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Adverse childhood experiences and biomarkers of inflammation in a diverse cohort of early school-aged children

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ABSTRACT

Objective: The objective of this study was to evaluate the relationship between ACEs and inflammatory profiles (i.e., pro- and anti-) in early childhood and to examine whether patterns differ for racial/ethnic subgroups.
Study design: Using longitudinal data from the Multidimensional Assessment of Preschoolers Study (MAPS) (N = 122), we examined the relationship between adverse childhood experiences (ACEs) beginning at birth, C -reactive protein (CRP), and both pro-inflammatory (i.e., IL-1 β, IL-6, TNF, and CRP) and anti-inflammatory (i.e. IL-4 and IL-10) biomarkers during early school age (ages 6–8 years).
Results: No children in the sample were reported to have experienced 0 ACES, 7% had 1 ACE, 51% had 2-3 ACEs, and 42% had 4 or more ACEs accumulated by the early school-age wave (ESA). There were no significant associations between cumulative ACEs and inflammatory markers. However, parental substance abuse, a specific ACE, was positively correlated with a pro-inflammatory profile at early school age ($r = 0.18$, $p < .05$). Specifically, substance abuse as an ACE was associated with higher levels of pro-inflammatory markers such as IL-1 β and IL-6. Additionally, Hispanics with ACEs had higher levels of CRP than Black and white individuals.
Conclusions: Children with histories of ACEs, especially those with parental substance abuse, may have higher levels of inflammation. Better understanding the role of inflammation in the development of chronic diseases for individuals with ACEs may allow earlier identification and prevention of disease during childhood for those at the highest risk.

1. Introduction

Exposure to psychosocial stress, particularly early in life, can alter brain development, promote chronic inflammation and increase susceptibility to a host of negative health outcomes, including both physical and mental health conditions (Lupien et al., 2000; Demir-Lira et al., 2016; Danese et al., 2007; Hughes et al., 2017). In particular, a robust literature has established that adverse childhood experiences (ACEs), retrospectively reported in adulthood, (Deighton et al., 2018) are linked to the development of chronic diseases. Adverse childhood experiences

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or ACEs, encompass a large breadth of experiences, including divorce, abuse, neglect, and household problems. There is growing literature linking ACEs to undesirable health outcomes, such as obesity, asthma, sleep disorders, diabetes, and cardiovascular disease, (Jakubowski et al., 2018; Huang et al., 2015) which may first appear in childhood (Oh et al., 2018) and then persist into adulthood (Anda et al., 2008; Llabre et al., 2017). Examining inflammatory biomarkers, which have been documented in childhood stress pathways, may be more easily identifiable because they are measurable from very early in life (O'Connor et al., 2014; Slopen et al., 2012).

The immune system – more specifically, pro-inflammatory markers – can be activated after experiencing a physical, emotional, or psychological stress such as ACE exposure, increasing and intensifying the body's physiological inflammatory response (Nusslock and Miller, 2016). The heightened inflammatory response and predisposition to chronic and systemic low-grade inflammatory state increases disease risk later in life. However, while the correlates of disease emerge in adulthood, the biological imprinting of ACEs and resultant pro-inflammatory state likely begins in early childhood. The presence of these adversities may overstimulate the immune system and predispose children to illnesses and diseases (Wyman et al., 2007; Caserta et al., 2008). With nearly half of children in the United States experiencing a serious adversity or an adverse childhood experience (ACE), (The prevalence of adverse, 2019) ACE-exposed children are at higher risk for greater systemic inflammation, and therefore suboptimal health conditions in adulthood (Felitti et al., 1998). Additionally, the influence of ACEs on inflammation in childhood may be integral to the understanding and reduction of disparities. Particularly as many chronic diseases, such as cardiovascular disease, continue to be a leading cause of mortality in racial/ethnic minority populations, and racial/ethnic minority children have disproportionately higher prevalence of ACEs (The prevalence of adverse, 2019; Loria and Caughy, 2018). However, there has been little focus on the relationship of inflammation in racial/ethnic minority children and ACEs.

In response to this gap in the literature, we sought to ascertain the relationship between ACEs and inflammation in early school-age among children from a diverse, racial/ethnic, and socioeconomic backgrounds. We draw on the early school-aged wave of a diverse sample of children followed from preschool age to early school-age. In this study, we preliminarily investigate the relationship between ACEs and inflammatory profiles (i.e., pro- and anti-) during early childhood, and examine whether patterns differ for racial/ethnic subgroups. We analyzed measures available for a diverse cohort of children followed since preschool, who were oversampled for racial and ethnicity minority groups and measured at serial time points regarding adverse childhood experiences, (Briggs-Gowan et al., 2019) to lay the foundation for subsequent population-based studies. We hypothesize that children with higher ACE scores (i.e., multiple types of ACE exposures) will have higher levels of inflammation as compared to their peers with fewer or no ACEs. We also hypothesized that racial/ethnic minority children will be more likely to demonstrate pro-inflammatory profiles. Our findings may more fully inform interventions that seek to ameliorate disparities in chronic disease.

2. Methods

2.1. Data

We used data from the Multidimensional Assessment of Preschoolers Study (MAPS), a racially/ethnically and socioeconomically diverse cohort recruited from pediatric clinics in an urban setting. The study began in 2011, with data collected for the early school age wave through 2017. MAPS was designed to characterize neurodevelopmental profiles of young children that predict the early onset of mental health problems, as well as to examine the role of violence exposure in these pathways (N = 497). As such, the MAPS oversampled families based on domestic violence exposure (Wakschlag et al., 2012). The MAPS cohort has been followed since preschool age (3–5 years) with follow-up waves at early school age (6–8 years) and ongoing data collection at pre-adolescence (9–10 years) (Briggs-Gowan et al., 2019; Greene et al., 2018; Wiggins et al., 2018; Wakschlag et al., 2015). To enhance the generalizability and avoid confounding of race/ethnicity and poverty, the MAPS sample was broadly stratified by demographic indicators to ensure that both poor and non-poor families of color were included. Written informed consent was obtained from the participants. A detailed description of MAPS is available elsewhere (Briggs-Gowan et al., 2019; Wakschlag et al., 2015; Nichols et al., 2015).

