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Stressful Life Events and Accelerated Biological Aging Over Time in Youths

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Abstract

Experiencing adversity in childhood and adolescence, including stressful life events (SLEs), may accelerate the pace of development, leading to adverse mental and physical health. However, most research on adverse early experiences and biological aging (BA) in youths relies on cross-sectional designs. In 171 youths followed for approximately 2 years, we examined if SLEs over follow-up predicted rate of change in two BA metrics: epigenetic age and Tanner stage. We also investigated if rate of change in BA was associated with changes in depressive symptoms over time. Youths aged 8-16 years at baseline self-reported Tanner stage and depressive symptoms at baseline and follow-up and provided saliva samples for DNA at both assessments. Horvath epigenetic age estimates were derived from DNA methylation data measured with the Illumina EPIC array. At follow-up, contextual threat interviews were administered to youths and caregivers to assess youths' experiences of past-year SLEs. Interviews were objectively coded by an independent rating team to generate a SLE impact score, reflecting the severity of all SLEs occurring over the prior year. Rate of change in BA metrics was operationalized as change in epigenetic age or Tanner stage as a function of time between assessments. Higher objective SLE impact scores over follow-up were related to a greater rate of change in epigenetic age ($\beta=0.21$, $p=.043$). Additionally, among youths with lower—but not higher—Tanner stage at baseline, there was a positive association of SLE impact scores with rate of change in Tanner stage (Baseline Tanner Stage x SLE Impact Score interaction: $\beta=-0.21$, $p=.011$). A greater rate of change in epigenetic age was also associated with higher depressive symptom levels at follow-up, adjusting for baseline symptoms ($\beta=0.15$, $p=.043$). Associations with epigenetic age were similar, although slightly attenuated, when adjusting for epithelial (buccal) cell proportions. Whereas much research in youths has focused on severe experiences of early adversity, we

demonstrate that more commonly experienced SLEs during adolescence may also contribute to accelerated BA. Further research is needed to understand the long-term consequences of changes in BA metrics for health.

Key words: Adolescents; Adversity; Depression; DNA methylation age; Epigenetic age; Pubertal stage

1. Introduction

Environmental experiences in childhood and adolescence are strongly related to health over the lifespan. In particular, early-life adversity (ELA) has powerful associations with negative mental and physical health (Nelson et al., 2020). Accelerated development—although a potentially advantageous short-term adaptation to ELA—may be one mechanism by which ELA contributes to deleterious health outcomes (Belsky and Shalev, 2016; Callaghan and Tottenham, 2016). ELA has been linked to advanced biological aging (BA) across cellular and reproductive strategy metrics in youths, including shorter telomere length (Belsky and Shalev, 2016; Coimbra et al., 2017), advanced epigenetic age relative to chronological age (Jovanovic et al., 2017; Marini et al., 2020), and earlier pubertal timing and age at menarche (Belsky et al., 1991; Mendle et al., 2016). These BA metrics have been associated with all-cause mortality and various aging-related health risk factors and conditions, including cardiometabolic risk and cognitive decline (e.g., Chen et al., 2016; Levine et al., 2015; Prentice and Viner, 2013; Roetker et al., 2018; Wang et al., 2018). To date, much research linking early adversity with indicators of BA in youths has focused on more severe experiences of ELA, including abuse, neglect, and institutional rearing. Further, this work suggests that adversity characterized by threat (reflecting potential for physical harm, e.g., abuse)—and not deprivation (involving the absence of expected environmental inputs, e.g., neglect)—are particularly associated with advanced BA (Colich et al., 2020a; Colich et al., 2020b; Sumner et al., 2019; Sun et al., 2020; Tang et al., 2020b).

Less is known about whether less severe, but more common, adverse experiences such as stressful life events (SLEs) relate to BA in youths. Early SLEs—such as interpersonal conflicts and academic stressors—are strong predictors of adverse health (Grant et al., 2003; Jenness et

al., 2019; Tang et al., 2020a; Wickrama et al., 2015), and these events become increasingly common during adolescence (Larson and Ham, 1993). Research in adults has linked a broad range of stressful life experiences with advanced BA (see Gassen et al., 2017 for a review). For example, greater SLEs and perceived stress have been associated with shorter telomere length (Epel et al., 2004; Parks et al., 2009; Schutte and Malouff, 2016) and advanced epigenetic age relative to chronological age (Zannas et al., 2015). Additionally, longitudinal research has demonstrated that SLEs early in life (e.g., prenatal stress exposure, discrimination during adolescence) predict epigenetic and telomere metrics of advanced BA in young adulthood (Brody et al., 2016; Entringer et al., 2011). However, less work has examined whether SLEs influence BA in youths, although maternal prenatal SLEs and adverse family contexts have been associated with earlier pubertal development (Bräuner et al., 2021; Pham et al., 2022). Furthermore, research in adolescent girls has linked greater diurnal cortisol production to advanced epigenetic age relative to chronological age (Davis et al., 2017), highlighting a potential mechanism by which SLEs may contribute to BA.

