitive period (7 years)	.21	<0.001
Family instability	sensitive period (1.5 years)	.93	<0.001	sensitive period (6.75 years)	.98	<0.001
Financial hardship	sensitive period (7 years)	**<.0001**	**0.05**	sensitive period (7 years)	.79	<0.001
Neighborhood disadvantage	sensitive period (7 years)	**0.0002**	**0.01**	sensitive period (7 years)	.68	<0.001

Models are based on multiply imputed data and are adjusted for sex, race, maternal smoking, birth weight, maternal education, pregnancy size, maternal marital status, home ownership, age of mother at child birth, parental job status, and number of previous pregnancies. Values that are statistically significant are denoted in bold. The Bonferroni-adjusted significance threshold is P=.004.

The R^2 values reported do not show the variance in age acceleration explained by a particular adversity exposure. Rather, the R^2 values generated using the SLCMA show the percentage of variation in the *residuals* of the outcome explained by *particular lifecourse theoretical model* of a particular adversity exposure. Thus, the R^2 values reported here can be interpreted as the percentage of variation in age acceleration that is explained by a given lifecourse theoretical model of exposure, after accounting for any variance explained by covariates.

Table 3. Results of LARS models showing the life course theoretical model that best explained the relationship between adversity and age acceleration, with Hannum's epigenetic clock, stratified by sex (n=973)

	Girls (n=488)			Boys (n=485)		
Adversities	Model selected	p-value	Improvement R^2	Model selected	p-value	Improvement R^2
Caregiver physical or emotional abuse	sensitive period (5 years)	**.027**	**0.012**	sensitive period (2.75 years)	.193	0.006
Sexual or physical abuse	sensitive period (3.5 years)	**.0004**	**0.027**	sensitive period (5.75 years)	.615	0.002
Maternal psychopathology	sensitive period (6 years)	**.0003**	**0.020**	sensitive period (1.75 years)	.578	0.002
One adult in the household	sensitive period (1.75 years)	.030	0.011	sensitive period (7 years)	.812	0.001
Family instability	sensitive period (4.75 years)	.923	<0.001	sensitive period (3.5 years)	.235	0.004
Financial hardship	recency	**<.0001**	**0.050**	sensitive period (7 years)	**<.0001**	**0.060**
Neighborhood disadvantage	sensitive period (7 years)	.108	0.008	sensitive period (7 years)	**.0005**	**0.022**

Models are based on multiply imputed data and are adjusted for sex, race, maternal smoking, birth weight, maternal education, pregnancy size, maternal marital status, home ownership, age of mother at child birth, parental job status, and number of previous pregnancies. Very early childhood=ages 8 months to 2.75 years. Values that are statistically significant are denoted in bold.

Supplemental Materials

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 (Boyd et al., 2012; Fraser et al., 2012; Paternoster et al., 2012). ALSPAC was designed to increase knowledge of the pathways to health across the lifespan, with an emphasis on genetic and environmental determinants. Approximately 85% of eligible pregnant women agreed to participate (N=14,541), and 76% of eligible live births (n=14,062) who were alive at 12 months 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. Please note that the study website contains details of all the data that is available through a fully searchable data dictionary (http://www.bris.ac.uk/alspac/researchers/data-access/data-dictionary/).

Several studies have assessed the representativeness of the ALSPAC cohort with regards to the total Avon area population, and the whole of Great Britain (Boyd et al., 2013; Fraser et al., 2012). Although the study population has been found to be broadly similar to the total Avon area population and to the rest of Great Britain, ALSPAC mothers generally had a higher socio-economic position (as indicated by fewer mothers being unmarried, not having a car, or living in rented accommodation) in comparison to the equivalent population of either Avon or Britain (Fraser et al., 2012). ALSPAC mothers were also more likely to be white than equivalent women in both Avon and Britain (Fraser et al., 2012).

The ARIES mother-child pairs were randomly selected out of those with complete data across at least five waves of data collection. In comparison to ALSPAC mothers not participating in ARIES, ARIES mothers had, on average, a higher socio-economic position (as indicated by being more highly educated, having higher ranking occupations, and being more likely to be married and a homeowner). ARIES mothers were also more homogenously White, and less likely to have smoked during their pregnancies (**Supplemental Table 1**).

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.

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 seven time-points: child ages 1.5 years, 2.5 years, 3.5 years, 4.75 years, 5.75 years, 6.75. Reports of sexual or physical abuse were not reported to child welfare services, consistent with the lack of mandatory reporting laws in the UK (Bell and Tooman, 1994; Khan, 2018). Parents were also informed prior to responding that all of their responses were confidential.

Maternal psychopathology. Maternal psychopathology was determined using data from: (1) the Crown-Crisp Experiential Index (CCEI), which includes separate subscales for anxiety and depression (Birtchnell et al., 1988; Crown and Crisp, 1979); (2) the Edinburgh Postnatal Depression Scale (EPDS)(Cox et al., 1987); and (3) a question asking about suicide attempts in the past 18 months. 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 (Enoch et al., 2010) 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 (Crown and Crisp, 1979); (2) mother had a CCEI anxiety score greater than 10(Birtchnell et al., 1988); (3) mother had an EPDS score greater than 12 (Cox et al., 1987); 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 six 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 been: (1) taken into care; (2) separated from their mother for two or more weeks; (3) separated from their father for two or more weeks; or (4) acquired a new parent. These items were completed at seven time-points: when children were ages 1.5 years, 2.5 years, 3.5 years, 4.75 years, 5.75 years, 6.75 years. Children were coded as exposed if any of these events occurred.

Financial hardship. 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 slight difficulty for three or more items at each time point; this cut-point roughly corresponded to the top quartile.

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.

Cellular Aging

Peripheral blood samples (whole blood or buffy coat) were collected according to standard procedures at age 7. After DNA extraction, the Zymo EZ DNA MethylationTM kit (Zymo, Irvine, CA) was used for the bisulfite-conversion process. DNAm was determined using the Illumina Human Methylation 450k BeadChip microarray, which captures DNAm at 99% of RefSeq genes (over 485,000 CpG sites). All procedures were performed at the University of Bristol (Relton et al., 2015). The level of methylation is expressed as a 'beta' value (β-value), representing the proportion of cells methylated at each interrogated CpG site, and ranges from 0 (no methylated dinucleotides observed) to 1 (all dinucleotides methylated).