Among the sample population, children were included if they had complete ACEs data at the preschool (PS) and early school age (ESA) waves and also had a venous blood draw at the ESA wave (N = 126). Four children were removed from the sample because of the following medical conditions: hemophilia (N = 1), sickle cell anemia (N = 1), cystic fibrosis (N = 1), and leukemia (N = 1). Our study sample consisted of 122 children who had venous blood draws at the ESA wave and complete ACE-relevant data. Sociodemographic data (i.e., gender, race/ethnicity) was drawn from the PS wave and combined with the ESA wave age, socioeconomic, inflammatory markers, ACE history, and medical conditions. The mean age for the sample was 7.6 years and girls represented nearly 48% of the sample at the ESA wave, which was not statistically different across the ACE groups. However, socioeconomic status and race and ethnicity were statistically different across ACE groups.

2.2. Measures

2.2.1. ACEs & early life stress exposure

MAPS has a wide breadth of data about stress and stressful events, using prospective and timeline follow-back methods to assess early life adverse exposures beginning from birth (Demir-Lira et al., 2016; O'Dor et al., 2017). While children at the PS wave were 3–5 years old, parent respondents reported events that occurred since birth, thus representing a lifetime history of adverse events. Traditionally-defined ACE measures (Felitti et al., 1998) were not administered; thus the ACEs measure used in this study was constructed from responses to existing survey and interview questions. The original ACE measures encompass three domains, which are abuse, neglect, and household dysfunction. Specifically, ACEs address physical and emotional abuse and neglect, sexual abuse, and household dysfunction, which includes living in a household with domestic violence, substance abuse, mental illness, divorce, and parental incarceration (Felitti et al., 1998). Children with reports of any adverse events in a particular domain are given a score of one, irrespective of the number of times a particular event was experienced. Thus, conventionally-scored ACEs are sum scores that range from zero to ten.

We identified 59 unique items from existing MAPS surveys and interviews that indicate one of the extant ACE domains. Based on these items, we developed and scored ACEs at both PS and ESA wave. We utilized the ACE scores at the ESA wave because they encompassed the cumulative number of ACEs that a child may have experienced over their lifetime. The measures used in MAPS to create our ACEs measure included the MAPS family history questionnaire. Family Socialization Interview, Conflict Tactics Scale, Stressful Life Events, Child Life Events Scale, Preschool Age Psychiatric Assessment, and Kiddie-SADS.

2.2.2. MAPS family history questionnaire

MAPS respondents were ask if "you or your partner had serious psychiatric/mental health problems." Respondents were also asked about drug or alcohol problems during 3 time points between the PS wave and the ESA wave.

2.2.3. Family Socialization Interview—Revised (FSI-R)

The *Family Socialization Interview* is an open-ended, semi-structured interview and a gold-standard interview method to assess parenting style and discipline through multiple developmental periods (O'Dor et al.,

2017; Dodge et al., 1994). The FSI-R was created to be used within families from a diverse set of backgrounds. The FSI-R also assesses child and family stressors. There are 42 stressful life events queried (e.g., extended parent-child separation, death of a close family member, family conflict, financial instability, medical problems, and homelessness), beginning at the child's birth to one year of age and within the last year (O'Dor et al., 2017). Five domains from the FSI-R were used to build the cumulative ACEs measure for the study. These included: parent separation, divorce, parent drug or alcohol problem, mother or father incarceration, and other parent-child separation.

2.2.4. Conflict Tactics Scale (CTS)

The *Conflict Tactics Scale* is a self-report survey that measures the presence of conflict within families and has been used to measure the presence of child maltreatment in a household. The CTS also assesses the frequency with which certain disciplinary tactics are used (Straus, 1979; Straus and Gelles, 1989; Straus et al., 1996, 1998). Within the MAPS cohort, intimate partner violence was assessed with the partner version of this measure (CTS2) (Straus et al., 1996). Respondents report tactics regarding conflict resolution with children and with their partners, using a Likert scale, from physical violence to discussion. Together these two measures contributed to multiple ACE domains, including verbal and physical abuse, emotional and physical neglect, and domestic violence.

2.2.5. Stressful life events (SLE) Child

Stressful Life Events (SLE) Child is an adaptation of the social readjustment rating scale with supplemental items of relevance to families with young children (SRRS) (Holmes and Rahe, 1967). This scale is a list of life events including divorce/separation, household drug or alcohol problems, and incarceration, which were used in the creation of the ACEs measure.

2.2.6. Child Life Events Scale (CLES)

Child Life Events Scale (CLES) assesses recent stressful life events in children (Carter and Briggs-Gowan, 1998; Briggs-Gowan et al., 2012). The CLES includes four items which contributed to assessment of the domestic violence ACE in the MAPS cohort. Specifically, the CLES asked "has your child seen someone: hit, push, or kick a family member, threaten a family member, use a weapon to hurt or threaten a family member, or seen family members arguing very loudly or fighting".

2.2.7. Preschool Age Psychiatric Assessment (PAPA)

The PAPA is a semi-structured diagnostic interview that assesses psychopathology in young children (Egger and Angold, 2004). For this study, questions from PAPA were used to capture sexual abuse and rape histories. The PAPA includes questions regarding sexual abuse such as: "has anyone ever touched him/her [child is the reference] in places where they shouldn't", "has anyone ever touched him/her in ways that made him/her feel uncomfortable?" The PAPA was also used to ascertain divorce and separation histories. Respondents were asked: "have you and your partner split up", "has s/he [child is the reference] ever had a parent get a divorce?" (Egger et al., 2004)

2.2.8. Kiddie-SADS (K-SADS)

The K-SADS is a semi-structured interview adapted from the Schedule for Affective Disorders and Schizophrenia (SADS), (Endicott and Spitzer, 1978) which characterizes symptoms over the last week and intense symptoms over the last 12 months in children 6–17 (Chambers et al., 1985). Questions from the K-SADS were used to identify sexual abuse and rape histories in addition to the PAPA.

2.2.9. Cumulative ACEs

Cumulative scores for ACEs in MAPS were derived from the aforementioned scales and interviews. In our sample the number of ACEs ranged from 1 to 8. The sample had a minimum ACE score of 1 because all but one parent reported yelling, shouting, or screaming at their child. Additionally, MAPS, oversampled families experiencing domestic violence (DV) and this household exposure to DV may have placed children at higher risk for experiencing the verbal abuse ACE. For each ACE, a score was calculated based on a dichotomous 0/1, if an ACE occurred or not. Individual ACEs were added to create a total ACE score. Notably, as this cohort has data on ACEs longitudinally, children may have experienced certain ACEs more than once, still, both similar experiences were counted as one because the ACE score is a cumulative lifetime score and does not account for frequency.

