

# **Early Experiences of Threat, but not Deprivation, Are Associated With Accelerated Biological Aging in Children and Adolescents**

## ***Supplemental Information***

### **Supplemental Methods**

#### **Participants**

Children aged 8-16 years and a parent or guardian were recruited to participate in a study examining early life adversity (ELA), emotion regulation, and psychopathology. Between January 2015 and January 2017, 262 children were enrolled from the community in Seattle, WA. Children and caregivers were recruited for participation at schools, after-school and prevention programs, adoption programs, food banks, shelters, parenting programs, medical clinics, and the general community. Recruitment efforts were targeted at recruiting a sample with variation in exposure to violence and other forms of ELA. To do so, we recruited from neighborhoods with high levels of violent crime, from clinics that served a predominantly low-socioeconomic status catchment area, and agencies that work with families who have been victims of violence (e.g., domestic violence shelters, programs for parents mandated to receive intervention by Child Protective Services).

#### **ELA Exposure**

Children completed two interviews with a trained member of our research team. The Childhood Experiences of Care and Abuse (CECA) (1) interview assesses caregiving experiences, including physical and sexual abuse and emotional neglect. We modified the interview to ask parallel questions about witnessing domestic violence (i.e., directly observing violence directed at a caregiver). Inter-rater reliability for maltreatment reports is excellent, and validation studies suggest high agreement between siblings on maltreatment reports (2). If

children reported exposure to physical abuse, sexual abuse, and/or domestic violence, they also reported the age at which they were first exposed to this experience. The Violence Exposure Scale for Children-Revised (VEX-R) (3,4) assesses the frequency of exposure to different forms of violence. Children are presented with a cartoon and caption depicting a child of the same sex witnessing a type of violence (e.g., “Chris sees a person slap another person really hard”) and experiencing that same type of violence (e.g., “A person slaps Chris really hard”). Children are then asked to report how frequently they have witnessed or experienced that type of violence (e.g., “How many times have you seen a person slap another person really hard?”; “How many times has a person slapped you really hard?”) on a Likert scale ranging from 0 (Never) to 3 (Lots of times). The VEX-R demonstrates good reliability and has been validated with children as young as second grade (3,4). We created a violence exposure composite on the VEX-R by summing the number of distinct forms of violence the child experienced or witnessed firsthand. Only items clearly reflecting violence were included (i.e., either experiencing or witnessing being pushed or shoved really hard, slapped really hard, beaten up, having a gun or knife pointed at them, and corporal punishment); items that do not clearly reflect violence were not included in the count (e.g., seeing someone selling drugs).

Children additionally completed two self-report measures assessing child maltreatment and trauma exposure. The Childhood Trauma Questionnaire (CTQ) is a 28-item scale that assesses the frequency of maltreatment during childhood, including physical, sexual, and emotional abuse and physical and emotional neglect. Validated thresholds for exposure to physical and sexual abuse (5) were applied here in evaluating abuse exposure based on the CTQ. The CTQ has excellent psychometric properties including internal consistency, test-retest reliability, and convergent and discriminant validity with interviews and clinician reports of

maltreatment (6,7). The UCLA PTSD Reaction Index (PTSD-RI) includes a trauma screen that assesses exposure to numerous traumatic events, including physical abuse, sexual abuse, and domestic violence and additionally assesses PTSD symptoms. The PTSD-RI has good internal consistency and convergent validity (8).

Caregivers completed three self-report measures of child maltreatment and trauma: the Conflict Tactics Scale-Parent Child Version (CTS) (9), the Juvenile Victimization Questionnaire (JVQ) lifetime caregiver report (10), the caregiver version of the PTSD-RI, and a measure assessing food insecurity. The CTS includes 22 items assessing caregiver responses to child disobedience or misbehavior in the past year. Caregivers indicate how frequently they have used each strategy (e.g., shook him/her) on a Likert scale ranging from 0 (“This has never happened”) to 6 (“More than 20 times in the past year”) and can also indicate if they have used the strategy in the past but not in the last year. The CTS has adequate reliability and good discriminant and construct validity (9). The JVQ includes 34 items assessing exposure to crime, child maltreatment, peer and sibling victimization, sexual victimization, and witnessing and indirect victimization and has excellent psychometric properties, including test-retest reliability and construct validity (10). Caregivers endorsed whether their child had experienced each event in his/her lifetime. Caregivers also completed the trauma screen included in the PTSD-RI, described above. A trained interviewer followed up with the caregiver if the endorsed any form of abuse or domestic violence to gather additional information about the experience.