Using the β-values for each participant in the sample, we generated two estimates of epigenetic age based on the approaches of Horvath (Horvath, 2013) and Hannum (Hannum et al., 2013). For each clock, we estimated age acceleration using a regression procedure in which epigenetic age was the outcome and chronological age was the independent variable. To derive the Hannum clock, we followed a well-established procedure (Simons et al., 2016; Wolf et al., 2016; Wolf et al., 2017): we summed the normalized β-values using the Touleimat method (Touleimat and Tost, 2012) and multiplied these summed values by the 71 respective regression coefficients obtained by Hannum and colleagues in their model (20). This regression procedure adjusted for blood cell composition (specifically percentage of CD8+,CD4+,CD56, CD19,CD14, and granulocytes). For the Horvath clock, we used the online epigenetic clock calculator (http://labs.genetics.ucla.edu/horvath/dnamage/) which calculates the "intrinsic epigenetic age acceleration" derived by regressing the epigenetic age against chronological age, adjusting for cell counts (Chen et al., 2016). In both the Horvath and Hannum epigenetic clocks, age acceleration or deceleration is represented by the residuals of the above described regression procedures (Marioni et al., 2015; Wolf et al., 2016). Positive residuals indicate accelerated aging, in which the child's chronological age is lower than their estimated methylation age (hereafter referred to as accelerated aging). Conversely, negative residuals indicate age deceleration, in which the child's estimated methylation age is lower than their actual chronological age

Data Analysis

Covariates

Beyond the technical adjustments described above, we additionally controlled for the following variables, measured at child birth: *child race/ethnicity* (0=non-White; 1=White); *child birth weight* (<3000=0, 3000-3500 = 1, 3500-4000=2, >4000=4); *pregnancy size* (0=single; 1=multiple); *number of previous pregnancies* (between 0-3+); *maternal age* (0=ages 15-19, 1=ages 20-35, 2=age>35); *highest level of maternal education* (1=less than O-level, 2=O-level, 3=A-level, 4=Degree or above; in the UK except Scotland, the lower and the higher two main levels of standardized examinations in secondary schools are the O level and the A level respectively); *parent job status* (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); *homeownership* (0=mortgage/own home; 1=rent home; 2=other); and *sustained maternal smoking during pregnancy* (0=non-smoker; 1=smoker in two or more trimesters, including the third trimester) (Richmond, 2015).

Justification for the Inclusion of Baseline SES Variables as Covariates. In the current study, the following baseline socioeconomic status (SES) related variables were included as covariates in the primary analysis: parent job status, maternal education, and home ownership. While there is concern that these covariates conceptually overlap with some of the childhood adversity types treated as exposure (namely, financial hardship and neighborhood disadvantage), we included these variables as potential confounders for on two primary reasons.

First, different dimensions of SES are associated with childhood adversity and have distinct effects on health outcomes (Glymour et al., 2014); the effect of SES captured by the baseline variables is conceptually separate from financial hardship and neighborhood disadvantage. Abundant research evidence has shown 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 job status), tend to be more fixed or stable across time. Other dimensions tend to be less stable, such as indicators of financial hardship or neighborhood disadvantage, which vary 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 that this temporal variation requires the separate consideration of different domains of SES (Braveman et al., 2005; Duncan et al., 2002; Rehkopf et al., 2016), as they each could have different links to health outcomes. In the current study, controlling for baseline SES would help tease apart the effects of subjective levels of poverty or neighborhood disadvantage experienced by the children throughout development from a less variable status of social disadvantage as captured by baseline maternal education, home ownership, and parent social class. In fact, the correlations between baseline SES variables and the more dynamic aspects of SES (i.e., financial hardship and neighborhood disadvantage) are moderate to low in the analytic sample ($|r| \leq 0.31$ for parent job status, $|r| \leq 0.53$ for maternal education, and $|r| \leq 0.53$ for home ownership). Inclusion of parent social class thus allowed us to control for the baseline environment into which children were born and better capture the effects of perceived level of stress during childhood.

Second, baseline SES variables are plausible suspects for confounding the relationship between exposure to other types of childhood adversity and epigenetic aging and the estimate of these types of adversity on epigenetic aging may be biased without adjusting for baseline SES. Confounding has been traditionally defined based on associational criteria; in the past decade, researchers in the field of causal inference (see for example: Greenland et al., 1999; Pearl, 1998; VanderWeele and Shpitser, 2013) have emphasized alternative strategies such as greater use of causal diagrams and careful assessment of theoretical evidence. As discussed above, there is theoretical evidence for suspecting a link between baseline SES variables and various forms of childhood adversity. There has also been growing research revealing associations between different indicators of SES and epigenetic aging. As an example, Fiorito et al. (Fiorito et al., 2017) found an association between SES trajectories (defined using occupational position) and accelerated aging in three prospective cohorts. Furthermore, Simons et al. (Simons et al., 2016) used a similar approach to tease apart the effects of fixed and dynamic aspects of SES; they showed that low income was linked to

epigenetic age acceleration after adjusting for baseline SES variables such as education. As baseline SES variables may affect exposure to childhood adversity and epigenetic aging *independent* of the exposure to childhood adversity while not being a downstream effect of the exposure, we decided to include baseline SES variables as covariates to control for confounding.

Justification for the Inclusion of the Other Baseline Variables as Covariates. In addition to variables capturing socioeconomic statues, we have included other variables that are known to be associated with exposures to childhood adversity as well as epigenetic aging. Failure to include these variables may lead to biased estimates of the exposure-outcome relationship. Some of the factors are known to confound genetic and epigenetic analyses if not properly accounted for, such as child race or ethnicity. The prevalence of trauma and childhood adversity is patterned by race and ethnicity (Roberts et al., 2011), and the rates of epigenetic aging were different across race and ethnicity groups as well (Horvath et al., 2016).