2.3. Inflammation

A health disparities supplement was designed to examine inflammation as a mechanism of stress on psychopathology risk. Of the 497 children who participated in the longitudinal sample, 24.5% of mothers and children mutually consented/assented for the inflammatory blood draw and were included in the analysis for an inflammatory sub-sample of 122. This sample did not differ from others in the larger sample in terms of sex ($p = 0.39$), race ($p = 0.35$), age ($p = 0.08$), or poverty, where data was available ($p = 0.946$). With regards to age, the study specifically collect age at the time of the blood draw for those in the sample, but not for the larger sample and thus was assessed at the closest assessment date. Also, due to the cumulative measure of ACEs, those in the inflammation sample were more likely to have complete data at all waves and therefore, more likely to have ACEs ($p < 0.0001$).

Pro-inflammatory markers (IL-1 β, IL-6, CRP, and TNF-α) and anti-inflammatory markers (IL-4, IL-10) were collected from serum and stimulated with peripheral blood mononuclear cells (PBMCs). Venous blood was collected from each of the 122 study participants and placed into tubes containing sodium heparin (for lymphocytes) or with nothing (for serum). Although assessment of circulating cytokines is a common approach, stimulated cytokine production from immune cell activation was used to better assess immune cell response to activation triggers and detect the presence of low-level inflammation to better characterize the Th1 vs.Th2 type response. PBMCs were stimulated in different cultures, with three different mitogenic agents targeted to three cell populations: CpG oligodeoxynucleotide (CpG) for B cells (monocytes), lipopolysaccharide (LPS) for B cells, and anti-CD3 antibody for T cells and harvest the cell supernatants after 48 h. The evaluation of cytokines in serum and cell supernatants (i.e., lipopolysaccharide, CpG oligodeoxynucleotide, and anti-CD-3 stimulation) was performed using commercial ultrasensitive solid phase ELISA kits, following manufacturer protocol (Bouchard et al., 2016). This study examined the pro-inflammatory and anti-inflammatory markers individually, as well as creating and examining both a pro-inflammatory and anti-inflammatory composite measure.

2.4. Covariates

Covariates in multivariate analyses were PS wave sociodemographic characteristics including, age, sex, race/ethnicity, and socioeconomic status, defined as poor, borderline, and non-poor using the federal poverty guidelines based on annual household income and household size (Wakschlag et al., 2012). We also controlled for factors from the ESA wave that could elevated pro-inflammatory profiles, such as mother-reported asthma and BMI (calculated, based on objectively measured height and weight).

2.5. Statistical analysis

Pro-inflammatory markers and two anti-inflammatory markers were analyzed as the primary outcome measurements. These markers were log-transformed prior to completing principal components analyses. Principal components analyses yielded three components: a pro-inflammatory component (IL-1 β, IL-6, and TNF), an anti-inflammatory component (IL-4 and IL-10), and a single pro-inflammatory marker that

was not associated with any other biomarkers (CRP). All biomarkers within a given component correlated greater than 0.90 with all other biomarkers within that component. Pro- and anti-inflammatory component scores accounted for 93.3% and 91.8% of variance in observed scores, respectively. Pro- and anti-inflammatory scores were correlated (r=.60). Inflammation variables showed highly skewed distributions, and generally an unexpectedly high positive correlation between pro- and anti-inflammatory markers. The correlations showed a range of min of -.03 to max of 0.86. Using Pearson correlation analyses, pro-inflammatory markers (IL-1 β, IL-6, and TNF) were combined to generate a composite measurement, anti-inflammatory markers (IL-4 and IL-10) were combined to generate a composite measurement, and CRP was analyzed independently. Regressions yielded similar patterns of results and statistical significance as compared to the correlations and thus the correlations are presented. This study was approved by the Northwestern University Institutional Review Board.

3. Results

Fig. 1a presents the ACEs reported by the sample at the ESA wave. This figure draws on data collected from the PS wave up to the ESA and represents a longitudinal and cumulative representation of the ACEs in this cohort. Given the timeline of data collection, this measure captures the lifetime number of ACEs experienced by children (Fig. 1a).

Table 1 illustrates the sociodemographic characteristics of our sample by ACEs, highlighting the relatively high amount of exposure in the MAPS sample. Of the 122 children, no children had experienced 0 ACES, 7% had 1 ACE, 51% had 2-3 ACEs, and 42% had 4 or more ACEs accumulated by the ESA wave. Non-Hispanic white children represented 21% of the sample population but accounted for nearly 67% of the children with 1 ACE and less than 10% of the 4 or more ACEs group. While Non-Hispanic Black children represented 51% of the sample, they had a disproportionally low reports of 1 ACE (11%) and higher reports of having 4 or more ACEs (63%). Hispanic children most commonly reported having 2–3 ACEs.

Children from non-poor households (67%) were more likely to report having 1 ACE as compared to their peers who were more likely to report 4 or more ACEs (51.1%). More than half of the children with 4 or more ACEs were from poor households. Also, about 9% of children in the sample had a serious medical condition, which included epilepsy, convulsions, genetic conditions, or other medical conditions. Parent-reported asthma diagnosis was examined separately from other medical conditions; 23% of parents reported their child had asthma, while over 30% of children with asthma were likely to report having two or three ACEs. Finally, BMI and BMI-z was also examined separately, showing an increase as ACE score increased (Table 1).

Fig. 1b illustrates the type of ACEs reported by the sample, with the most common ACEs being verbal abuse (99%), physical abuse (75%), and separation/divorce (57%).

Table 2 displays the correlations for inflammation by individual and cumulative ACEs in the ESA wave. Overall, there were no significant correlations between cumulative ACEs and inflammatory markers. However, parental substance abuse, an individual ACE, was positively correlated with a pro-inflammatory profile at the ESA wave ($p<.05$). Specifically, having a substance abuse ACE was associated with higher levels of pro-inflammatory markers – IL-1 β, IL-6, and the pro-inflammation biomarker composite measure. Although, not statistically signifcant, sexual abuse was cooorrelated with CRP at p=.10 and any history of parental incarceration was correlated with the anti-inflammatory marker, IL-4 at $p = 0.08$. Verbal abuse was positively correlated with all the pro-inflammatory biomarkers, IL-10 (an anti-inflammatory biomarker) and both the pro-inflammatory and anti-inflammatory composite biomarker measurement. However, 99% of the analytic sample reported verbal abuse; therefore, the distribution of this particular ACE was skewed and not included in the results. While the correlation between substance abuse and pro-inflammatory markers was statistically significant, cumulative ACEs at the ESA wave did not demonstrate a statistically significant relationship with either the inflammatory biomarkers or the pro- or anti-inflammatory composite measures.