Food insecurity in the past 12 months was assessed using a set of 4 items drawn from the short form of the U.S. Department of Agriculture’s Food Security Scale (11). These four items constitute a validated measure of food insecurity that has been used in epidemiological surveys of youth psychopathology (e.g., the National Comorbidity Survey Replication – Adolescent

Supplement) (12). Children and their parent completed two dichotomous items indicating whether they had ever been hungry but did not eat because they could not afford enough food and whether they had ever eaten less than they thought they should because there was not enough money to buy food. Two additional items assessed how often adolescents and parents did not have enough money to buy food and could not afford to buy balanced meals in the past 12 months.

To assess the degree of cognitive stimulation in the home environment, parents completed the Home Observation for Measurement of the Environment-Short Form (HOME-SF) (13). The HOME-SF has slightly different versions for children aged 6-9 and 10-15 years, with 16 items that are identical across these age ranges. We used only the 16 questions that are present in the HOME-SF for both younger and older children as our sample spanned both of these age windows. This assessment included items that assess cognitive stimulation and exposure to varied learning experiences. Example items included: “About how many books does your child have?”; “How many times does your child get out of the house per week for activities (e.g., sports, extracurricular activities, activities with the family)?”; and “Did you and/or your partner teach your child numbers at home?”. The measure was scored using the cut-offs used in the original HOME assessment, where one point is assigned for each item where age-appropriate experiences are met (e.g., 1 point is assigned if a child leaves the house at least once a week for an activity; 0 is assigned if the child leaves the house less than once a week for an activity); for a total possible score of 16.

Child Protective Services were alerted to any cases of child abuse or neglect that had not previously been reported to the proper authorities.

We created a threat exposure composite by summing the total number of threat experiences endorsed by the child and/or caregiver. Child and caregiver reports were combined using an “or” rule (i.e., a particular ELA was coded present if either the caregiver or the child endorsed it). The threat experiences composite included physical abuse, sexual abuse, emotional abuse, domestic violence, and exposure to other forms of interpersonal violence. Physical and sexual abuse were coded present if the child endorsed these experiences in the CECA interview, scored above a validated threshold on the CTQ sub-scales for physical and sexual abuse (5), or if the parent endorsed these experiences on the JVQ, CTS, or PTSD-RI. Emotional abuse was coded based on child-report only and was considered present if the child scored above a validated threshold on either the CECA interview or CTQ for emotional abuse (5). Domestic violence was considered present if it was endorsed by the child on the CECA interview or the PTSD-RI or by the parent on the JVQ or PTSD-RI. Finally, the number of forms of interpersonal violence reported by the child on the VEX-R were summed.

We created a deprivation exposure composite using the same procedures. The deprivation experiences composite included emotional neglect, physical neglect, food insecurity, and an absence of cognitive stimulation (i.e., cognitive deprivation). Emotional neglect was coded based on the CECA interview using a validated threshold (14). We elected to use the CECA rather than the CTQ for assessing emotional neglect as this measure more closely aligns with accepted definitions of neglect (15) by assessing neglectful behaviors (e.g., “She would leave me unsupervised before the age of 10”) as compared to the CTQ, which focuses largely on appraisals (e.g., “My family was a source of strength and support”). Physical neglect was coded present if children scored above a validated threshold on the CTQ (5). Food insecurity was coded as present if either the child or caregiver endorsed being hungry but not eating because they

could not afford food or eating less than they thought they should because there was not enough money to buy food. Cognitive stimulation was assessed using the HOME-SF, which does not have an established cut-point for stimulation low enough to be considered deprivation. As such, we coded children in the bottom quartile of this measure in our sample as experiencing cognitive deprivation.