Many studies of ELA and BA in youths have been cross-sectional or included only one aging-related assessment. Accordingly, it is unclear whether early experiences are related to *change* in BA. Some longitudinal studies of adults have investigated how trauma and related psychopathology are associated with change in BA (e.g., greater rate of change of the epigenetic clock; Boks et al., 2015; Wolf et al., 2019). Recently, Copeland and colleagues (2022) examined whether a range of ELA, including dimensions of threat, material deprivation, loss, and unpredictability, related to change in epigenetic age from childhood to adulthood. Greater cumulative ELA was associated with accelerated epigenetic aging, but robust links between different ELA dimensions and change in epigenetic aging were not detected. However, research

examining how SLEs influence the pace of BA in childhood and adolescence is largely lacking—a notable gap, as these BA indicators often exhibit substantial change during these developmental periods. For example, not only does pubertal development occur during this time, but the rate of change in the epigenetic clock is faster earlier in life compared to after puberty (Marioni et al., 2019; Raj and Horvath, 2020).

If SLEs relate to accelerated BA, this process could underlie links between stress and psychopathology. Substantial research indicates that earlier pubertal development predicts incident psychopathology in adolescence (Colich and McLaughlin, 2022), and emerging evidence in youths has revealed cross-sectional associations between advanced epigenetic age and depressive symptoms (Sumner et al., 2019) and internalizing disorders (Dammering et al., 2021). Additionally, in older adults, advanced epigenetic aging metrics partially accounted for the association of ELA with depressive symptoms (Klopach et al., 2022). However, longitudinal research investigating whether change in BA relates to psychopathology in youths—and whether accelerated aging may underlie associations of SLEs with psychopathology—is needed.

Additionally, although research in adults has identified relatively low concordance between different BA measures (Belsky et al., 2018), understanding of whether repeated measures of cellular and reproductive BA metrics relate to one another in youths is limited. Cross-sectional research has demonstrated a positive association between epigenetic age and pubertal stage in youths (Sumner et al., 2019), but evidence from longitudinal studies is mixed. In adolescent girls, greater epigenetic age advancement over puberty was related to younger age at menarche and a faster pubertal tempo (Binder et al., 2018). However, there was limited evidence that advanced epigenetic age at birth or in childhood or adolescence were related to pubertal development in a large birth cohort (Simpkin et al., 2017).

In this study, we addressed these limitations by examining rate of change in cellular and reproductive strategy BA measures—epigenetic age and pubertal stage—in children and adolescents followed over approximately 2 years. In 171 youths aged 8-16 years at baseline, we examined how BA metrics related to one another within and across time, and whether SLEs occurring between baseline and follow-up predicted differential change in these metrics. Additionally, we investigated whether changes in BA were related to changes in depressive symptoms. We hypothesized that experiences of SLEs would be positively associated with rate of acceleration in BA which, in turn, would be positively associated with change in depressive symptoms.

2. Materials and Methods

2.1 Participants and Procedure

Youths between 8 and 16 years of age and a caregiver (parent/guardian) were recruited to participate in a study of ELA, emotion regulation, and psychopathology (see Weissman et al., 2019 for details). Between January 2015 and January 2017, 262 children were enrolled from the community in Seattle, WA. Participants in the parent study completed a baseline assessment and were invited to return for a follow-up assessment approximately two years later (Weissman et al., 2019). Of the 262 youths enrolled in the parent study, 161 participants were included in a sub-sample that provided neuroimaging data and saliva samples for epigenetic analysis (Jenness et al., 2021); 121 youths had measures of epigenetic age at baseline and follow-up. Participants who were not in this sub-sample were still invited to complete measures of mental health symptoms and pubertal stage at the follow-up assessment; 140 youths in the parent study had reliable information on Tanner stage at both assessments (4 youths were excluded due to having unreliable reports indicating meaningful decreases in Tanner stage from baseline to follow-up).

Study procedures were approved by the University of Washington Institutional Review Board, and research was carried out in accordance with the Declaration of Helsinki. Caregivers provided written informed consent and youths provided written assent. The analytic sample for the current study comprised 171 participants who had measures of at least one BA metric at both baseline and follow-up.

2.2 SLE Measure

We used a multi-informant contextual threat interview-based approach to measure SLEs that occurred between the baseline and follow-up assessments. SLEs occurring in the past year of the follow-up period were queried with the UCLA Life Stress Interview (LSI; Hammen, 1988, 1991), a semi-structured interview developed to characterize SLEs as objectively as possible. The UCLA LSI was administered to youths and a caregiver. This interview has been validated for use in youth and adult samples, and it is a gold-standard method for assessing SLEs (Monroe, 2008). The UCLA LSI utilizes a series of prompts to assess various domains of a youth's life (e.g., school, peers, parents), and youths and caregivers each completed the interview and reported on events occurring in the past year. With this interview, an independent research team determines objective ratings indicating the likely impact of each event for a youth of that age and sex on a scale from 1 (no negative impact) to 5 (extremely severe negative impact). As in prior research (Hammen et al., 2000; Weissman et al., 2020), an objective SLE impact score was calculated by summing the impact scores for all events (excluding those scored as 1, as these have no likely negative impact). This total score provides a weighted average of the number and severity of SLEs in the past year. As in previous work in the parent study (Weissman et al., 2020), the highest score reported by youths or caregivers was used in analyses, reflecting the multi-informant method.