We next explored whether there were differences in magnitude and direction of these associations by gender and race (Table 3). ACEs and CRP were positively associated in Hispanic children ($p < 0.05$). Exploratory analyses revealed apparent differences in magnitude and directionality exist among subgroups. Specifically, ACEs had a negative correlation with CRP in females ($p<.05$), while the correlation was positive in males ($p = NS$) and positively associated in Hispanic children ($p < 0.05$). ACEs had a negative correlation with IL-1 β and TNF-alpha in males, while those correlations were positive in females (all values, $p = NS$).

Additionally, similar differences existed for race and ethnicity. ACEs were negatively correlated to CRP for both Blacks and Whites, but was 2.5 times higher and positively correlated in Hispanics. Also, ACEs were negatively correlated with IL-1 β in both Hispanics and whites, but the correlation was positive in Blacks. Additionally, correlations were examined for children with low ACEs (1–3) versus high ACEs (4 or more). These analyses did not demonstrate any statistically significant correlations between high ACE score and inflammation (results not shown).

4. Discussion

This study was a preliminary investigation of the relationship between ACEs and inflammatory profiles in a demographically diverse cohort of early school-aged children with cumulative ACE histories from birth. We found expected associations between specific ACEs and inflammatory markers but not an overall association between lifetime ACEs and inflammation overall. There was a correlation between household substance abuse and pro-inflammatory biomarkers in childhood. Our findings suggest that living in a household with substance abuse may begin to influence the inflammatory profiles of individuals before 8 years old. The exposure to ACEs may contribute to higher levels of inflammation that may place children at risk for the development of chronic diseases later in life (Egger et al., 2004). This study contributes to the literature seeking to describe the biological and physiological embedding of ACEs in the body (Berens et al., 2017).

ACEs may serve as a persistent stimulus giving rise to the production of cytokines, generally produced in response to tissue damage or injury and activates the immune system. Cytokines can also be activated after experiencing a physical, emotional, or psychological stress, such as an ACE exposure, increasing and intensifying the body's physiological inflammatory response (Nusslock and Miller, 2016). The ongoing production of cytokines disseminates the immune response diffusely, resulting in a low-grade inflammatory state (Lacy and Stow, 2011) and subsequently, the development of illnesses and diseases (Wyman et al., 2007; Caserta et al., 2008) in childhood and throughout the life course (Hostinar et al., 2018). Particularly, cytokines such as IL-1 β, TNF-alpha, CRP (Danese et al., 2007), and IL-6 have been implicated in extant studies as markers of chronic inflammation and may have a role in the development of undesirable health, including poor physical, mental, (Dowlati et al., 2010; Baker et al., 2012; Valkanova et al., 2013; Howren et al., 2009) and neurodevelopmental outcomes (Nikas, 2013; Wright et al., 2006; Ganguly and Brenhouse, 2015).

ACEs may cause both immunologic activation and impairment leading to chronic disease; however, the influence of ACEs may also be amplified during more developmentally-sensitive time periods (Berens et al., 2017). Specifically, Measelle et al. conducted a study of nearly 50 primarily white infants to examine the relationship between early adversity and pro-inflammatory cytokines and found a positive correlation (Measelle and Ablow, 2018). As the study focused on infants, it examined adversity within the contexts of socioeconomic status, familial stress, maternal depression, and secure attachment. Maternal stress and socioeconomic disadvantage predicted higher CRP levels in infancy

