BIOLOGICAL EMBEDDING OF CHILD MALTREATMENT

BIOLOGICAL EMBEDDING OF CHILD MALTREATMENT: A SYSTEMATIC REVIEW OF BIOMARKERS AND RESILIENCE IN CHILDREN AND YOUTH

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TITLE: Biological Embedding of Child Maltreatment: A Systematic Review of Biomarkers and Resilience in Children and Youth

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LAY ABSTRACT

Child maltreatment is a serious problem linked to poor mental and physical health outcomes. The mechanisms of these links are not always clear, however biological changes observed in some maltreated individuals may play a role. Here, we systematically review literature related to two biomarkers of interest in maltreated children, telomere length and DNA methylation. Findings are varied; however, overall, they support an association between child maltreatment and changes in both biomarkers. We additionally discuss factors that may confer resilience related to these changes to highlight potential targets for future study and interventions.

ABSTRACT

Objective: Child maltreatment (CM) is a widespread problem associated with poor mental and physical health outcomes. The underlying mechanisms of this link are not always well understood, however certain biological changes observed in maltreated individuals may play a role in connecting experience and outcome. This review specifically focuses on two markers of biological embedding, DNA methylation (DNAm) and telomere length (TL) in maltreated children and youth. As biomarker changes are not uniform among maltreated children, we additionally discuss biological and environmental resilience factors that may contribute to variability. *Methods:* We conducted a systematic review of Medline, Embase and PsycInfo databases for studies examining DNAm and/or TL in maltreated children and youth. Methodological quality of the included articles was assessed using the Scottish Intercollegiate Guidelines Network (SIGN) checklists for cohort studies and randomized control trials. Data extraction focused on various factors including population and CM (type, chronicity, severity, and duration) characteristics. Results: The initial search returned 1,688 non-duplicate results, with 417 full text articles reviewed. Twenty-six articles from 16 studies were ultimately included of which 8 examined telomere length and 18 examined DNA methylation. *Conclusions:* While some heterogeneity of findings was found, evidence supports differential changes in both biomarkers associated with CM. This review enhances understanding of the constellation of biological changes related to CM and consideration of the important role of resilience factors in mitigating risk. Elucidating these factors may highlight targets for future study and intervention development.

Keywords: child maltreatment, telomere length, epigenetics, methylation, resilience

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LIST OF ABBREVIATIONS

ALSPAC	Avon Longitudinal Study of Parents and Children
BEIP	Bucharest Early Intervention Project
BMI	Body Mass Index
CEA	Child emotional abuse
CM	Child maltreatment
CPA	Child physical abuse
CpG	Cytosine Guanine site
CSA	Child sexual abuse
DMR	Differentially methylated region
DNAm	DNA methylation
EWAS	Epigenome-wide association study
FSIQ	Full Scale Intelligence Quotient
IPV	Intimate partner violence
QA	Quality assessment
RCT	Randomized controlled trial
SES	Socioeconomic status
SIGN	Scottish Intercollegiate Guidelines Network
TL	Telomere length

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Introduction

The ramifications of child maltreatment (CM) are serious and far reaching, affecting over 300 million children annually worldwide (Butchart et al., 2006). The World Health Organization recognizes four domains of CM, including physical, sexual and emotional abuse, and neglect (Butchart et al., 2006). Due to the frequency with which exposure to intimate partner violence (IPV) co-occurs with CM types and its documented negative effects on children, many researchers include IPV exposure as an additional CM subtype (Hamby et al., 2010). A 2014 meta-analysis by Stoltenborgh et al. found high prevalence of CM, ranging from 163/1000 to 363/1000 when assessed via self-report, and 3/1000 to 4/1000 when assessed via informant-report, depending on CM type (Stoltenborgh et al., 2014). These findings indicate that cases of CM substantiated through child welfare services grossly underrepresent true prevalence globally (Stoltenborgh et al., 2014; Cicchetti & Toth, 2005). Most children experiencing CM will experience more than one type (Hamby et al., 2010). Stoltenborgh and colleagues (2014) indicate a significant gap in the literature regarding published prevalence rates of concurrent CM types, despite this significant evidence that CM types often co-occur. Several factors have been associated with increased risk of CM. Low socioeconomic status (SES), parents with substance use disorders or mental health challenges, and parents who have been maltreated themselves are among several factors that increase risk of CM (Butchart et al., 2006).