2.3 BA Measures

2.3.1 Epigenetic age. Youths provided saliva samples at baseline and follow-up using Oragene® kits (DNA Genotek, Ontario, Canada). DNA extraction and methylation profiling were conducted by AKESOgen (Atlanta, GA). The Illumina Infinium MethylationEPIC array was used to assess DNA methylation levels at >850,000 cytosine-guanine dinucleotides across the genome at single-nucleotide resolution. Samples were plated such that a participant's baseline and follow-up samples were on the same chip. Our plating strategy also minimized correlations between technical variables (chip, position, plate) with participant demographics and history of ELA. Additionally, two participants were randomly selected as technical replicates; duplicate baseline and follow-up samples for each individual were assayed on different chips. After measurement, data were cleaned following the pipeline of Illumina and processed using “minfi” (Fortin et al., 2017). CpGs with detection p -values $>.01$ in $>5\%$ of individuals were removed. Cross-hybridizing, genetically confounded, and sex chromosome probes were removed. DNA methylation data were pre-processed using the Illumina-type background correction, dye-bias adjustment, and normal-exponential out-of-band normalization.

We examined the pan-tissue Horvath clock trained on chronological age (Horvath, 2013) as our measure of epigenetic age in analyses. Given that most other DNA methylation age estimators have been constructed based on blood samples from adults, we selected this epigenetic clock because it was built and calibrated across various tissues, including saliva, and in younger individuals (Horvath, 2013). The CpG sites for the estimations of Horvath epigenetic age were retrieved from the whole epigenome data. Horvath epigenetic age estimates were calculated at baseline and follow-up based on raw (non-normalized) probe data according to

instructions on the Horvath website (<https://dnamage.genetics.ucla.edu>). There was evidence of high reproducibility for the Horvath epigenetic age estimates for the technical replicates (intraclass correlation coefficient(3,1)=.88).

2.3.2 Pubertal stage. At baseline and follow-up assessments, pubertal stage was determined using self-report Tanner staging (Marshall and Tanner, 1969, 1970; Morris and Udry, 1980). Using schematics of two secondary sex characteristics (pubic hair and breast/testes development), youths reported their developmental stage on a scale of 1-5. A Tanner stage of 1 indicates no pubertal development has begun, whereas a stage of 5 indicates adult levels of pubertal maturation. The ratings for these two secondary sex characteristics were averaged to generate a Tanner stage score at each assessment. Self-report Tanner stage scores correlate with physicians' physical examinations of pubertal status (Chavarro et al., 2018; Coleman and Coleman, 2002; Morris and Udry, 1980).

2.4 Depressive Symptoms

At baseline and follow-up, youths completed the Children's Depression Inventory-2, a widely used self-report measure of depressive symptoms in children and adolescents with sound psychometric properties (Kovacs, 1992; Reynolds, 1994). This scale has 27 items; for each item, individuals choose between 3 statements corresponding to a 3-point scale. Responses were summed to create a total depressive symptoms score; higher scores indicate greater symptom severity. Due to positive skew in the total scores (skewness=1.44 at baseline, 1.18 at follow-up), we analyzed natural log-transformed depressive symptom variables.

2.5 Covariates

Given the wide range in participants' chronological age, analyses adjusted for chronological age at baseline, in addition to sex and race/ethnicity. Additionally, as poverty is a context that can increase the likelihood of experiencing stress, adversity, and other environmental risks that can influence BA (e.g., exposure to toxins, differences in parenting, crowding; Johnson et al., 2016), family income-to-needs ratio was included as a covariate. Caregivers reported household income at baseline and follow-up; income-to-needs ratio was calculated by dividing household income by the U.S. census-defined poverty line for their family size. As in prior longitudinal research in this cohort (Sumner et al., 2022), the income-to-needs ratio covariate was calculated by averaging baseline and follow-up reports to reflect circumstances over the study period.

2.6 Analytic Approach

We first calculated the correlations of 1) epigenetic age and 2) Tanner stage with chronological age at each time point. We then investigated the stability of epigenetic age and Tanner stage by calculating correlations across baseline and follow-up for each BA metric. We also examined the degree of correlation across the BA metrics by computing correlations of epigenetic age and Tanner stage both within and across time.