Various factors related to pregnancy and the prenatal environment have also been linked to epigenetic changes and differential profiles of epigenetic aging in the offspring. Simpkin et al. systematically investigated associations between prenatal and antenatal experiences and accelerated aging using data from the same sample (i.e., ARIES) and a replication sample, and found that parity (or number of previous pregnancies), child birth weight, and maternal smoking during pregnancy were robustly related to accelerated aging (Simpkin et al., 2016). Other prior research has also provided suggestive evidence of links between other pregnancy related factors (such as maternal age and pregnancy size) and differences in epigenetic marks (Markunas et al., 2016). Characteristics of the prenatal and antenatal environment may also index adverse experiences in early life that are related to childhood adversity. Therefore, we adjusted for these factors in our analyses to ensure that the observed associations between childhood adversity and epigenetic aging were not induced by differences in children's experiences before or at birth.

In summary, we included a series of covariates in the analyses based on the conventions in the field as well as theoretical knowledge about potential confounding mechanisms.

Multiple Imputation

Although missing data were limited overall (**Supplemental Table 3**), to reduce potential bias and minimize loss of power due to attrition, we performed multiple imputation, separately for each exposure, using logistic regression in 20 datasets with 25 iterations each among all children with complete data on the outcome. Variables were included in the imputation models following the guidance of van Buuren and colleagues (van Buuren et al., 1999; van Buuren and Groothuis-Oudshoorn, 2011) as well as prior research with imputation in the ALSPAC dataset (Evans et al., 2012; Ramchandani et al., 2008). All covariates and exposures were allowed to enter the imputation models. Variables uncorrelated with the missing variable ($r<0.10$) were excluded from the imputation model (van Buuren et al., 1999; van Buuren and Groothuis-Oudshoorn, 2011). Imputation was performed with chained equations (Azur et al., 2011) with the mice package in R (van Buuren and Groothuis-Oudshoorn, 2011). To reduce noise in estimation of effect estimates, we did not impute the outcome (White et al., 2011). For each adversity, we assessed the convergence of the imputation model and the distribution of imputed data as compared to the observed data.

After imputation, there were 973 children in the analytic sample. We then achieved a single dataset for analysis by implementing LARS on the covariance structure among all variables, estimated by averaging the covariance structure across all multiply imputed datasets. This allowed us to avoid potential problems arising from different model selections across multiply imputed datasets (Wood et al., 2008).

Exposed vs. Unexposed Modeling

To explore the association between adversity exposure and epigenetic age without accounting for the timing of exposure, we began by running simple ever vs. never exposed models for each adversity type (**Supplemental Tables 6 and 7**).

LARS Regression Modeling

To explore the role of timing of exposure, for each type of adversity, we generated three sets of encoded variables: (1) a set of variables indicating presence vs. absence of the adversity at a

specific developmental 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 (coded as 0-6); and (3) a single variable denoting the total number of developmental periods of exposure, with each exposure linearly weighted by the age (in months) of the child during the measurement time period, to test the recency hypothesis; this variable assumed a linear increase in the effect of exposure over time and weighted more recent exposures more heavily than distally-occurring ones, allowing us to determine whether more recent exposures were more impactful. This weighted recency variable is distinguished from the last sensitive period model, which captures only the most recent exposure.

We then evaluated the relative importance of these variables using a two-stage structured lifecourse modeling approach (SLCMA) originally developed by Mishra (Mishra et al., 2009) for analyzing repeated, binary exposure data across the lifecourse. Relative to a more traditional regression model, the main advantage of the SLCMA is that it provides a structured and unbiased way to compare multiple competing theoretical models simultaneously and identify the most parsimonious explanation for the observed outcome variation.

In the first stage, we followed the approach of Smith (Simpkin et al., 2015) and entered the set of variables described previously into a Least Angle Regression (LARS) procedure (Efron et al., 2004) in order to identify, separately for each type of adversity, the single theoretical model (or potentially more than one models working in combination) that explained the most variability in child age acceleration. We used a covariance test (Lockhart et al., 2014) and examined elbow plots to determine whether the selected variables were supported by the ALSPAC data. The covariance test provides a p-value for the selected variable that accounts for the selective nature of the LARS procedure; in other words, the test statistic is conditioned on the fact that LARS has selected the predictor with the largest correlation with the response. Therefore, the common issue of "cherry-picking" of model fitting following selection is resolved. Compared to other variable selection procedures, including stepwise regression, the SLCMA has been shown to not over-inflate effect size estimates (Efron et al., 2004) or bias hypothesis tests (Lockhart et al., 2014). Compared to other methods for the structured approach, LARS has been shown to have greater statistical power and not bias subsequent stages of analysis (Simpkin et al., 2015). To adjust for potential confounding, we regressed each encoded variable on the covariates and implemented LARS on the regression residuals (Smith et al., 2016).

In the second stage, the theoretical models determined by a covariance test p-value threshold of 0.05 in the first stage (which appeared before the elbow; see Figure 1) were carried forward to a single multiple regression framework, where measures of effect would have been estimated for all selected hypotheses. The goal of this second stage is to determine the contribution of a selected theoretical model after adjustment for covariates as well as other selected theoretical models, in instances where more than one theoretical model is chosen in the first stage. Of note, the p-value from the covariance test (i.e., adjusting for testing multiple models), instead of the p-value we would have obtained in a linear regression, is reported. As the covariance test does not provide a confidence interval (CI) directly, the CIs were calculated using a method proposed by Smith et al. (Simpkin et al., 2015), which accounts for the selective nature of the LARS as well and has been shown to have desirable coverage probability in simulations. Since the inference adjusts for the fact that selection has already been performed in the same sample, over-fitting is not of concern in the current study.

Secondary Analysis

Two additional sets of analyses were performed following the primary analyses described above. First, to explore the possibility that a broader definition of sensitive periods would yield comparable results, and to facilitate interpretation of our findings in comparison to prior studies (Andersen et al., 2008; Dunn et al., 2018; Kaplow and Widom, 2007; Slopen et al., 2014), we re-analyzed our data focusing on three sensitive periods: very early childhood (ages 8 months – 2.75 years); early childhood (ages 3.5 – 5.75 years); and middle childhood (ages 6 – 7 years) (**Supplemental Table 8**). These time periods have been used to define potential sensitive periods in previous studies by our research group (Dunn et al., 2017; Dunn et al., 2018) and others (Andersen et al., 2008; Kaplow and Widom, 2007; Slopen et al., 2014).