CM has been associated with a variety of poor mental and physical health outcomes in childhood and across the lifespan (Hughes et al., 2017; Ridout et al., 2018). Elucidating the mechanisms that link CM to adverse health outcomes is an ongoing effort, however biological changes observed in some maltreated individuals may be salient factors in the association. Many researchers consider biological changes in maltreated individuals as evidence that early

experience "gets under the skin" – a phenomenon referred to as biological embedding (Berens et al., 2017). Two biomarkers that have been shown to demonstrate changes in normal function related to CM are telomere length (TL) and DNA methylation (DNAm). Both biomarkers are associated with the negative downstream physical and mental health effects of CM exposure, and thus show promise as mediators of experience and outcome. It is important to note that not all children who are maltreated will experience negative outcomes or biological changes. Understanding what makes some individuals resilient to negative outcomes or biological embedding, can provide valuable insight into factors that may help break the cycle of CM, mitigate risk, and maximize wellbeing for maltreated children. This review focuses on two biomarkers of CM, telomere length (TL) and DNA methylation (DNAm).

Telomere Length

Telomeres are non-coding DNA sequences at the ends of chromosomes that act as a buffer to protect coding genomic information during cell division (Frenck et al., 1998). Over a lifetime, they shorten at an expected rate as cells divide. When they become critically short, they enter senescence or undergo apoptosis (Frenck et al., 1998). Length is typically correlated to chronological age, disease morbidity and mortality, and plays a causal role in aging cells, which is unique compared to other biomarkers (Ridout et al., 2018). Telomere shortening is a natural and necessary process, but issues begin to arise if it is accelerated. TL is influenced by biological stress and inflammation and accelerated shortening has been associated with certain types of adverse experience (Colich et al., 2020). When telomeres are shortened prematurely, cellular age becomes incongruous with chronological age (Ridout et al., 2018). Given that telomere shortening often precedes chronic disease development and is associated with negative mental

and physical health outcomes, it may represent a mechanism connecting early adversity and later health; however, the evidence relating TL to maltreatment is mixed.

Epigenetics – DNA methylation

The first step in gene expression is the copying of genetic information from DNA in a process called transcription. Transcription begins at specific sites along a gene sequence called transcription start sites. DNAm is an epigenetic process that acts primarily on cytosine-guanine (CpG) sites that are typically located near transcription sites (Cecil et al., 2020; Horvath & Raj, 2018). These CpG sites are usually unmethylated to allow transcription factors to bind to the DNA sequence and activate gene expression. When they become methylated, transcription factor binding is impeded. This subsequently affects gene activity by depressing or activating gene expression. While DNAm is an important and natural part of epigenetic processes, it can be up- or down-regulated as a result of experience. Exposure to chronic stress, poor diet, disease, and various adversities has been linked to changes in methylation levels (Cecil et al., 2020). DNAm is a well-studied biomarker of CM exposure, however the literature includes variable findings, in part due to varying study designs that examine epigenome-wide methylation, methylation in candidate genes, or both (Cecil et al., 2020). Epigenome-wide assessment studies (EWAS) examine DNAm levels across the genome to identify global differences in methylation and specific differentially methylated regions (DMRs). Candidate gene analyses examine methylation in specific genes of interest typically associated with mental and physical health.

An additional type of DNAm assessment, and a fairly recent concept in epigenetic research, is the Epigenetic Clock. Epigenetic Clock models suggest that there are sets of fixed DNAm markers that can be used to estimate chronological age with a high degree of accuracy (Hannum et al., 2013; Horvath & Raj, 2018). This measurement is called the epigenetic age, and

as with accelerated telomere shortening, can become incongruous with chronological age in some circumstances (Hannum et al., 2013; Horvath & Raj, 2018). It is unique to other DNAm assessments, as EWAS and candidate gene studies do not provide an epigenetic age measurement.

Though changes to both biomarkers have been associated with CM, there is also evidence to suggest that certain biological and environmental factors may confer resilience to these changes, and that DNAm may even be reversible in some cases (Cecil et al., 2020). Given the link between these potential biomarkers of CM and disease morbidity and mortality, elucidating factors that may confer resilience to these biological changes is imperative in improving outcomes for maltreated children and youth.