### **DNA Methylation (DNAm) Age**

Saliva samples were collected using Oragene® kits (DNA Genotek, Ontario, Canada). DNA extraction and methylation profiling were conducted by AKESOgen (Atlanta, GA). The Illumina Infinium MethylationEPIC BeadChip kit was used to assess methylation levels at over 850,000 methylation sites. Prior to calculation of DNAm age estimates, poor performing probes ( $\geq 10\%$  detection  $p \leq 0.001$ ) and samples ( $\geq 50\%$  missing) were filtered out using CpGAssoc. Horvath DNAm age estimates were then calculated based on raw (non-normalized) probe data according to the instructions on the Horvath website (<https://labs.genetics.ucla.edu/horvath/dnamage/>). This algorithm was developed on the Illumina HumanMethylation450 BeadChip, and it uses information from 353 probes. Sixteen of the 353 sites for the Horvath epigenetic clock (4.5%) were not included on the MethylationEPIC chip, although initial work suggests congruence between DNAm levels from both chips (16, 17). Horvath DNAm age estimates predict age across a range of tissues, including blood and saliva (18), and they are well calibrated in children and adolescents [other DNAm age algorithms (e.g., the Hannum et al. (19) estimates) have not been found to perform well in individuals younger than 20 years of age (20)]. As in prior research (21, 22), we regressed DNAm age on chronological age; the unstandardized residuals were our indicator of epigenetic age acceleration and were used as the dependent variables in analyses. Positive residuals indicate that DNAm is

overestimated (i.e., accelerated), whereas negative residuals indicate that DNAm age is underestimated compared to chronological age (i.e., decelerated epigenetic aging) (21).

**Table S1.** Regression parameters and 95% confidence intervals for associations of early life adversity individual experiences of threat and deprivation with biological aging metrics.

	DNAm Age Residual				Tanner Stage Residual			
	<i>b</i> (95% CI)	$\beta$	<i>p</i>	<i>n</i>	<i>b</i> (95% CI)	$\beta$	<i>p</i>	<i>n</i>
<u>Threat exposure</u>								
Physical abuse	0.82 (-0.40–2.03)	0.11	.186	190	0.07 (-0.18–0.32)	0.04	.600	208
Sexual abuse	-0.34 (-1.52–0.85)	-0.04	.575	190	0.03 (-0.23–0.28)	0.02	.833	208
Emotional abuse	0.89 (-0.23–2.02)	0.12	.120	190	-0.08 (-0.32–0.17)	-0.04	.535	208
Domestic violence	1.41 (0.24–2.57)	0.19	.018	190	0.07 (-0.18–0.31)	0.04	.582	208
Number of types of directly experienced interpersonal violence	0.22 (0.003–0.43)	0.16	.047	186	0.07 (0.02–0.11)	0.21	.003	204
<u>Deprivation exposure</u>								
Physical neglect	0.87 (-0.29–2.03)	0.11	.141	190	-0.02 (-0.26–0.23)	-0.01	.904	208
Emotional neglect	0.08 (-1.14–1.29)	0.01	.902	190	-0.11 (-0.37–0.15)	-0.06	.415	208
Food insecurity	0.82 (-0.90–2.54)	0.08	.345	190	-0.17 (-0.55–0.21)	-0.07	.385	208
Cognitive deprivation	-0.22 (-1.57–1.13)	-0.03	.745	188	-0.06 (-0.35–0.24)	-0.03	.695	205

Note. DNAm=DNA methylation. CI=confidence interval. Models adjusted for sex, race/ethnicity, and family poverty status.



**Table S2.** Regression parameters and 95% confidence intervals for associations of early life adversity experiences of threat (alternate operationalization) with biological aging metrics.