Second, we performed sex-stratified secondary analyses, given that adversity exposure (Koenen et al., 2010) varies between males and females and males overall have higher IEAA than females ((Horvath et al., 2016) (**Table 3**).

Sensitivity Analysis: Adjusting for Exposure to Other Types of Adversity

In the primary analyses, we examined the time-dependent effects of exposure to childhood adversity on epigenetic aging. The results suggested that exposure to sexual or physical abuse at 3.5 years, exposure to financial hardship at 7 years, and exposure to neighborhood disadvantage at 7 years were each independently associated with an acceleration in epigenetic aging. While examining individual types of childhood adversity in separate regression models allowed us to identify and interpret meaningful differences in the associations between distinct adversity types and accelerated aging, we recognize the possibility that the observed effects may be partially explained by other types of adversity, since different types of adverse experiences likely co-occur.

We were unable to test this hypothesis in the primary analyses due to the limitations of LARS/LASSO modeling. Although all exposures to adversity were repeatedly measured, the LARS/LASSO modeling approach currently does not allow for inclusion of time-varying covariates. It was therefore impossible to adjust for other forms of exposure appropriately without running into the issue of inappropriately adjusting for a mediator. For example, for financial hardship to confound the relationship between the association between sexual or physical abuse and epigenetic aging, it would have to occur before *all* occasions of exposure to sexual or physical abuse; otherwise, it could mediate the relationship and hence adjusting for it would lead to bias towards the null (Schisterman et al., 2009). Thus, due to the nature of model selection in the SLCMA, we included all encoded variables in the model simultaneously.

However, one alternative approach is to further assess the effects of the selected hypotheses from the SLCMA in a multiple linear regression framework while preserving the temporality of exposures, which we performed here as a sensitivity analysis. Briefly, for each of the three selected hypotheses (sexual or physical abuse at 3.5 years, exposure to financial hardship at 7 years, and exposure to neighborhood disadvantage at 7 years), we fitted a linear regression model adjusting for *any* exposure to other types of adversity strictly before the time point of the selected hypothesis (i.e., not concurrent exposure). All other baseline covariates in the primary analyses were also included. As shown in **Supplemental Figure 3**, the point estimates of the effects were relatively stable before and after adjusting for exposure to other adversity that preceded the examined the hypothesis. The confidence intervals were tightened for exposure to sexual or physical abuse and neighborhood disadvantage with the additional adjustment. There was no statistical evidence for an effect of exposure to other types of adversity. Therefore, we concluded that although the observed effects may be attenuated after adjusting for other adversity, the general patterns of effects remained the same.

We would like to note that there are a few limitations to this alternative approach. First, because the linear regression approach did not take model selection into account, the inference may not be valid and should not be interpreted independent of the primary analyses. Second, although we attempted to preserve temporality by only adjusting for other types of exposure that occurred before the selected hypothesis in each model, the underlying causal relationships among these factors are unclear — different types of adversity may work jointly and interactively to affect outcomes such as epigenetic aging. Third, although the aggregate measure of any other exposure that we included may be too crude and the confounding mechanism may be both adversity- and time point-specific, we were unable to test all possibilities given the number of possible combinations and our sample size.

In conclusion, we emphasize the need to focus on overall patterns of associations between time-dependent effects of exposure to childhood adversity and epigenetic aging. An important goal of future analyses will be to develop new ways to examine multiple adversities simultaneously, taking different causal mechanisms into consideration (McLaughlin and Sheridan, 2016).

Supplemental Tables

Supplemental Table 1. Distribution of covariates in the ARIES and ALSPAC samples			
	ARIES (n=973)	ALSPAC (n=15445)	p-value (χ2 test)
Female (%)	488 (50.2)	7152 (48.7)	0.356
White (%)	911 (97.2)	11488 (94.9)	0.001
Maternal smoking during pregnancy (%)	99 (10.7)	2577 (21.2)	<0.001
Birth weight, g (%)			<0.001
<3000	128 (13.4)	2760 (20.0)	
3000 - 3499	346 (36.2)	4924 (35.7)	
3500 - 3499	336 (35.2)	4382 (31.8)	
>= 4000	145 (15.2)	1735 (12.6)	
Maternal education (%)			<0.001
Less than O-level	154 (16.2)	3735 (30.0)	
O-level	325 (34.1)	4303 (34.6)	
A-level	279 (29.3)	2795 (22.5)	
Degree or Above	195 (20.5)	1603 (12.9)	
Maternal marital status (%)			<0.001
Never Married	118 (12.3)	2522 (19.2)	
Widowed/Divorced/Separated	49 (5.1)	787 (6.0)	
Married	791 (82.6)	9838 (74.8)	
Home ownership (%)			<0.001
Mortgage/own home	835 (88.3)	9579 (73.2)	
Rent home	92 (9.7)	3046 (23.3)	
Other	19 (2.0)	462 (3.5)	
Age of Mother at Child Birth (%)			<0.001
Ages 15-19	9 (0.9)	650 (4.6)	
Ages 20-35	866 (89.5)	12363 (88.4)	
Age >35	93 (9.6)	968 (6.9)	
Parental social class (%)			<0.001
Professional	175 (18.0)	1419 (9.6)	
Managerial and technical	377 (38.7)	4288 (29.0)	
Skilled, non-manual	204 (21.0)	2623 (17.8)	
Skilled, manual	54 (5.5)	909 (6.2)	
Semi-skilled, manual	18 (1.8)	270 (1.8)	
Unskilled, manual/other	145 (14.9)	5254 (35.6)	
Number of previous pregnancies (%)			0.01
0	439 (46.7)	5800 (44.7)	
1	346 (36.8)	4550 (35.0)	
2	119 (12.7)	1860 (14.3)	
3+	36 (3.8)	772 (5.9)	

Supplemental Table 2. Description of theoretical models used in the analysis, with exposure to abuse as an example			
Life course model tested	Definition	Variables	Specific variables entered into the LARS model

Sensitive period	A single timepoint at which there can be exposure to adversity. To test if a single adversity experience at a specific timepoint explains the most variance in epigenetic age residuals.	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)