The Present Review

Heterogeneity in the CM literature related to TL and DNAm poses a distinct challenge in elucidating the effects of early adversity on biology, and the possibility that these biomarkers may represent causal links between adversity and outcome (Colich et al., 2020). Adversity is a broad concept, and often lacks a common taxonomy. Differences in adversity reporting, use of measurement tools, and varying timing of exposure and assessment collectively creates a muddied landscape from which it is difficult to define clear associations. A 2020 review by Colich et al. outlined the links between threat and deprivation, indicating TL and DNAm age as indices of biological age acceleration across the lifespan. More specifically, exposure to threat was significantly associated with accelerated biological age, whereas experiences of deprivation were not (Colich et al., 2020). Importantly, this review used a broad definition of adversity and studies measuring DNAm outside the context of an epigenetic clock model were excluded (Colich et al., 2020).

The present review extends prior work by examining the specific link between CM and TL and DNAm. As our focus is biological embedding of CM, we will include studies that assess DNAm age as well as those that examine global methylation and candidate gene effects in participants under the age of 18. Factors that may mitigate risk are discussed using Bronfenbrenner's socioecological framework for human development. This model provides a nested framework for understanding the impact of various internal and external factors and their interactions on child development (Bronfenbrenner & Morris, 2006). We aim to clarify some of the heterogeneity of findings and identify areas for future investigation.

Methods

To identify studies relevant to CM and biological aging in children and youth, a systematic review was conducted using PRISMA guidelines (see Figure 1). A search of Embase, Medline, and PsycInfo databases was conducted using search terms and strategy outlined in Table 1. The search was run through to May 15, 2020. Review articles were manually searched for additional inclusions.

To meet inclusion criteria, studies must have been written in or translated to English and examine TL and/or DNAm before the age of 18 in human children and youth with a history of CM. Retrospective studies examining these biomarkers in adults who experienced CM as children were excluded. CM was defined as sexual, physical, or emotional abuse, neglect, and exposure to IPV. Studies that examined other adversities but did not specifically parse out CM or its subtypes were excluded. All articles were assessed by two reviewers to ensure inclusion criteria were met. Conflicts were resolved via discussion, and outside input by the senior author as needed.

For the purposes of understanding potential resilience factors, special attention was paid to extraction of family and parenting characteristics, socioeconomic information, and other relevant aspects. Depending on study design, all included papers were assessed for quality using the Scottish Intercollegiate Guidelines Network (SIGN) checklist for cohort studies (n=22) or the SIGN checklist for randomized control trials (n=4). Quality assessment was carried out by the first author and verified by the second reviewer. Most studies did not include follow up periods, thus, SIGN checklist questions around outcome presence at time of enrolment and adequacy of follow up period did not apply and were not assessed.

Table 1.

Search Terms

CM terms		Telomere terms		DNA Methylation terms	
1	exp child abuse/ or exp child abuse, sexual/	8	telomer*.tw	13	((DNA) adj3 (methyl*)).tw
2	exp Child, Abandoned/	9	Biomarker.tw	14	epigenetic.tw
3	exp Shaken Baby Syndrome/	10	((telomer* attrition) or (telomer* length) or (telomer* adj3 erosion) or (cell* age) or (cell* aging)).tw	15	((glucocort* adj3 (receptor)).tw
4	((child* or infant* or toddler* or early or early-life or preschool* or pre-school* or youth* or preteen*) adj5 (abus* or maltreat* or depriv* or (intimate partner violence) or (intimate partner abuse) or (domestic abuse) or (domestic violence) or stress or neglect* or rape? or raping or molest* or (sex* adj2abus*))).tw	11	or/8-10	16	(methylome).tw
5	((Child maltreatment) or (child neglect)).tw	12	6 and 7 and 11	17	or/13-16
6	or/1-5			18	6 and 7 and 17
7	(infan* or newborn* or new-born* or neonat* or baby or babies or child* or youth or toddler* or pre-school* or preteen* or early-life or adolescen*).tw				

Results

Study Characteristics

Following full text review, 26 articles from 16 studies were included (see Figure 1). Of these, eight examine TL and 18 examine DNAm. Papers were published between 2012 and 2020.

Most papers were published in the United States (n=18), while the remainder were published in Europe, Brazil, Egypt, and Japan. Quality assessment using the SIGN checklist for cohort studies (n=22) and SIGN checklist for Randomized Control Trials (n=4) returned scores of 'Acceptable' quality or better for all inclusions (detailed results of quality assessment for all inclusions available as a supplementary table on request from the author). For the purposes of comparison and examining associations between CM and biological embedding in youth, we extracted cross sectional data at various time points from the included articles that collected longitudinal data (n=10) as indicated in Table 2.