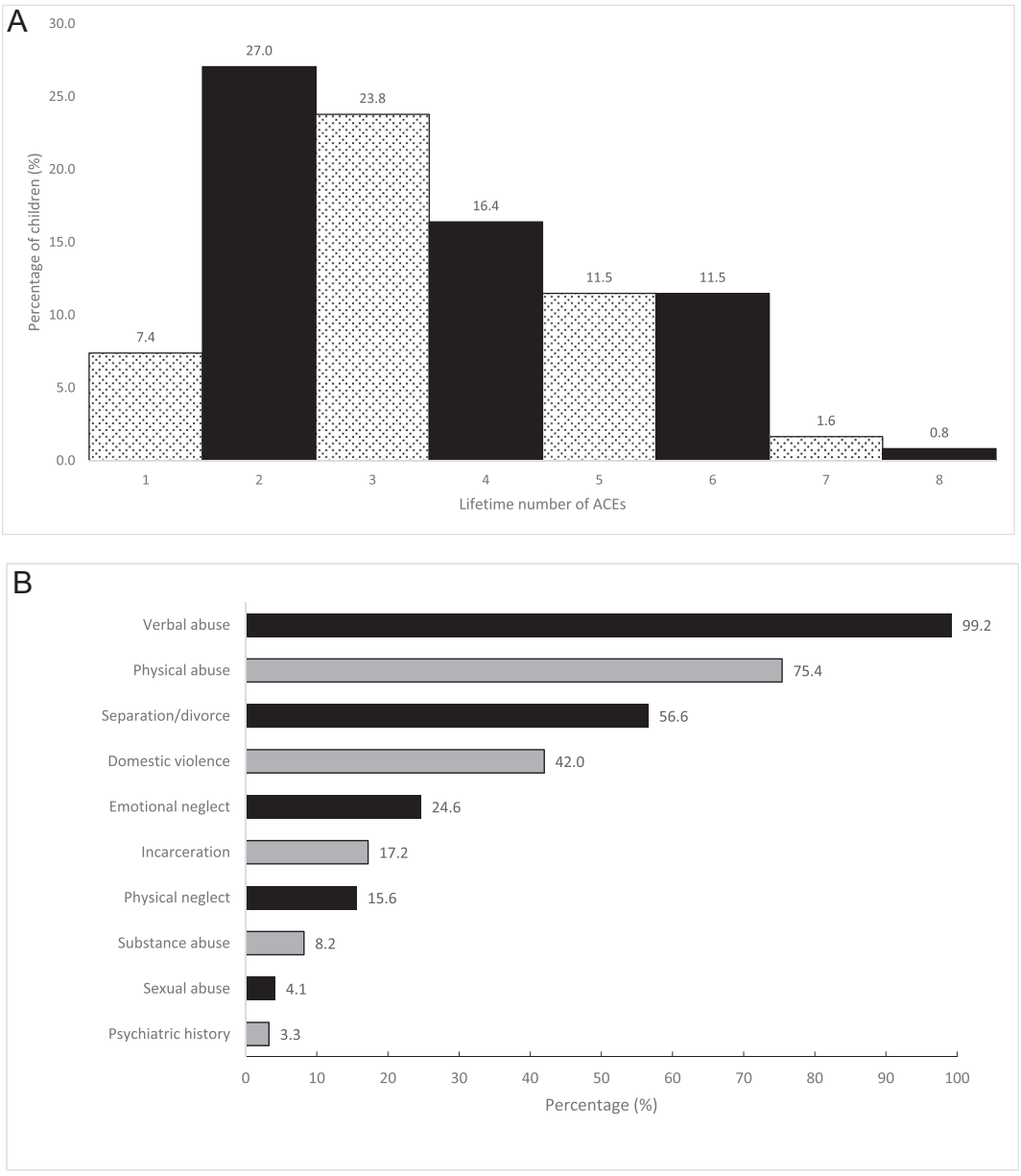

Fig. 1. A: Cumulative Number of ACEs reported by MAPS Analysis Sample (From Birth to Early School Age Wave).
B: Type of ACEs reported by MAPS Analysis Sample (Early School Age Wave).

(David et al., 2017). Another study that examined early adversity and inflammation (i.e., IL-1 β and CRP) in preschool-aged children found a relationship between lifetime stressors and traumatic life events and IL-1 β, but no relationship was seen with CRP (Tyrka et al., 2015). Similar findings have been reported among racial/ethnic minorities. A study of 112 school-aged Hispanic children suggested that adversity was an independent predictor of TNF-alpha, another inflammatory cytokine (Dixon et al., 2009). Similarly, a review by Slopen et al. found an overall positive trend between adversity and inflammation in children; however, there was heterogeneity among the studies' findings and variable study quality (Slopen et al., 2012).

Our study examined multiple domains of adversity and did not find strong or consistent relationships with either cumulative ACEs or inflammation among ACE groups (low versus high). We also found significant heterogeneity among subgroups, namely gender and race/ethnicity. Although these findings must be replicated and extended in larger samples with strong representation of these subgroups, these findings suggest the possibility that these mechanistic pathways may differ based on race/ethnicity and gender. Our sample of early school-aged children may not have yet developed the expectant pro-inflammatory profile, due to timing after ACE exposure or being outside of a sensitive period. Also, our findings suggest that there are differences by gender and by race and ethnicity that may be masking the overall relationships between ACEs and inflammation in childhood that are often reported in aggregate.

Though we did not find a relationship between ACEs and inflammation overall, parental substance abuse was positively correlated with both IL-1 β and IL-6. These findings are consistent with other studies that have shown a relationship between specific ACEs and inflammation. A recent study of Tanzanian children found a relationship between maternal domestic violence and CRP levels in children with active or recent infections (Slopen et al., 2018). Though our study did not demonstrate a statistically significant relationship between cumulative ACEs and CRP, sexual abuse had a positive correlation with CRP approaching statistical significance ($p = 0.10$). Broadly, our study provides supporting evidence for the presence of immune system activation and the presence of

Table 1
Study sample characteristics by adverse childhood experiences, MAPS (2011–2017) (N = 122).

	Total Sample N = 122		1 ACE N = 9		2-3 ACEs N = 62		4 + ACEs N = 51		P-value
	N	%	N	%	N	%	N	%	%
Age, mean (SD)	7.6 (7.4,7.8)<		7.5 (6.8,8.1)		7.5 (7.2,7.7)		7.8 (7.5,8.1)		0.14
Gender, % Female	58	47.5	5	55.6	32	51.6	21	41.2	0.48
Race/Ethnicity									0.01**
Non-Hispanic White	25	20.5	6	66.7	14	22.6	5	9.8	
Non-Hispanic Black	62	50.8	1	11.1	29	46.8	32	62.7	
Hispanic	35	28.7	2	22.2	19	30.6	14	27.5	
Socioeconomic Status									0.02*
Poor	45	38.8	2	22.2	19	31.7	24	51.1	
Borderline	23	19.8	1	11.1	10	16.7	12	25.5	
Not Poor	48	41.4	6	66.7	31	51.7	11	23.4	
Medical Conditions	10	8.8	0.0	0.0	12.8	10.5	10.1	8.3	0.58
Asthma	28	23.0	0.0	0.0	17	27.4	11	21.6	0.18
BMI, mean [95% CI]	17.8 [17.1,18.5]		16.0 [14.8,17.2]		17.6 [16.7, 18.6]		18.4 [17.3,19.5]		0.18
BMI z-score, mean [95% CI]	0.55 [0.35, 0.76]		.04 [-0.56, 0.64]		0.51 [0.22,0.80]		0.70 [0.36, 1.04]		0.26

P-values reflect statistical comparisons across the categories of ACEs.
**Comparison between 1 ACE and 0 ACE p = 0.06; between 2 + ACE and 0 ACE p < 0.001.

Table 2
Unadjusted Correlations between individual and cumulative ACEs‡ and Inflammation‡‡ at the Early School Age Wave.

Type of ACE	CRP	IL-1 β	IL-6	TNF-a	Pro-	IL4	IL10	Anti-
Physical Abuse	−0.02	0.03	0.07	0.01	0.04	−0.07	−0.10	−0.09
Sexual Abuse	0.15	0.04	0.04	0.10	0.08	0.05	0.09	0.07
Emotional Neglect	0.09	0.07	0.0	0.03	0.06	−0.14	−0.07	−0.11
Physical Neglect	0.01	−0.11	−0.06	−0.08	−0.09	−0.14	−0.15	−0.15
Parental Separation or Divorce	0.09	−0.05	0.03	0.03	0.00	−0.06	−0.07	−0.07
Domestic Violence	0.05	0.03	0.09	0.10	0.07	0.10	0.09	0.10
Substance Abuse	0.04	0.17*	0.19*	0.14	0.18*	0.06	0.03	0.05
Parental Incarceration	−0.14	0.03	0.10	0.13	0.09	0.16	0.08	0.13
Parental psychiatric history	0.01	0.12	0.11	0.10	0.11	0.07	0.03	0.05
High (4 + ACEs) vs. Low (1–3 ACEs)	0.03	−0.00	0.08	0.07	0.05	−0.01	−0.04	−0.03
Cumulative ACEs	0.05	0.05	0.15	0.12	0.11	−0.02	−0.02	−0.02

‡ The verbal abuse ACE was removed from the table due to the skewed distribution, 99% of participants (N = 121) reported verbal abuse.
‡‡ Values Log transformed.
*p<.05.