Accumulation	Sum of the number of times exposed to a given type of adversity across all time periods. To test whether the cumulative impact of each adversity experience explains the most variance in epigenetic age residuals.	1	abuse_accumulation=count of the number of time periods exposed to abuse (range 0-6)
Recency	Sum of the number of times exposed to each adversity reported across all time periods, with each time period of exposure weighted by the age in years of the child during the exposure. To test if temporal proximity to adversity events explains the most variance in epigenetic age residuals.	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)

Supplemental Table 3. Missing data for each adversity exposure and covariate		
Variable	Timing of assessment	n (%) missing
maternal smoking during pregnancy	Baseline	52 (5.34)
child birth weight	Baseline	18 (1.85)
child race/ethnicity	Baseline	36 (3.7)
sex	Baseline	0 (0)
maternal age	Baseline	5 (0.51)
parent job status	Baseline	0 (0)
highest level of maternal education	Baseline	20 (2.06)
number of births in the pregnancy	Baseline	0 (0)
parental homeownership	Baseline	27 (2.77)
maternal marital status	Baseline	15 (1.54)
number of previous pregnancies	Baseline	33 (3.39)
caregiver physical or emotional abuse	8 months	45 (4.62)
	1.75 years	57 (5.86)
	2.75 years	79 (8.12)
	4 years	76 (7.81)
	5 years	77 (7.91)
	6 years	89 (9.15)
sexual or physical abuse (by anyone)	1.5 years	40 (4.11)
	2.5 years	78 (8.02)
	3.5 years	64 (6.58)
	4.75 years	79 (8.12)
	5.75 years	97 (9.97)
	6.75 years	91 (9.35)
maternal psychopathology	8 months	45 (4.62)
	1.75 years	66 (6.78)
	2.75 years	93 (9.56)
	5 years	89 (9.15)
	6 years	101 (10.38)
one adult in the household	8 months	47 (4.83)
	1.75 years	71 (7.3)
	2.75 years	88 (9.04)
	4 years	87 (8.94)
	7 years	91 (9.35)
family instability	1.5 years	40 (4.11)
	2.5 years	78 (8.02)
	3.5 years	64 (6.58)
	4.75 years	79 (8.12)
	5.75 years	97 (9.97)
	6.75 years	91 (9.35)
financial hardship	8 months	49 (5.04)
	1.75 years	60 (6.17)
	2.75 years	82 (8.43)
	5 years	5 (0.51)
	7 years	5 (0.51)
neighborhood disadvantage	1.75 years	72 (7.4)
	2.75 years	88 (9.04)
	5 years	88 (9.04)
	7 years	94 (9.66)

Supplemental Table 4. Distribution of covariates in the total sample and by exposure to any childhood adversity, and epigenetic age measures (n=973)												
	Total Sample		Exposure to any adversity				Hannum's clock age ^			Horvath's clock age ^		
	%	N	%	N	χ^2	*p*-value	Mean	SD	*p*-value#	Mean	SD	*p*-value#
Gender					1.41	.23			.04			.72
Boys	49.8	485	74.8	363			-0.010	0.136		-0.023	2.019	
Girls	50.2	488	78.3	382			0.010	0.157		0.023	2.000	
Race					4.71	.029			.88			.46
White	97.2	911	75.9	691			-0.008	0.094		-0.043	1.983	
Non-White	2.8	26	96.2	25			-0.003	0.138		0.246	1.801	
Maternal Smoking					0.82	.36			.74			.72
Smoker	10.7	99	80.8	80			0.005	0.139		0.030	2.047	
Non-smoking	89.3	822	76.1	626			-0.001	0.149		-0.046	1.701	
Birth weight category (in grams)					3.81	.28			.25			.30
<3000	13.4	128	80.5	103			0.014	0.176		0.238	1.991	
3000-3500	36.2	346	77.7	269			-0.012	0.127		0.051	2.134	
3500-4000	35.2	336	73.2	246			0.004	0.157		-0.137	1.928	
>4000	15.2	145	78.6	114			0.006	0.135		0.063	1.955	
Maternal Education*					10.41	.015			.05			.41
less than O-level	16.2	154	85.1	131			0.017	0.139		0.109	2.179	
O-level	34.1	325	72.0	234			0.005	0.157		-0.175	1.880	
A-level	29.3	279	76.3	213			-0.017	0.105		0.022	2.027	
Degree or Above	20.5	195	78.5	153			-0.012	0.136		0.035	1.957	
Pregnancy Size					0.61	.43			.65			.37
Single	99.8	971	76.5	743			0.000	0.147		0.003	2.009	
Multiple (2+)	0.2	2	99.9	2			0.004	0.002		-1.279	1.240	
Maternal Marital Status					29.70	<.001			<.001			.41
Never Married	12.3	118	91.5	108			0.013	0.147		0.186	2.366	
Widowed/Divorced/ Separated	5.1	49	95.9	47			0.070	0.263		-0.221	2.401	
Married	82.6	791	73.3	580			-0.009	0.121		-0.033	1.914	

Home Ownership					20.66	<.01			.18		.56
Mortgage/own home	88.3	835	74.4	621			-0.004	0.135		-0.036	2.015
Rent home	9.7	92	94.6	87			0.022	0.159		0.090	2.005
Other	2	19	89.5	17			-0.021	0.094		0.393	1.801
Age of Mother at Child Birth					2.85	.24			.06		<.001
Ages 15-19	0.9	9	100	9			0.103	0.132		2.195	2.733
Ages 20-35	89.5	866	76.9	666			-0.002	0.136		-0.068	1.930
Age >35	9.6	93	75.3	70			-0.011	0.136		0.33	2.470
Parental job status					12.45	.029			<.001		.14
Foreman	18	175	76	133			-0.012	0.119		0.045	1.916
Manager	38.7	377	76.4	288			-0.017	0.115		-0.161	1.883
Supervisor	21	204	69.6	142			0.009	0.166		-0.062	1.913
Lending Hand	5.5	54	83.3	45			0.008	0.011		0.335	2.402
Self-Employed	1.8	18	77.8	14			0.138	0.321		0.646	2.187
None of these	14.9	145	84.8	123			0.002	0.185		0.247	2.338
Number of previous pregnancies					9.29	.026			.44		.22
0	46.7	439	78.8	346			-0.003	0.136		0.048	2.035
1	36.8	346	71.7	248			0.000	0.152		-0.157	1.795
2	12.7	119	79	94			-0.015	0.092		0.251	2.532
3+	3.8	36	88.9	32			0.026	0.117		0.180	1.828