As in recent work by Wolf et al. (2019), we operationalized accelerated aging over time in terms of the rate of change in raw BA metrics. Although research has examined the residual of BA on chronological age as a measure of BA advancement relative to chronological age (e.g., Sumner et al., 2019), in the current longitudinal study, we were interested in the rate with which BA metrics themselves changed over time. Specifically, we computed the rate of epigenetic age change per calendar year by calculating the difference in epigenetic age estimates (follow-up minus baseline) as a function of the time in years between the baseline and follow-up

assessments (time between assessments was calculated to the day rather than averaged up to a whole year value). A value of 1.0 suggests that for each intervening calendar year, epigenetic age increases by one year. Values greater than 1.0 indicate an accelerated rate of epigenetic age change relative to the intervening time, whereas values less than 1.0 indicate a slower rate of epigenetic age change relative to the intervening time. Similarly, we estimated the rate of Tanner stage change per calendar year by calculating the difference in Tanner stage scores (follow-up minus baseline) as a function of follow-up time. Although this does not have the same clear interpretation as change in epigenetic age per calendar year above, it does signal important information about pubertal tempo, or the rate of change in pubertal stage over time.

We examined if objective SLE impact scores over follow-up were associated with rate of change in our BA metrics using linear regression. Models adjusted for chronological age at baseline, sex, race/ethnicity, and average family income-to-needs ratio. Additionally, we tested for interactions of SLE impact scores with sex, as some research has identified sex differences in associations of early adverse experiences with BA (Tang et al., 2020b). Given that youths varied across the full Tanner stage 1-5 range at baseline, we also tested for an interaction of SLE impact scores with baseline Tanner stage to investigate whether SLEs might be more associated with rate of change in Tanner stage primarily among youths who had more of an opportunity to change. For this analysis examining rate of change in Tanner stage, the continuous Tanner stage and SLE impact score variables were z-scored and then multiplied to create the interaction term. Further, we did not include baseline chronological age as a covariate given its high positive correlation with Tanner stage at baseline ($r=.82, p<.001$).

To examine links between rate of change in BA metrics with change in depressive symptoms, we conducted linear regression models with follow-up depressive symptoms as the

outcome. Models adjusted for chronological age at baseline, sex, race/ethnicity, average family income-to-needs ratio, length of follow-up in years, and baseline depressive symptoms. We also considered whether rate of change in BA might explain an association between SLEs and change in depressive symptoms. If a significant relation was observed between a BA rate of change measure and change in depressive symptoms, we first tested the total effect of objective SLE impact scores on change in depressive symptoms. We then utilized the PROCESS macro (Hayes, 2022) in SPSS Version 28.0 (IBM Corp, Armonk, NY) to test for an indirect effect using bootstrapping with percentile-based confidence intervals.

We conducted three sets of sensitivity analyses. First, given prior cross-sectional research in this sample linking threat-related ELA to advanced epigenetic age and Tanner stage relative to chronological age at baseline (Sumner et al., 2019), we conducted an analysis to examine whether associations between objective SLE impact scores and rate of change in BA remained when accounting for threat-related experiences at baseline. As described previously, a multi-informant, multi-method approach was used to assess youths' lifetime threat-related experiences, including emotional, physical, and sexual abuse, domestic violence, and other forms of interpersonal violence (see Sumner et al., 2019 and Supplementary Methods for details). These experiences were summed to create a lifetime composite of threat-related ELA, which was included as a covariate in the sensitivity analysis.

Second, we conducted analyses that included the estimated proportion of epithelial (buccal) cells in each sample at baseline and follow-up as covariates in analyses of change in epigenetic age. The proportion of epithelial cells in saliva exhibits inter-individual variability and can influence DNA methylation levels (Smith et al., 2015). As with prior research (Jovanovic et al., 2017; Smith et al., 2015; Sumner et al., 2019), we estimated the proportion of epithelial

(buccal) cells using the method of Houseman and colleagues (Houseman et al., 2012; Koestler et al., 2013) and a reference from buccal cells (GSE46573) from the Gene Expression Omnibus. The median proportion of epithelial (buccal) cells was 29.0% (interquartile range=22.6%–35.4%) at baseline and 31.3% (interquartile range=23.6%–37.8%) at follow-up. These ranges overlap with other estimates of epithelial (buccal) cells in saliva samples (Eipel et al., 2016; Theda et al., 2018).

Third, although longitudinal research has found that epigenetic aging metrics are positively associated with depressive symptoms (Klopach et al., 2022), other longitudinal research has found that depressive symptoms were positively associated with change in epigenetic aging (Copeland et al., 2022). Thus, we tested whether baseline depressive symptoms were related to rate of change in the BA metrics, adjusting for chronological age at baseline, sex, race/ethnicity, and average family income-to-needs ratio.

All analyses were performed with SPSS Version 28.0 (IBM Corp., Armonk, NY), and p -value $<.05$ indicated statistical significance.