Table 3
Correlations between cumulative ACEs and Inflammation by gender and race.

	CRP	IL-1 β	IL-6	TNF-a	Pro-	IL4	IL10	Anti-
Gender								
Female	−0.02	0.12	0.24	0.20	0.19	−0.08	−0.02	−0.05
Male	0.13	−0.03	0.05	−0.01	0.01	−0.03	−0.06	−0.05
Race/Ethnicity								
Black	−0.14	0.18	0.20	0.17	0.19	0.04	0.08	0.06
Hispanic	0.35*	−0.03	0.13	0.12	0.08	−0.09	−0.04	−0.07
White	−0.04	−0.17	−0.17	0.01	−0.12	0.18	0.04	0.11

‡ Values Log transformed; *p<.05.

inflammation after experiencing specific ACEs during childhood. Further, this study demonstrates that while currently ACEs are generally considered equally in the literature, specific ACEs may have a differential impact on inflammation and possibly disease in childhood as well as in later life. This is aligned with extant work regarding the variation in inflammatory response following adversities. A study based in Germany found a relationship between IL-4 with parental separation; however, this relationship was not evident with other adversities (Herberth et al., 2008).

An important limitation of this study is its sample size; in addition, the MAPS cohort was not formed to study the influence of ACEs or adversities on inflammation as a primary outcome. However, this sample has collected rigorous, longitudinal data from the prenatal period about stressors and adversities. Additionally, this cohort has substantial racially and socioeconomic diversity, which allow us to preliminarily examine the influence of stress on minority children who are often understudied and in whom it is difficult to disentangle socioeconomic status. Another limitation is that we collected data on 6 inflammatory biomarkers, and ACEs may influence additional biomarkers that were not captured in this study (Wolf et al., 2008). Timing could be another important consideration for this study, as we found a relationship with inflammation and the household substance abuse ACE during early school age; however, other ACEs may influence inflammation at older ages, (Slopen et al., 2013) which this study did not capture. Further, the severity and chronicity of the ACEs may drive the inflammatory pathway. For this study, our threshold of abuse may have been too low, leading to conservative

correlations between ACEs and inflammation in childhood. However, measuring ACE severity and chronicity is a limitation with the research that utilizes the traditional ACE measure, as severity and chronicity are not examined. Finally, ACEs were parent-reported, and given the sensitive nature of the questions, ACEs may have been under-reported.

5. Implications

Children with histories of ACEs, especially with familial substance abuse, may be at increased risk of having elevated pro-inflammatory markers. Inflammation is an important mechanism in the development of chronic diseases, such as asthma, diabetes and cardiovascular disease and understanding the relationship between ACEs and inflammation may be important factor in the prevention, especially those that have a disproportionate impact on racial and ethnic minorities.

Future studies may benefit from focusing on older children and adolescents, collecting self-reported ACEs, and directly assessing cardiometabolic health. Screening children and families within medical visits may be an effective way to identify children at the highest risks for ACEs (Selvaraj et al., 2018). Identifying the children at highest risk for ACEs and those specific ACEs that influence systemic inflammation may allow future research to develop interventions to best treat and ultimately, prevent ACE exposures and the inflammatory sequelae. In doing so, ACEs may provide a pathway towards the prevention of chronic diseases and reducing disparities.

Declaration of competing interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbih.2019.100006.

References

Anda, R.F., Brown, D.W., Dube, S.R., Bremner, J.D., Felitti, V.J., Giles, W.H., 2008. Adverse childhood experiences and chronic obstructive pulmonary disease in adults. Am. J. Prev. Med. 34 (5), 396–403. https://doi.org/10.1016/j.amepre.2008.02.002.

Baker, D.G., Nievergelt, C.M., O'Connor, D.T., 2012. Biomarkers of PTSD: neuropeptides and immune signaling. Neuropharmacology 62 (2), 663–673. https://doi.org/10.1016/j.neuropharm.2011.02.027.

Berens, A.E., Jensen, S.K.G., Nelson 3rd, C.A., 2017. Biological embedding of childhood adversity: from physiological mechanisms to clinical implications. BMC Med. 15 (1), 135. https://doi.org/10.1186/s12916-017-0895-4.

Bouchard, L.C., Antoni, M.H., Blomberg, B.B., et al., 2016. Postsurgical depressive symptoms and proinflammatory cytokine elevations in women undergoing primary treatment for breast cancer. Psychosom. Med. 78 (1), 26–37. https://doi.org/10.1097/PSY.0000000000000261.

Briggs-Gowan, M.J., Carter, A.S., Ford, J.D., 2012. Parsing the effects violence exposure in early childhood: modeling developmental pathways. J. Pediatr. Psychol. 37 (1), 11–22. https://doi.org/10.1093/jpepsy/jsr063.

Briggs-Gowan, M.J., Estabrook, R., Henry, D., et al., 2019. Parsing dimensions of family violence exposure in early childhood: shared and specific contributions to emergent psychopathology and impairment. Child Abuse Negl. 87, 100–111. https://doi.org/10.1016/j.chiabu.2018.06.006.

Carter, A.S., Briggs-Gowan, M.J., 1998. Child Life Events Screener.

Caserta, M.T., O'Connor, T.G., Wyman, P.A., et al., 2008. The associations between psychosocial stress and the frequency of illness, and innate and adaptive immune function in children. Brain Behav. Immun. 22 (6), 933–940. https://doi.org/10.1016/j.bbi.2008.01.007.

Chambers, W.J., Puig-Antich, J., Hirsch, M., et al., 1985. The assessment of affective disorders in children and adolescents by semistructured interview: test-retest reliability of the Schedule for affective disorders and Schizophrenia for school-age children, present episode version. Arch. Gen. Psychiatr. 42 (7), 696–702. https://doi.org/10.1001/archpsyc.1985.01790300064008.

Danese, A., Pariante, C.M., Caspi, A., Taylor, A., Poulton, R., 2007. Childhood maltreatment predicts adult inflammation in a life-course study. Proc Natl Acad Sci U A 104 (4), 1319–1324. https://doi.org/10.1073/pnas.0610362104.

David, J., Measelle, J., Ostlund, B., Ablow, J., 2017. Association between early life adversity and inflammation during infancy. Dev. Psychobiol. 59 (6), 696–702. https://doi.org/10.1002/dev.21538.