*See supplemental methods for categorization of maternal education. # t-tests and ANOVAs. ^ These represent the residuals of the regression procedure in which epigenetic age was the outcome and chronological age was the independent variable. Median (IQR) for Hannum's clock age: -0.031 (-0.091 - 0.05); Median (IQR) for Horvath's clock age: -0.12 (-1.29 – 1.10)

Supplemental Table 5. Tetrachoric correlations between developmental time periods exposure for each adversity

Caregiver physical or emotional abuse					
Age (years)	8 months	1.75	2.75	4	5
8 months					
1.75	.796				
2.75	.679	.788			
4	.590	.721	.765		
5	.552	.535	.622	.638	
6	.428	.471	.478	.553	.700

Sexual or physical abuse (by anyone)					
Age (years)	1.5	2.5	3.5	4.75	5.75
1.5					
2.5	.472				
3.5	.019	.279			
4.75	.297	.369	.653		
5.75	.366	.440	.572	.507	
6.75	.259	.359	.234	.443	.510

Maternal psychopathology				
Age (years)	8 months	1.75	2.75	5
8 months				
1.75	.697			
2.75	.577	.691		
5.08	.652	.643	.665	
6	.469	.553	.599	.707

One adult in the household				
Age (years)	8 months	1.75	2.75	4
8 months				
1.75	.908			
2.75	.811	.938		
4	.707	.859	.941	
7	.600	.793	.832	.814

Family instability					
Age (years)	1.5	2.5	3.5	4.75	5.75
1.5					
2.5	.558				
3.5	.543	.660			
4.75	.073	.197	.378		
5.75	.168	.260	.351	.515	
6.75	.136	.246	.251	.455	.647

Financial hardship				
Age (years)	8 months	1.75	2.75	5
8 months				
1.75	.472			
2.75	.019	.279		
5	.297	.369	.653	
7	.366	.440	.572	.507
Neighborhood disadvantage				
Age (years)	1.75	2.75	5	
1.75				
2.75	.758			
5	.760	.808		
7	.701	.782	.879	

Supplemental Table 6. Results of linear regression analysis of exposed vs. non-exposed on Hannum's epigenetic clock (n=973**)**

	Beta (years)	se	p-value	95% C.I.
Caregiver physical or emotional abuse	0.012	0.0145	.428	-0.018 - 0.041
Sexual or physical abuse	0.027	0.015	.078	-0.003 - 0.056
Maternal psychopathology	0.003	0.011	.812	-0.019 - 0.025
One adult in the household	0.029	0.017	.092	-0.005 - 0.063
Family instability	0.003	0.011	.748	-0.018 - 0.024
Financial hardship	**0.047**	**0.010**	**<.0001**	**0.027 - 0.068**
Neighborhood disadvantage	0.004	0.019	.822	-0.033 - 0.041

All the models are adjusted for the covariates measured at child birth specified in the manuscript (namely: child race/ethnicity; number of births in the pregnancy; number of previous pregnancies; maternal marital status; highest level of maternal education; maternal age; maternal smoking during pregnancy; child birth weight; parental homeownership; and parent job status)

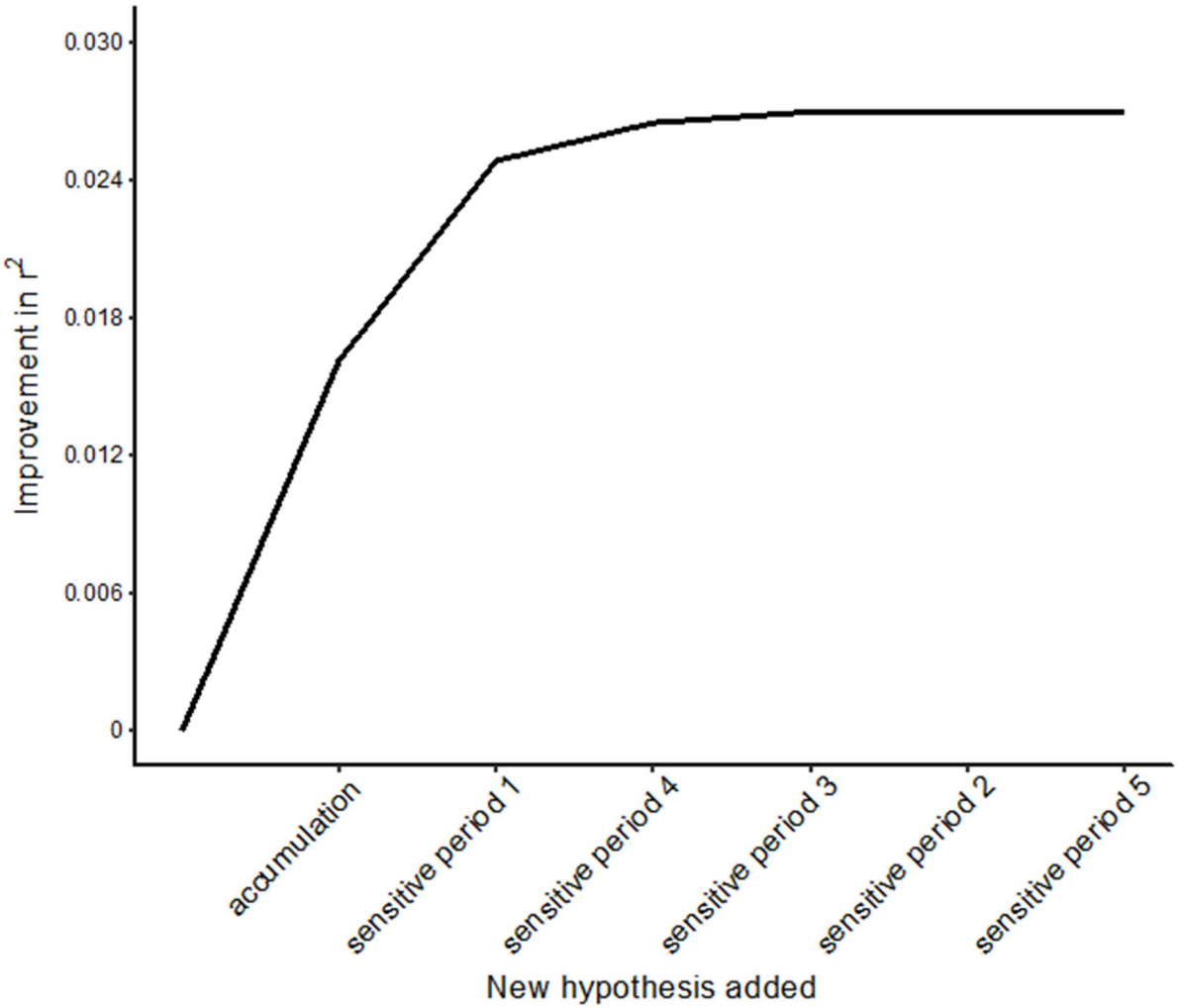
Supplemental Table 7. Results of linear regression analysis of exposed vs. non-exposed on Horvath's epigenetic clock (n=973)				
	Beta (years)	se	p-value	95% C.I.
Caregiver physical or emotional abuse	-0.030	0.181	.869	-0.386 - 0.326
Sexual or physical abuse	-0.120	0.201	.551	-0.515 - 0.275
Maternal psychopathology	-0.035	0.149	.814	-0.328 - 0.258
One adult in the household	0.098	0.194	.614	-0.283 - 0.480
Family instability	0.040	0.138	.771	-0.230 - 0.310
Financial hardship	0.095	0.148	.523	-0.196 - 0.385
Neighborhood disadvantage	-0.153	0.191	.424	-0.529 - 0.223