3. Results

3.1 Participant Characteristics

Descriptive statistics for the analytic sample are presented in Table 1. Participants had a mean age of 12.5 years at baseline, and 44.4% of the analytic sample was female. Additionally, participants were diverse with respect to race/ethnicity and family income-to-needs ratio, and there was wide variability in past-year SLEs and depressive symptom levels. Consistent with research demonstrating that experiences of SLEs increase during adolescence relative to childhood (Ge et al., 2001; Larson and Lampman-Petratis, 1989; Rudolph and Hammen, 1999), chronological age at baseline was positively associated with SLE impact scores at follow-up

($r=.18, p=.049$). Chronological age was also positively correlated with depressive symptoms at baseline ($r=.21, p=.006$) and follow-up ($r=.34, p<.001$). The average time between baseline and follow-up assessments was 1.82 years. Baseline demographics for participants in the analytic sample were very similar to those for the larger group of participants in the parent study (Supplementary Table 1).

3.2 BA Metrics Within and Across Time

Descriptive statistics for raw BA metrics and their correlations within and across time are presented in Table 2. Epigenetic age estimates and Tanner stage scores were strongly positively correlated with chronological age at baseline (epigenetic age: $r=.70$, Tanner stage: $r=.82$, $ps<.001$) and follow-up (epigenetic age: $r=.69$, Tanner stage: $r=.78$, $ps<.001$). There was also evidence of considerable stability over time for both epigenetic age ($r=.73, p<.001$) and Tanner stage ($r=.86, p<.001$). Additionally, the two BA metrics exhibited moderate positive correlations with one another both within and across time (Table 2).

With respect to rate of change in BA metrics, epigenetic age estimates increased at a rate, on average, of 1.02 years per calendar year ($SD=1.84$, range= -3.90–6.15). The mean rate of change per calendar year for Tanner stage was 0.39 ($SD=0.46$, range= -0.39–2.99). Rates of change for epigenetic age and Tanner stage were not significantly correlated ($r=.10, p=.338$). Rate of change for Tanner stage was negatively correlated with chronological age at baseline ($r=-.38, p<.001$), but rate of change for epigenetic age was not significantly associated with chronological age ($r=.05, p=.575$).

3.3 SLEs and Change in BA

Higher objective SLE impact scores were significantly associated with a greater rate of change in epigenetic age per calendar year [$b=0.04$ (95% confidence interval [CI]: 0.00–0.08),

$\beta=0.21$, $p=.043$, $n=115$; see Figure 1 and Supplementary Table 2 for results for full regression model]. There was no evidence of association between SLEs and rate of change in Tanner stage [$b=0.01$ (95% CI: -0.00–0.02), $\beta=0.14$, $p=.187$, $n=95$; see Supplementary Table 2 for results for full regression model]. However, there was a significant interaction of baseline Tanner stage and SLE impact score when examining rate of change in Tanner stage [$b=-0.10$ (95% CI: -0.18–0.02), $\beta=-0.21$, $p=.011$, $n=95$]. As shown in Figure 2, analyses of simple slopes indicated that higher objective SLE impact scores were associated with a greater rate of change in Tanner stage per calendar year for youths with lower—but not higher—Tanner stage scores at baseline.

There were no statistically significant Sex x SLE Impact Score interactions for rate of change in epigenetic age or Tanner stage ($ps>.191$).

3.4 Rate of Change in BA and Change in Depressive Symptoms

A greater rate of change in epigenetic age per calendar year was associated with higher depressive symptom levels at follow-up, adjusting for depressive symptoms at baseline [$b=0.07$ (95% CI: 0.00–0.14), $\beta=0.15$, $p=.043$, $n=119$; see Supplementary Table 3 for results for full regression model]. In contrast, rate of change in Tanner stage was not significantly associated with depressive symptom levels from baseline to follow-up [$b=0.10$ (95% CI: -0.20–0.39), $\beta=0.05$, $p=.525$, $n=136$; see Supplementary Table 3 for results for full regression model]. In light of our finding that SLEs were associated with rate of change in Tanner stage only for youths with lower Tanner stage at baseline, we also tested the interaction of baseline Tanner stage with rate of change in Tanner stage when examining change in depressive symptoms over time. However, there was no evidence of significant moderation [$b=0.05$ (95% CI: -0.09–0.18), $\beta=0.06$, $p=.490$, $n=136$].

Given the significant relation between rate of change in epigenetic age and change in depressive symptoms over follow-up, we explored whether accelerated epigenetic aging was a potential pathway between SLEs and change in depressive symptoms. Objective SLE impact scores were positively associated with depressive symptoms at follow-up, adjusting for baseline symptom levels, but this association did not reach the level of statistical significance [$b=0.01$ (95% CI: -0.00–0.03), $\beta=0.14$, $p=.079$, $n=124$]. Furthermore, the indirect effect was small (0.003) and not statistically different from zero (95% CI: -0.00–0.01)].

3.5 Sensitivity Analyses

The mean of the composite score reflecting lifetime experiences of threat-related ELA at baseline in the analytic sample was 5.0 ($SD=3.7$, range= 0–14), and lifetime threat and objective SLE impact scores were positively correlated ($r=.57$, $p<.001$). Higher objective SLE impact scores remained associated with a greater rate of change in epigenetic age per calendar year when adjusting for lifetime threat-related ELA at baseline [$b=0.04$ (95% CI: 0.00–0.08), $\beta=0.22$, $p=.050$, $n=115$].