Although inclusions were limited to samples <18 years old, a wide range of ages were represented in the included studies. TL samples varied from 3-5 years to 5-15 years old. DNAm samples varied from 8-35 months old to 6-20 years old. Socioeconomic status (SES) was evaluated in 15 papers using a variety of characteristics. SES measurement tools are described in Appendix B. Additional factors that have been associated with biomarker changes such as existing diagnoses, body mass index and low birthweight were assessed in a minority of studies (Table 2).

Telomere length was primarily assessed via buccal swab samples, although one study used saliva (Ridout et al., 2019) and one used a peripheral blood sample (Xavier et al., 2018). Buccal swabs (n=2), saliva samples (n=9), and blood samples (n=5), or a combination (n=2) were used in DNAm studies to assess methylation samples. DNAm measures included epigenome-wide assessments, methylation of individual candidate genes, and analyses using Horvath and Hannum epigenetic clock models (see Table 2). Functions of all included genes are described in Appendix A.

CM exposure was assessed in one of three ways: 1) exposure to institutional care, 2) self- or parent-report measures, and 3) child welfare or protective services records. Three articles investigated the impact of institutional care on TL. One utilized a combination of protective services record review, semi-structured interview, and parent-report. Three used a variety of self- and parent-report measures. One article noted some history of welfare involvement from record review without further elaboration. DNAm papers (n=18) had equally varied CM assessments, including institutionalization records alone (n=3), protective services record review (n=3), self- and parent-report (n=5), and some combination (n=7).

CM measurement tools and subtypes varied significantly between studies. Many studies included additional adversity types (e.g., bullying, traumatic life events, parental death or incarceration). Most studies included at least sexual, physical, and emotional abuse and neglect as CM subtypes. Exposure to IPV was less commonly included. One TL study assessed IPV exposure only (Drury et al., 2014). Onset, duration, chronicity, and severity of CM were assessed in a minority of studies. When these characteristics were included, this was summarized in the findings (Table 2). Comprehensive details of study characteristics extracted from each paper is available as a supplementary table on request from the author.

Telomere Length

Institutionalized Populations

The Bucharest Early Intervention Project (BEIP) is a longitudinal randomized control trial that investigates the effects of early institutional rearing on child development, and tests quality foster care as an intervention (Zeanah et al., 2003). The ever-institutionalized group included children with a history of institutionalized care. These participants were randomized into care as usual and foster care groups. An additional control group with no history of

institutional care was included. Percent time in institutional care was assessed at multiple time points over the course of the study, and biological samples were obtained at the 8- and 12-year follow up timepoints. Using data from the BEIP, three articles focused on TL and institutionalization and found significant associations between length of time in institutional care and TL. Humphreys et al. (2016) reported a negative association between TL and percent time in institutional care at age eight in the care as usual group. Wade et al. (2020) did not find TL differences between care as usual or foster groups, or between the ever institutionalized and control groups, however when stratified by sex, amount of time spent in institutions by 54 months and eight years of age was negatively associated with TL at age 8-10 in boys. A similar effect was found in girls, where a higher percentage of time spent in institutions at baseline and 54 months of age was associated with shorter telomeres at age 12-14 (Wade et al., 2020). Drury et al. (2012) reported a significant negative association between percent time in institutional care at baseline and 54 months and TL, which remained significant when including group assignment and other covariates. When stratified by sex, TL was significantly associated with exposure to institutional care before baseline assessment but not at the 54-month assessment in females, however in male participants, percent institutionalization at 54-months but not at baseline was most predictive of TL in childhood (Drury et al., 2012). Taken together, these findings suggest that increased time in institutional care is associated with shorter telomeres, and that sex, age and length/onset of institutionalization play a moderating role in this association.

Maltreatment

The remaining five papers used subtypes of CM and composite scores to assess associations between CM and TL. In a study by Xavier et al. (2018) using a large sample of N = 561 from the Brazilian High-Risk Cohort study, a CM composite score comprised of physical

abuse, neglect, emotional maltreatment and sexual abuse was significantly associated with TL and moderated by sex such that the effect of CM on TL was only observed in males (Xavier et al., 2018). A smaller study compared a group of children with child welfare involvement (considered high-risk for CM) to children with no history of welfare involvement (low-risk for CM) and found that children in the high-risk group had significantly shorter telomeres than low-risk children (Asok et al., 2013). Contrary to these findings, a child welfare sample of children aged 3-5 years and with documentation of moderate to severe CM within the past six months had a strong positive correlation with TL (Ridout et al., 2019).