All the models are adjusted for the covariates measured at child birth specified in the manuscript (namely: child race/ethnicity; number of births in the pregnancy; number of previous pregnancies; maternal marital status; highest level of maternal education; maternal age; maternal smoking during pregnancy; child birth weight; parental homeownership; and parent job status)

Supplemental Table 8. Results of LARS models showing the life course theoretical model that best explained the relationship between adversity and age acceleration, using Hannum's and Horvath's clocks, with sensitive periods collapsed into three categories: very early, early, and middle childhood (n=973)

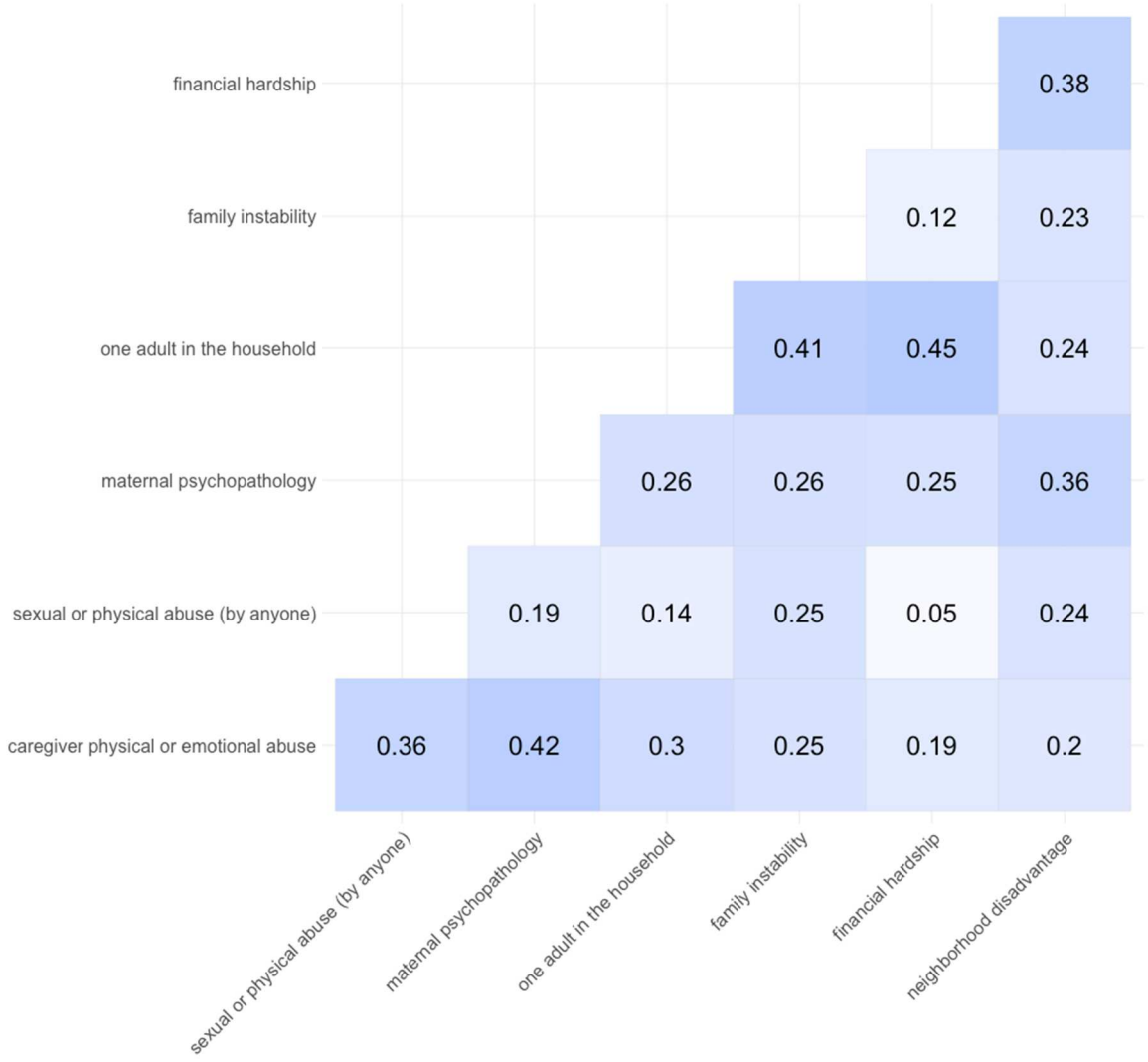
Adversity	Hannum's clock			Horvath's clock		
	Model selected	p-value	Improvement R^2	Model selected	p-value	Improvement R^2
Caregiver physical or emotional abuse	Exposure middle childhood	.5567	0.001	Exposure very early childhood	.7684	<0.001
Sexual or physical abuse	Exposure very early childhood	**.0038**	**0.007**	Exposure early childhood	.671	<0.001
Maternal psychopathology	Exposure middle childhood	**.0478**	**0.004**	Exposure very early childhood	.505	<0.001
One adult in the household	Exposure early childhood	**.038**	**0.004**	Exposure middle childhood	.121	0.002
Family instability	Exposure very early childhood	.814	<0.001	Exposure middle childhood	.393	0.001
Financial hardship	Exposure middle childhood	**<.0001**	**0.05**	Exposure middle childhood	.739	<0.001
Neighborhood disadvantage	Exposure middle childhood	**.012**	**0.01**	Exposure middle childhood	.260	0.001