Additionally, although a similar pattern of results for associations of SLE impact scores with epigenetic aging was obtained when adjusting for the proportions of epithelial (buccal) cells at baseline and follow-up, results were slightly attenuated with respect to effect size and did not reach the threshold of statistical significance [$b=0.04$ (95% CI: -0.00–0.07), $\beta=0.18$, $p=.057$, $n=115$]. In addition, rate of change in epigenetic age per calendar year remained associated with higher depressive symptom levels at follow-up with a nearly identical effect size in the cell type-adjusted model, but it did not reach the level of statistical significance [$b=0.07$ (95% CI: -0.01–0.15), $\beta=0.15$, $p=.072$, $n=119$].

In analyses of baseline depressive symptoms and the rate of change in BA metrics, we did not find that depression at baseline predicted an accelerated rate of change in epigenetic age [$b = -0.43$ (95% CI: -0.87 – 0.01), $\beta = -0.20$, $p = .053$, $n = 119$] or Tanner stage [$b = -0.02$ (95% CI: -0.11 – 0.07), $\beta = -0.03$, $p = .701$, $n = 137$].

4. Discussion

In this study, we conducted a longitudinal examination of epigenetic and reproductive strategy indicators of BA in youths over approximately 2 years, and we investigated whether experiences of SLEs over follow-up related to changes in these metrics. There was evidence of stability over time for epigenetic age and Tanner stage, and both metrics were positively correlated with one another within and across time. Furthermore, exposure to greater stressful experiences over time was associated with differential rate of change in BA. Specifically, higher objective SLE impact scores reflecting past-year experiences at follow-up were significantly associated with a greater rate of change in epigenetic age per calendar year. SLE impact scores were also positively associated with rate of change in Tanner stage, but only for youths with lower Tanner stage at baseline who had more of an opportunity to change over follow-up. Furthermore, a greater rate of change in epigenetic age—but not in Tanner stage—was associated with an indicator of poor mental health, namely increased depressive symptom levels over follow-up. Results for epigenetic age were very similar in effect size, yet did not reach the threshold for statistical significance, when adjusting for cell types relevant to saliva, namely the estimated proportions of epithelial (buccal) cells. Given that DNA methylation differs across cell types, further research with larger samples is needed to ascertain the degree to which these associations are robust to cell type variation.

Our finding of correspondence between our two BA metrics (epigenetic age and Tanner stage), both within and across time, is consistent with prior literature demonstrating that greater average epigenetic age advancement is associated with faster pubertal development in adolescent girls (Binder et al., 2018). However, we did not always observe this same degree of concordance for other associations with measures of change in the two BA metrics. Indeed, rate of change in these two BA measures did not correlate significantly. Additionally, higher objective SLE impact scores were associated with a greater rate of change in epigenetic age and Tanner stage, but the latter relation was observed only among youths with lower Tanner stage at baseline who had more potential to change over the follow-up period. Furthermore, only rate of change in epigenetic age was related to change in depressive symptom levels. Together, these findings suggest that experiences of SLEs may relate to accelerated BA across cellular and reproductive strategy metrics. However, given the wide age range of youths at baseline in the current study, further testing is required in a larger sample of youths who have not yet undergone puberty at the start of follow-up. Including three or more assessments of pubertal stage, along with measures of adrenal and gonadal hormones that may capture more nuanced changes in pubertal status, will also permit investigations of potential nonlinear patterns of change.

To our knowledge, we are the first to demonstrate that SLEs are significantly associated with a greater rate of change in epigenetic age in youths. This finding is consistent with previous reports demonstrating that psychosocial stress is related to advanced epigenetic age in adults (Brody et al., 2016; Zannas et al., 2015). Furthermore, in the current study, we measured these experiences of intervening stress using a gold-standard interview and an objective rating system that integrates information about the frequency and severity of SLEs. This finding thus suggests that normative experiences of SLEs (e.g., an argument with a close friend, the break-up of a

romantic relationship, failing an exam) may contribute to a greater rate of change in epigenetic age over time. This result is notable, as much research on early adversity and BA has focused on more severe forms of ELA, such as abuse, neglect, and institutional rearing (Colich et al., 2020b). Our work suggests that accelerated epigenetic aging may be a process that is influenced by less severe, yet more common, experiences of SLEs in addition to more severe forms of ELA. Furthermore, research suggests that accelerated development may result in greater experiences of ELA and SLEs; these findings are particularly strong for the associations between early menarche and sexual assault (Mendle et al., 2019). Thus, longitudinal research that examines bidirectional associations of change in SLEs and in BA over time is required to understand these relations more comprehensively.