Deighton, S., Neville, A., Pusch, D., Dobson, K., 2018. Biomarkers of adverse childhood experiences: a scoping review. Psychiatry Res. 269, 719–732. https://doi.org/10.1016/j.psychres.2018.08.097.

Demir-Lira, Ö.E., Voss, J.L., O'Neil, J.T., Briggs-Gowan, M.J., Wakschlag, L.S., Booth, J.R., 2016. Early-life stress exposure associated with altered prefrontal resting-state fMRI connectivity in young children. Dev Cogn Neurosci 19, 107–114. https://doi.org/10.1016/j.dcn.2016.02.003.

Dixon, D., Meng, H., Goldberg, R., Schneiderman, N., Delamater, A., 2009. Stress and body mass index each contributes independently to tumor necrosis factor-alpha production in prepubescent Latino children. J. Pediatr. Nurs. 24 (5), 378–388. https://doi.org/10.1016/j.pedn.2008.02.034.

Dodge, K.A., Pettit, G.S., Bates, J.E., 1994. Socialization mediators of the relation between socioeconomic status and child conduct problems. Child Dev. 65 (2 Spec No), 649–665.

Dowlati, Y., Herrmann, N., Swardfager, W., et al., 2010. A meta-analysis of cytokines in major depression. Biol. Psychiatry 67 (5), 446–457. https://doi.org/10.1016/j.biopsych.2009.09.033.

Egger, H.L., Angold, A., 2004. The Preschool Age Psychiatric Assessment (PAPA): A Structured Parent Interview for Diagnosing Psychiatric Disorders in Preschool Children. in: Handbook of Infant, Toddler, and Preschool Mental Health Assessment. Oxford University Press, New York, NY, US, pp. 223–243.

Egger, H.L., Ascher, B., Angold, A., 2004. The electronic preschool age psychiatric assessment (ePAPA). Print version 2.0.7. http://devepi.duhs.duke.edu/eMeasures/PAPA%20(for%20review%20only).pdf.

Endicott, J., Spitzer, R.L., 1978. A diagnostic interview: the Schedule for affective disorders and Schizophrenia. Arch. Gen. Psychiatr. 35 (7), 837–844. https://doi.org/10.1001/archpsyc.1978.01770310043002.

Felitti, V.J., Anda, R.F., Nordenberg, D., et al., 1998. Relationship of childhood abuse and household dysfunction to many of the leading causes of death in adults. The Adverse Childhood Experiences (ACE) Study. Am. J. Prev. Med. 14 (4), 245–258.

Ganguly, P., Brenhouse, H.C., 2015. Broken or maladaptive? Altered trajectories in neuroinflammation and behavior after early life adversity. Dev Cogn Neurosci 11, 18–30. https://doi.org/10.1016/j.dcn.2014.07.001.

Greene, C.A., Chan, G., McCarthy, K.J., Wakschlag, L.S., Briggs-Gowan, M.J., 2018. Psychological and physical intimate partner violence and young children's mental health: the role of maternal posttraumatic stress symptoms and parenting behaviors. Child Abuse Negl. 77, 168–179. https://doi.org/10.1016/j.chiabu.2018.01.012.

Herberth, G., Weber, A., Röder, S., et al., 2008. Relation between stressful life events, neuropeptides and cytokines: results from the LISA birth cohort study. Pediatr Allergy Immunol Off Publ Eur Soc Pediatr Allergy Immunol 19 (8), 722–729. https://doi.org/10.1111/j.1399-3038.2008.00727.x.

Holmes, T.H., Rahe, R.H., 1967. The social readjustment rating scale. J. Psychosom. Res. 11 (2), 213–218. https://doi.org/10.1016/0022-3999(67)90010-4.

Hostinar, C.E., Nusslock, R., Miller, G.E., 2018. Future directions in the study of early-life stress and physical and emotional health: implications of the neuroimmune network hypothesis. J. Clin. Child Adolesc. Psychol. 47 (1), 142–156. https://doi.org/10.1080/15374416.2016.1266647.

Howren, M.B., Lamkin, D.M., Suls, J., 2009. Associations of depression with c-reactive protein, IL-1, and IL-6: a meta-analysis. Psychosom. Med. 71 (2), 171–186. https://doi.org/10.1097/PSY.0b013e3181907c1b.

Huang, H., Yan, P., Shan, Z., et al., 2015. Adverse childhood experiences and risk of type 2 diabetes: a systematic review and meta-analysis. Metabolism 64 (11), 1408–1418. https://doi.org/10.1016/j.metabol.2015.08.019.

Hughes, K., Bellis, M.A., Hardcastle, K.A., et al., 2017. The effect of multiple adverse childhood experiences on health: a systematic review and meta-analysis. Lancet Public Health 2 (8), e356–e366. https://doi.org/10.1016/S2468-2667(17)30118-4.

Jakubowski, K.P., Cundiff, J.M., Matthews, K.A., 2018. Cumulative childhood adversity and adult cardiometabolic disease: a meta-analysis. Health Psychol Off J Div Health Psychol Am Psychol Assoc 37 (8), 701–715. https://doi.org/10.1037/hea0000637.

Lacy, P., Stow, J.L., 2011. Cytokine release from innate immune cells: association with diverse membrane trafficking pathways. Blood 118 (1), 9–18. https://doi.org/10.1182/blood-2010-08-265892.

Llabre, M.M., Schneiderman, N., Gallo, L.C., et al., 2017. Childhood trauma and adult risk factors and disease in hispanics/latinos in the US: results from the hispanic community health study/study of latinos (HCHS/SOL) sociocultural ancillary study. Psychosom. Med. 79 (2), 172–180. https://doi.org/10.1097/PSY.0000000000000394.

Loria, H., Caughy, M., 2018. Prevalence of adverse childhood experiences in low-income latino immigrant and nonimmigrant children. J. Pediatr. 192, 209–215. https://doi.org/10.1016/j.jpeds.2017.09.056 e1.

Lupien, S.J., King, S., Meaney, M.J., McEwen, B.S., 2000. Child's stress hormone levels correlate with mother's socioeconomic status and depressive state. Biol. Psychiatry 48 (10), 976–980.

Measelle, J.R., Ablow, J.C., 2018. Contributions of early adversity to pro-inflammatory phenotype in infancy: the buffer provided by attachment security. Attach. Hum. Dev. 20 (1), 1–23. https://doi.org/10.1080/14616734.2017.1362657.