Model fully adjusted for sex, race, maternal smoking, weight at birth, maternal education, pregnancy size, maternal marital status, home ownership, age of mother at child birth, parental job status, and number of previous pregnancies. Very early childhood=ages 8 months to 2.75 years. Values that are statistically significant are denoted in bold.



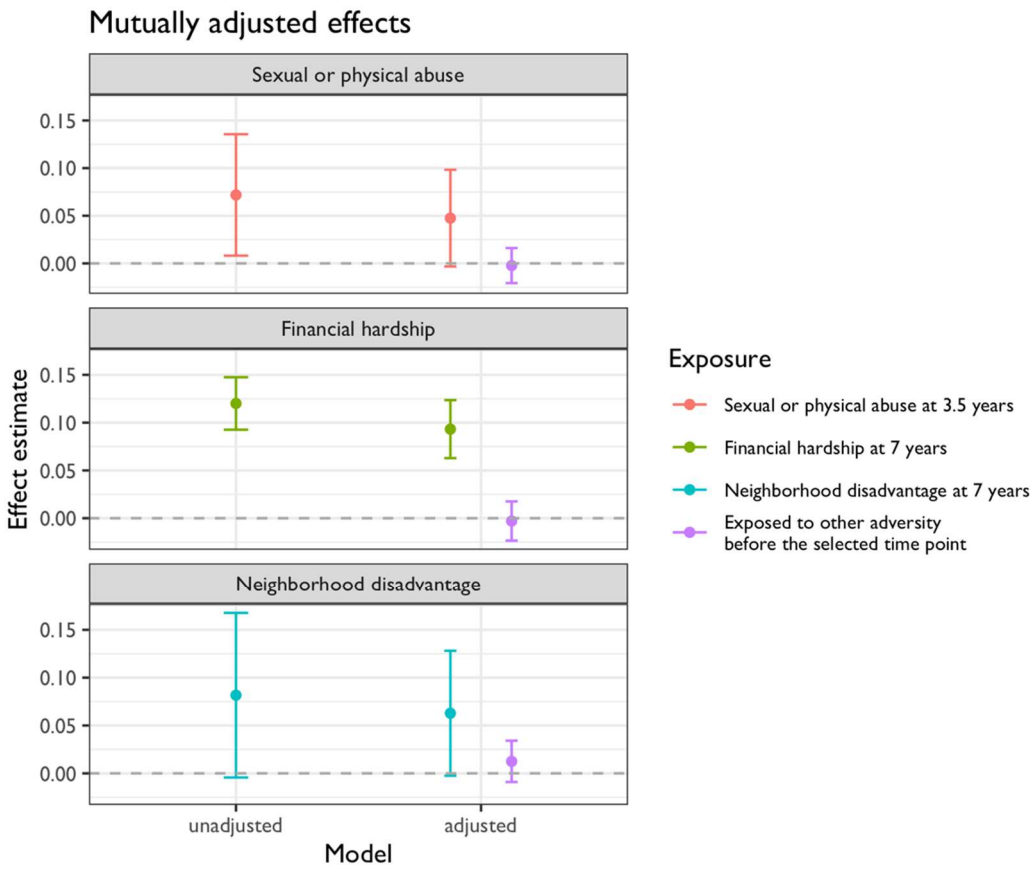
Supplemental Figure 1. Elbow plot illustrating LARS variable selection procedure

LARS begins by first identifying the single variable with the strongest association to the outcome; it then identifies the combination of two variables with the strongest association, followed by three variables, and so on, until all variables are included. LARS therefore achieves parsimony by identifying the smallest combination of encoded variables that explain the most amount of outcome variation. In addition to a covariance test, which is calculated at each stage of the LARS procedure and tests the null hypothesis that adding the next encoded variable does not improve r2, results can also be summarized in an "elbow plot," showing the increase in overall model r2 as additional predictors were added to the model. The point where this plot levels off indicates the point of diminishing marginal improvement to the model goodness-of-fit from adding additional predictors, suggesting that the predictors included in the model at this point represent an optimal balance of parsimony and thoroughness. In this example, both accumulation and sensitive period 1 were selected in the best fitting model.



Supplemental Figure 2. Graphical depiction of tetrachoric correlations between exposed vs. nonexposed to different adversity types

The heat map indicates the strength of the correlations between adversity exposures throughout childhood, with stronger positive correlations denoted in dark blue. No negative correlation was present. As shown, most of the heat map is pale blue (indicating low positive correlation). The strongest observed correlation was between financial hardship and having one adult in the household (r=0.45). The weakest observed correlation was between financial hardship and sexual or physical abuse (r=0.05).



Supplemental Figure 3. Effect estimates of the selected hypotheses after adjusting for the presence versus absence of any of the other six types of adversity that occurred before the examined time point.

The point estimates of the effects were relatively stable before and after adjusting for exposure to any other adversity that preceded the examined hypothesis, with exposure status coded as a binary indicator (1=exposed to any of the other types of adversity before the occurrence of the examined exposure, 0=unexposed). The confidence intervals were tightened for exposure to sexual or physical abuse and neighborhood disadvantage. There was no statistical evidence for an effect of exposure to other types of adversity. All other baseline covariates in the primary analyses were also included.

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