We also examined the extent to which accelerated BA over time was associated with an indicator of mental health: change in depressive symptoms. Here, we extended our previous cross-sectional findings linking advanced epigenetic age to higher depressive symptom levels (Sumner et al., 2019) by demonstrating that a greater rate of change in epigenetic age predicted higher depressive symptom levels at follow-up, adjusting for baseline symptoms. In that baseline study, we also only detected an association between epigenetic age—and not Tanner stage—with depressive symptoms, which parallels our findings in the current study. The distinct methods for measuring epigenetic age (assayed from biological samples) and Tanner stage (self-reported by youths) may have resulted in differential measurement error for the BA metrics, and this may explain—in part—the differential associations observed with change in depressive symptoms over time. Nevertheless, our findings are consistent with previous research in adults linking advanced epigenetic age to depression diagnoses (Han et al., 2018) and symptoms (Klopach et al., 2022) and to reductions in the positive affect domain of a measure of depressive symptoms

(Beydoun et al., 2019). However, there has been limited demonstration of predictions of change in depression over time (Beydoun et al., 2019), as we show here.

Mechanism-focused research is needed to better understand how accelerated BA may lead to increases in depressive symptoms over time. For example, accelerations in BA indicators may lead to neuronal changes such as neuroinflammation and neurodegeneration (either directly or through other physiological processes, including oxidative stress and mitochondrial dysfunction) in brain areas relevant to depression (e.g., hippocampus), and these brain changes may increase susceptibility to depression (Epel and Prather, 2018; Hawn et al., 2022; Wolf et al., 2021). Changes in systemic inflammation and behavioral risk factors (e.g., smoking) may also contribute to depression risk in the context of accelerated BA (Klopach et al., 2022; Miller et al., 2009). Preclinical studies utilizing experimental designs, in addition to further longitudinal research in humans, may help to elucidate key molecular and behavioral mechanisms.

Additionally, recent longitudinal research found that depressive symptoms during childhood partially explained the link between ELA and accelerated epigenetic aging from childhood to adulthood (Copeland et al., 2022). Even though we focused on the extent to which change in BA indicators was associated with mental health (here, depressive symptoms) over time, it is worth noting that mental health may also impact BA, although sensitivity analyses did not provide robust support for the notion that baseline depressive symptoms predicted an accelerated rate of change in the BA metrics. Further, we note that the timeframes for assessing BA and depressive symptoms were overlapping; thus, our analyses cannot address causality. Additional longitudinal research with multiple repeated assessments is needed to better understand the bidirectionality of these associations and how they may unfold over the life course. Furthermore, we did not observe that accelerated epigenetic aging was a potential path

linking experiences of SLEs with change in depressive symptoms over time. This finding contrasts with a study in older adults indicating that epigenetic aging indicators partially accounted for associations of ELA with depressive symptoms (Klopack et al., 2022). Differences in the nature of the early-life experiences examined (self-reported ELA vs. objectively rated SLEs) and in the developmental stage of participants (older adults vs. youths) may explain the lack of concordance in results between the investigation by Klopack and colleagues and the current study. Moreover, numerous mechanisms spanning multiple levels of analysis underlie the link between early-life stressors and psychopathology (Grant et al., 2003; McLaughlin et al., 2020). Longitudinal research in large samples of youth is required to elucidate the most important mechanisms across these levels of analysis.

Several limitations need to be considered when interpreting the findings of the current investigation. We used saliva-based measures of DNA methylation, and we estimated Horvath epigenetic age, as this has been validated in saliva and in children and adolescents (Horvath, 2013). Numerous epigenetic age algorithms have been developed (e.g., Alisch et al., 2012; Hannum et al., 2013; Wu et al., 2019), but not all are available for saliva samples collected from younger individuals. Some clocks have also been trained on morbidity and mortality (e.g., Levine et al., 2018; Lu et al., 2019), which may not be as relevant for youths. Furthermore, this approach extended our prior cross-sectional research with Horvath epigenetic age in this sample (Sumner et al., 2019). Additionally, we used the MethylationEPIC array to assess DNA methylation levels, and the Horvath epigenetic clock was developed using the HumanMethylation27 and HumanMethylation450 arrays. However, even though 19 of the 353 CpG sites used to calculate Horvath epigenetic age are missing from the EPIC array, research suggests that this has a minimal effect on epigenetic age estimates (McEwen et al., 2018). In

addition, although we used a multi-informant interview approach and an objective rating method for assessing SLE impact, experiences of SLEs were retrospectively reported, which has established limitations (Baldwin et al., 2019; Hardt and Rutter, 2004). We also examined self-reported Tanner stage. Despite high correlations between self-report and physical exam measures of pubertal development, variation exists in the accuracy of self-reporting (Chavarro et al., 2018; Coleman and Coleman, 2002; Dorn and Biro, 2011; Morris and Udry, 1980). We also acknowledge that Tanner stage (and pubertal development more broadly) is a developmentally constrained BA indicator. Although appropriate for youths, this marker loses value as a marker of BA in young adulthood and beyond. Additionally, our sample was diverse with respect to race/ethnicity. Although this increases the generalizability of our findings, race and ethnicity have been linked to differences in epigenetic age and pubertal stage (Herman-Giddens, 2006; Horvath et al., 2016; Kaplowitz, 2006; Liu et al., 2019). However, we adjusted for self-reported race/ethnicity in our analyses. Even with these limitations, our study has a number of strengths that allow it to make a unique contribution to the literature, including 1) utilizing a longitudinal design to examine rate of change in cellular and reproductive strategy metrics of BA, and 2) including a gold-standard interview-based measure and objective rating of SLEs.