Studies investigating individual CM types indicate varied findings. In a study examining three types of violence exposure and TL in children from ages 5 to 10, only physical abuse was significantly associated with telomere shortening (Shalev et al., 2013). Exposure to IPV showed some association with telomere shortening, however this association was not statistically significant (Shalev et al., 2014). Notably, Shalev and colleagues reported telomere lengthening in a small number of participants between ages 5 and 10. Specifically, longer TL at age 10 was predicted by shorter TL at baseline (Shalev et al., 2013). In another study, children exposed to IPV demonstrated significantly more telomere shortening than unexposed children (Drury et al., 2014). This relationship remained significant after controlling for relevant covariates. When stratified by sex, a significant negative association between IPV and TL was found in girls, while the association met only trend level in boys (Drury et al., 2014). None of the included articles examined associations between TL and sexual abuse or emotional abuse specifically or neglect outside of institutional care. Overall, findings support an association between CM and TL shortening, however differences in CM types, age ranges, and other study characteristics make direct comparisons difficult.

DNA Methylation

Epigenome-wide associations test global methylation levels, while candidate gene assessments investigate methylation levels of specific genes. As highlighted earlier, epigenetic clock assessments provide a measure of accelerated cellular aging based on methylation levels. Five studies that examined epigenome-wide methylation additionally went on to discuss and/or examine specific genes of interest that emerged from their assessments.

Institutional Rearing – Epigenome-Wide Associations

Two papers examined effects of institutional rearing using EWAS. Several DMRs emerged that were linked to multiple genes. In an investigation using data from the English and Romanian Adoptee study, methylation differences between participants with extended (> 6 months) and limited (< 6 months) institutional care were compared. Initial probe-wise assessment of ~400,000 sites across the genome did not show differences between the groups; however, methylation differences in specific regions were highly correlated to exposure duration (Kumsta et al., 2016). A significant DMR on the *CYP2E1* gene involved in fatty-acid metabolism was identified, which was hypermethylated in the extended institutional care group (Kumsta et al., 2016). Another epigenome-wide study comparing children in institutional care to children raised in biological families found 164 DMRs associated with 172 genes (Naumova et al., 2019). Several genes associated with immune function were among those identified: *RB1*, *GNB1*, *ENO1*, *SOCS3*, *HSPA8*, and *MAPK14.* Except for *MAPK14,* these were hypomethylated in the institutional care group. Examination of *NRC31,* a specific candidate gene related to regulation of the stress response, indicated seven hypermethylated CpGs and one hypomethylated CpG in the institutional care group (Naumova et al., 2019). These findings

suggest that institutional care in early life has significant differential methylation effects on genes related to critical somatic and stress-related processes.

Institutional Rearing - Candidate Gene Analyses

Using data from the BEIP, significant negative associations were found between percent time in institutional care and methylation patterns in stress-related genes *FKBP5* and *SLC6A4* at age 12, after accounting for covariates (Non et al., 2016). Methylation differences in the Oxytocin Receptor gene *OXTR* between maltreated and non-maltreated children was examined within an institutionalized context in Japan (Fujisawa et al., 2019). *OXTR* is implicated in social interactions, bonding, maternal behaviour, stress and anxiety. The CM group evidenced significantly higher methylation at CpG 5,6 of the OXTR gene than did the non-maltreated group. Sex and age were not associated with methylation at these sites (Fujisawa et al., 2019).

Maltreatment – Epigenome-Wide Studies

In the following studies, participants were considered maltreated or not, though parameters defining CM exposure as a criterion differed between studies. While some include all five subtypes, others used CM measures that include fewer than five subtypes as indicated.