	DNAm Age Residual				Tanner Stage Residual			
	<i>b</i> (95% CI)	$\beta$	<i>p</i>	<i>n</i>	<i>b</i> (95% CI)	$\beta$	<i>p</i>	<i>n</i>
Threat exposure score								
Model 1 <sup>a</sup>	0.26 (0.001–0.53)	0.16	.050	186	0.05 (-0.01–0.10)	0.13	.100	204
Model 2 <sup>b</sup>	0.28 (-0.05–0.61)	0.18	.093	184	0.09 (0.02–0.15)	0.24	.010	201

*Note.* DNAm=DNA methylation. CI=confidence interval.

<sup>a</sup>Model adjusted for sex, race/ethnicity, and family poverty status.

<sup>b</sup>Model 1 further adjusted for deprivation exposure score.

**Table S3.** Regression parameters and 95% confidence intervals for associations of early life adversity experiences of threat (alternate operationalization) and biological aging metrics with depressive symptoms and externalizing problems.

	Depressive Symptoms				Externalizing Problems			
	<i>b</i> (95% CI)	$\beta$	<i>p</i>	<i>n</i>	<i>b</i> (95% CI)	$\beta$	<i>p</i>	<i>n</i>
Threat exposure score <sup>a</sup>	1.77 (1.36–2.18)	0.52	<.0001	225	2.80 (2.21–3.39)	0.57	<.0001	225
DNAm age Model 1 <sup>b</sup>	0.27 (0.02–0.52)	0.17	.036	186	0.14 (-0.22–0.50)	0.06	.447	186
Tanner stage Model 1 <sup>b</sup>	-0.32 (-1.45–0.81)	-0.06	.579	204	-0.26 (-1.69–1.19)	-0.03	.728	204

Note. CI=confidence interval. DNAm=DNA methylation.

<sup>a</sup>Model adjusted for age, sex, race/ethnicity, and family poverty status.

<sup>b</sup>Model adjusted for age, sex, race/ethnicity, family poverty status, and threat exposure.

**Table S4.** Regression parameters and 95% confidence intervals for associations of early life adversity experiences of threat and deprivation with accelerated DNA methylation age in models adjusting for the proportion of epithelial (buccal) cells.

	DNAm Age Residual			
	<i>b</i> (95% CI)	$\beta$	<i>p</i>	<i>n</i>
Threat exposure score				
Model 1 <sup>a</sup>	0.14 (-0.02–0.29)	0.14	.085	180
Model 2 <sup>b</sup>	0.16 (-0.03–0.35)	0.16	.097	178
Deprivation exposure score				
Model 1 <sup>a</sup>	0.20 (-0.35–0.76)	0.06	.474	182
Model 2 <sup>c</sup>	-0.14 (-0.83–0.54)	-0.05	.682	178

*Note.* DNAm=DNA methylation. CI=confidence interval.

<sup>a</sup>Model adjusted for sex, race/ethnicity, and family poverty status.

<sup>b</sup>Model 1 further adjusted for deprivation exposure score.

<sup>c</sup>Model 1 further adjusted for threat exposure score.

**Table S5.** Regression parameters and 95% confidence intervals for associations of DNA methylation age with depressive symptoms and externalizing problems in models adjusting for the proportion of epithelial (buccal) cells.

	Depressive Symptoms				Externalizing Problems			
	<i>b</i> (95% CI)	$\beta$	<i>p</i>	<i>n</i>	<i>b</i> (95% CI)	$\beta$	<i>p</i>	<i>n</i>
DNA <sub>m</sub> age								
Model 1 <sup>a</sup>	0.44 (0.15–0.73)	0.27	.003	184	0.35 (-0.08–0.78)	0.15	.110	184
Model 2 <sup>b</sup>	0.30 (0.04–0.56)	0.19	.022	180	0.16 (-0.23–0.55)	0.07	.409	180

*Note.* DNA<sub>m</sub>=DNA methylation. CI=confidence interval.

<sup>a</sup>Model adjusted for age, sex, race/ethnicity, and family poverty status.

<sup>b</sup>Model 1 further adjusted for threat exposure.

**Supplemental References**

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