4.1 Conclusions

Despite considerable stability in measures of BA in youths over approximately 2 years, we found that greater exposure to objectively rated SLEs was significantly associated prospectively with accelerated epigenetic aging and—among those with lower Tanner stage at baseline—rate of change in Tanner stage. Additionally, a greater rate of change in epigenetic age predicted higher levels of depressive symptoms at follow-up, shedding light on how accelerated BA may impact mental health. Further research is needed to understand the long-term

consequences of changes in these metrics of BA for mental and physical health, and whether the process of accelerated aging can be altered through psychosocial intervention.

CRedit Authorship Contribution Statement

Jennifer A. Sumner: Conceptualization, Data curation, Funding acquisition, Analysis, Writing – original draft, Writing – review and editing; **Xu Gao:** Data curation, Writing – review and editing; **Simone Gambazza:** Data curation, Writing – review and editing; **Christian K. Dye:** Data curation, Writing – review and editing; **Natalie L. Colich:** Data curation, Writing – review and editing; **Andrea A. Baccarelli:** Writing – review and editing; **Monica Uddin:** Writing – review and editing; **Katie A. McLaughlin:** Conceptualization, Funding acquisition, Supervision, Writing – review and editing.

Declarations of Interest

None

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Data Sharing

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Table 1. Participant characteristics for the analytic sample ($N=171$).

Characteristic	<i>M</i> (<i>SD</i>)	Range	% (<i>n</i>)	Valid <i>n</i>
Age at baseline, years	12.50 (2.63)	8.00–16.95		171
Female sex			44.4 (76)	171
Race/ethnicity				171
White			44.4 (76)	
Black			25.1 (43)	
Latino			11.1 (19)	
Other			19.3 (33)	
Average family income-to-needs ratio	3.58 (2.68)	0.09–10.35		167
Time between baseline and follow-up, years	1.82 (0.65)	0.33–3.43		171
Stressful life events				
Objective stressful life events impact score, follow-up	11.49 (9.98)	0–44.0		126
Depressive symptoms				
Symptom levels, baseline	8.94 (7.44)	0–39		171
Symptom levels, follow-up	8.41 (7.55)	0–35		170

Note. *M*=mean; *SD*=standard deviation. Although we analyzed natural log-transformed depressive symptom variables due to positive skew, we present descriptive statistics based on the original scale.

Table 2. Raw estimates of biological age and correlations within and across time.

Biological Age Metric	Descriptive Statistics			Correlations			
	<i>M (SD)</i>	Range	<i>n</i>	1.	2.	3.	4.
1. Baseline Horvath epigenetic age	11.48 (3.53)	4.02–20.89	121	---			
2. Follow-up Horvath epigenetic age	13.24 (4.13)	4.63–25.76	121	.73	---		
3. Baseline Tanner stage	3.19 (1.35)	1–5	140	.53	.46	---	
4. Follow-up Tanner stage	3.85 (1.12)	1–5	140	.51	.48	.86	---

Note. All correlations significant at $p < .001$. Sample size for correlations for the Horvath epigenetic age = 121 and for Tanner stage = 140. Sample size for correlations across Horvath epigenetic age and Tanner stage = 90. Abbreviations: *M* = mean, *SD* = standard deviation.

Figure Legends

Figure 1. Scatter plot with regression line for the association of objective stressful life event impact scores with rate of change in epigenetic age per calendar year. For the rate of change in epigenetic age variable, a value of 1.0 suggests that for each intervening calendar year, epigenetic age increases by one year. Values greater than 1.0 indicate an accelerated rate of epigenetic age change relative to the intervening time, whereas values less than 1.0 indicate a slower rate of epigenetic age change relative to the intervening time.

Figure 2. Moderation of the relation between objective stressful life event (SLE) impact scores and rate of change in Tanner stage per calendar year by Tanner stage at baseline; regression lines and 95% confidence intervals are presented in the plot. Past-year SLEs at follow-up were significantly positively associated with rate of change in Tanner stage, adjusting for sex, race/ethnicity, and average family income-to-needs ratio, in participants with lower—but not higher—Tanner stage at baseline. Objective stressful life event impact scores and Tanner stage at baseline were z-scored for testing the interaction but variables are plotted in their raw scale for ease of interpretability. Larger positive values for rate of change in Tanner stage indicate a faster pubertal tempo over time.