Two papers from the same parent study examined genome-wide associations using the Illumina 450k BeadChip and included all subtypes in their CM assessment (Yang et al., 2013; Weder et al., 2014). One study found significant differences in methylation values of the CM group at 2868 CpG sites across all 23 chromosomes, after controlling for relevant demographic factors (Yang et al., 2013). These differences were found in several genes associated with biomarkers of disease risk, outlined in Table 2 (Yang et al., 2013). In the second study, differences in methylation values of maltreated and comparison children were significant at 11 sites after statistical correction within three genes that emerged from the EWAS as predictors of

depression: *ID3, TPPP,* and *GRIN1* (Weder et al., 2014). CpG sites in the promoter regions of *SLC6A4* and *NR3C1* are not included in the array used for assessment in both articles, so direct comparison to existing candidate gene findings that focus on promoter regions in these genes was not possible, however Yang et al. (2013) reported that one CpG site on the gene body of NR3C1 was significantly hypomethylated in maltreated children. Weder et al. (2014) also reported significantly different methylation at one site in NR3C1 in the CM group after corrections.

An additional two studies carried out EWAS using a CM measure that included all subtypes except exposure to IPV (Cicchetti et al., 2016; Seif et al., 2019). Cicchetti et al. found differential methylation patterns between maltreated and non-maltreated children at 1,876 CpG sites with a 6.2% mean difference between groups. Further analyses conducted to examine interactions between CM exposure and known disease biomarkers identified differentially methylated sites associated with mental health, cancer, cardiovascular systems, and immune function, suggesting that maltreated children are at greater risk for adverse physical and mental health outcomes associated with these methylation differences (Cicchetti et al., 2016). The second study stratified children by co-occurrence of CM subtypes, dividing groups into no CM, one type, two types, three types, or four types and found statistically significant differences in methylation between all five groups (Seif et al., 2019). Children who experienced three types of CM had the lowest mean methylation values. This was followed by children who experienced four types, and two types, with the highest mean methylation values measured in children exposed to one type of CM or no CM (Seif et al., 2019). When included CM types were assessed separately, level of DNAm was significantly correlated with physical abuse only (Seif et al., 2019). This paper additionally reported on a variety of CM experience

characteristics, including age of onset, frequency, and duration of exposure. Age of onset was significantly associated with methylation level, with lower methylation found in children exposed before the age of 9. Additionally, duration of CM exposure was significantly associated with methylation values, with shorter duration (1-3 years) predicting higher methylation values. Frequency was significantly and inversely associated with DNAm levels, with highest mean methylation in participants with a frequency of exposure of 1-2 times and lowest methylation in children exposed more than ten times. Using multivariate linear regression to examine interactions between CM, methylation, and CM characteristics, a significant association between duration and frequency of exposure and DNAm level was found (Seif et al., 2019). Taken together, these findings suggest CM is associated with differential methylation, and that experiencing multiple types of CM, more frequent CM, and CM over a longer period may amplify these effects. Additionally, methylation differences in genes associated with negative mental and physical health outcomes may contribute to risk of these outcomes for maltreated children.

Maltreatment - Candidate Gene Analyses

In studies that examined *NR3C1* methylation and utilized a CM assessment that included all subtypes except for exposure to IPV, three papers from the same parent study reported hypermethylation in one exon of *NR3C1* in the CM group only (Parade et al., 2016; Parent et al., 2017; Tyrka, 2015a). In another study, CM was associated with hypermethylation at one exon of *NRC31.* Additionally, early exposure, more CM types and chronic CM exposure was positively associated with *NRC31* methylation (Cicchetti & Handley, 2017). An additional study found no association between CM and methylation levels in maltreated and non-maltreated groups, however *NRC31* was hypermethylated in maltreated girls at two sites (Cicchetti et al., 2016). An

additional *NRC31* site showed a CM by race interaction, where maltreated children with African ancestry evidenced hypomethylation (Cicchetti et al., 2016).

Physical and emotional abuse and methylation in HPA-axis genes *NR3C1, POMC, CRH* and *AVP* were investigated via blood and saliva samples in a group of school children from Tanzania (Hecker et al., 2016). Due to high incidence of abuse exposure in the study population, a sufficiently sized control group with no history of abuse was not possible. As such, participants were instead divided into high- and low-exposure groups. Between groups, no clear difference in methylation levels was found for *NR3C1*, however four sites were found to be differently methylated with a moderate effect size in saliva only (Hecker et al., 2016). Differences in methylation between groups were not found in *CRH* or *AVP*, however the high-exposure group evidenced more methylation in *POMC* than the low-exposure group. In blood samples, one of the investigated CpG sites in *CRH* evidenced significant hypermethylation with a large effect size. In saliva, one of the investigated CpG sites in *CRH* evidenced significant hypermethylation with a medium effect size. No significant effects were found for *AVP* (Hecker et al., 2016).