Nichols, S.R., Briggs-Gowan, M.J., Estabrook, R., et al., 2015. Punishment insensitivity in early childhood: a developmental, dimensional approach. J. Abnorm. Child Psychol. 43 (6), 1011–1023. https://doi.org/10.1007/s10802-014-9950-1.

Nikas, J.B., 2013. Inflammation and immune system Activation in aging: a mathematical approach. Sci. Rep. 3 https://doi.org/10.1038/srep03254.

Nusslock, R., Miller, G.E., 2016. Early-life adversity and physical and emotional health across the lifespan: a neuroimmune network hypothesis. Biol. Psychiatry 80 (1), 23–32. https://doi.org/10.1016/j.biopsych.2015.05.017.

Oh, D.L., Jerman, P., Silverio Marques, S., et al., 2018. Systematic review of pediatric health outcomes associated with childhood adversity. BMC Pediatr. 18 (1), 83. https://doi.org/10.1186/s12887-018-1037-7.

O'Connor, T.G., Moynihan, J.A., Caserta, M.T., 2014. Annual research review: the neuroinflammation hypothesis for stress and psychopathology in children–developmental psychoneuroimmunology. JCPP (J. Child Psychol. Psychiatry) 55 (6), 615–631. https://doi.org/10.1111/jcpp.12187.

O'Dor, S.L., Grasso, D.J., Forbes, D., et al., 2017. The family socialization interview-revised (FSI-R): a comprehensive assessment of parental disciplinary behaviors. Prev Sci Off J Soc Prev Res 18 (3), 292–304. https://doi.org/10.1007/s11121-016-0707-7.

Selvaraj, K., Ruiz, M.J., Aschkenasy, J., et al., 2018. Screening for toxic stress risk factors at well-child visits: the addressing social key questions for health study. J. Pediatr. 0 (0) https://doi.org/10.1016/j.jpeds.2018.09.004.

Slopen, N., Koenen, K.C., Kubzansky, L.D., 2012. Childhood adversity and immune and inflammatory biomarkers associated with cardiovascular risk in youth: a systematic review. Brain Behav. Immun. 26 (2), 239–250. https://doi.org/10.1016/j.bbi.2011.11.003.

Slopen, N., Kubzansky, L.D., McLaughlin, K.A., Koenen, K.C., 2013. Childhood adversity and inflammatory processes in youth: a prospective study. Psychoneuroendocrinology 38 (2), 188–200. https://doi.org/10.1016/j.psyneuen.2012.05.013.

Slopen, N., Zhang, J., Urlacher, S.S., De Silva, G., Mittal, M., 2018. Maternal experiences of intimate partner violence and C-reactive protein levels in young children in Tanzania. SSM Popul Health 6, 107–115. https://doi.org/10.1016/j.ssmph.2018.09.002.

Straus, M.A., 1979. Measuring intrafamily conflict and violence: the conflict tactics (CT) scales. J. Marriage Fam. 41 (1), 75–88.

Straus, M.A., Gelles, R.J., 1989. Physical Violence in American Families: Risk Factors and Adaptations to Violence in 8,145 Families, Revised edition. Transaction Publishers, New Brunswick.

Straus, M.A., Hamby, S.L., Boney-McCoy, S., Sugarman, D.B., 1996. The revised conflict tactics scales (CTS2) development and preliminary psychometric data. J. Fam. Issues 17 (3), 283–316. https://doi.org/10.1177/019251396017003001.

Straus, M.A., Hamby, S.L., Finkelhor, D., Moore, D.W., Runyan, D., 1998. Identification of child maltreatment with the Parent-Child Conflict Tactics Scales: development and psychometric data for a national sample of American parents. Child Abuse Negl. 22 (4), 249–270.

The prevalence of adverse childhood experiences, nationally, by state, and by race or ethnicity. Child Trends. https://www.childtrends.org/publications/prevalence-adverse-childhood-experiences-nationally-state-race-ethnicity. (Accessed 4 January 2019).

Tyrka, A.R., Parade, S.H., Valentine, T.R., Eslinger, N.M., Seifer, R., 2015. Adversity in preschool-aged children: effects on salivary interleukin-1beta. Dev. Psychopathol. 27 (2), 567–576. https://doi.org/10.1017/s0954579415000164.

Valkanova, V., Ebmeier, K.P., Allan, C.L., 2013. CRP, IL-6 and depression: a systematic review and meta-analysis of longitudinal studies. J. Affect. Disord. 150 (3), 736–744. https://doi.org/10.1016/j.jad.2013.06.004.

Wakschlag, L.S., Choi, S.W., Carter, A.S., et al., 2012. Defining the developmental parameters of temper loss in early childhood: implications for developmental psychopathology. JCPP (J. Child Psychol. Psychiatry) 53 (11), 1099–1108. https://doi.org/10.1111/j.1469-7610.2012.02595.x.

Wakschlag, L.S., Estabrook, R., Petitclerc, A., et al., 2015. Clinical implications of a dimensional approach: the normal:abnormal spectrum of early irritability. J. Am. Acad. Child Adolesc. Psychiatry 54 (8), 626–634. https://doi.org/10.1016/j.jaac.2015.05.016.

Wiggins, J.L., Briggs-Gowan, M.J., Estabrook, R., et al., 2018. Identifying clinically significant irritability in early childhood. J. Am. Acad. Child Adolesc. Psychiatry 57 (3), 191–199. https://doi.org/10.1016/j.jaac.2017.12.008 e2.

Wolf, J.M., Miller, G.E., Chen, E., 2008. Parent psychological states predict changes in inflammatory markers in children with asthma and healthy children. Brain Behav. Immun. 22 (4), 433–441. https://doi.org/10.1016/j.bbi.2007.10.016.

Wright, C.B., Sacco, R.L., Rundek, T.R., Delman, J.B., Rabbani, L.E., Elkind, M.S.V., 2006. Interleukin-6 is associated with cognitive function: the Northern Manhattan Study. J Stroke Cerebrovasc Dis Off J Natl Stroke Assoc 15 (1), 34–38. https://doi.org/10.1016/j.jstrokecerebrovasdis.2005.08.009.

Wyman, P.A., Moynihan, J., Eberly, S., et al., 2007. Association of family stress with natural killer cell activity and the frequency of illnesses in children. Arch. Pediatr. Adolesc. Med. 161 (3), 228–234. https://doi.org/10.1001/archpedi.161.3.228.