Two studies examined the effects of physical maltreatment on *NRC31* methylation. Romens et al. (2015) reported a positive association between physical maltreatment and methylation at several CpG sites in *NRC31*. Van der Knaap et al. (2014) identified three amplicons of interest within the *NR3C1* promoter region. At amplicon 1, they found a positive association between single and repeat exposure to sexual abuse, as well as repeated exposure to physical abuse and DNAm (Van der Knapp et al., 2014). At amplicon two, single exposure to sexual abuse was associated with increased methylation, however in post-hoc analysis this finding was not replicated (Van der Knapp et al., 2014). While there was some variation in findings, hypermethylation of the *NRC31* gene in maltreated children was well supported overall.

Tyrka and colleagues examined methylation levels in candidate gene *FKBP5,* a gene implicated in stress response modulation, inflammation, and immune function (Tyrka et al., 2015b). This investigation considered all CM types except exposure to IPV in their assessment. At two CpG sites on *FKBP5*, maltreated children demonstrated significant hypomethylation (Tyrka et al., 2015b).

One study investigated DNAm in two genes associated with alcohol metabolism, mood and cognition*, ALDH2 and ANKK1* (Cicchetti et al., 2016). All subtypes except exposure to IPV were included in the CM assessment. Significant associations were found between CM and hypermethylation on one site for each of the genes (Cicchetti et al., 2016). Further analyses were performed examining the effects of sex, race, and age of onset and recency of CM. Three distinct onset/recency combinations: early onset/recent, early onset/not recent, late onset/recent were created. Within the maltreated group, maltreated girls showed significant hypomethylation compared to non-maltreated girls at one site on *ALDH2* (Cicchetti et al., 2016). At the same location, maltreated boys had significant hypermethylation compared to non-maltreated boys. No significant differences were found among the non-maltreated group. Considering age at onset and recency of CM, significant hypermethylation at *ALDH2* was found among boys with early onset, but not recent CM compared to boys with no CM history (Cicchetti et al., 2016). Girls had significant hypermethylation compared to boys at one *ANKK1* site. Similarly, African ethnicity was correlated to higher methylation levels than non-African ethnicity. Maltreated children with early onset and not recent CM had significant hypermethylation compared to the control group (Cicchetti et al., 2016). Taken together, significant differences in methylation in candidate genes associated with a variety of mental and

physical health outcomes in children with a history of CM may demonstrate epigenetic risk factors for negative downstream effects.

Horvath and Hannum Epigenetic Clocks

Two included papers used data from the Avon Longitudinal Study of Parents and Children (ALSPAC) to investigate associations between CM and DNAm age using the Horvath and Hannum Epigenetic clock models. Both models provide an epigenetic age measurement based on expected vs. actual methylation levels at specific CpG sites. The Horvath clock uses 353 CpG sites, while the Hannum model uses 71 CpG sites identified through analysis of existing methylation data from healthy subjects. There are 6 common CpG sites between the models. Though they use different sites and different number of CpGs to evaluate epigenetic age, both models provide reliable age measures (Hannum et al., 2013; Horvath & Raj, 2018). In both papers, CM assessment included physical, sexual, and emotional abuse (Marini et al., 2020, Tang et al., 2020). Marini and colleagues assessed cumulative and timing effects of adversity by summing the total number of time periods in which a child experienced adversity and the total number of developmental periods in which adversity was experienced. Using Hannum's model and accounting for both recency and accumulation, exposure to sexual or physical abuse at 3.5 years was associated with epigenetic age acceleration (Marini et al., 2020). When considering child sex in this association, any type of abuse exposure was associated with increased epigenetic age in girls. The acceleration was quantified with the example that girls exposed to abuse in the 3.5-year sensitive period will experience epigenetic age acceleration of almost two months by age 7.5. No associations were found in this paper using the Horvath epigenetic clock (Marini et al., 2020). Tang et al. reported exposure to emotional or physical abuse before age 14 was associated with Horvath accelerated aging at age 17 in maltreated girls but not in boys. Abuse

was associated with an adjusted mean difference in DNAm age of 1.20 years for emotional abuse, and 1.22 years for physical abuse (Tang et al., 2020). Although these investigations reported effects using different epigenetic clock models, they produce compelling evidence that experience of abuse in combination with other factors may accelerate epigenetic aging processes in children exposed to maltreatment.

Figure 1.

PRISMA with Details

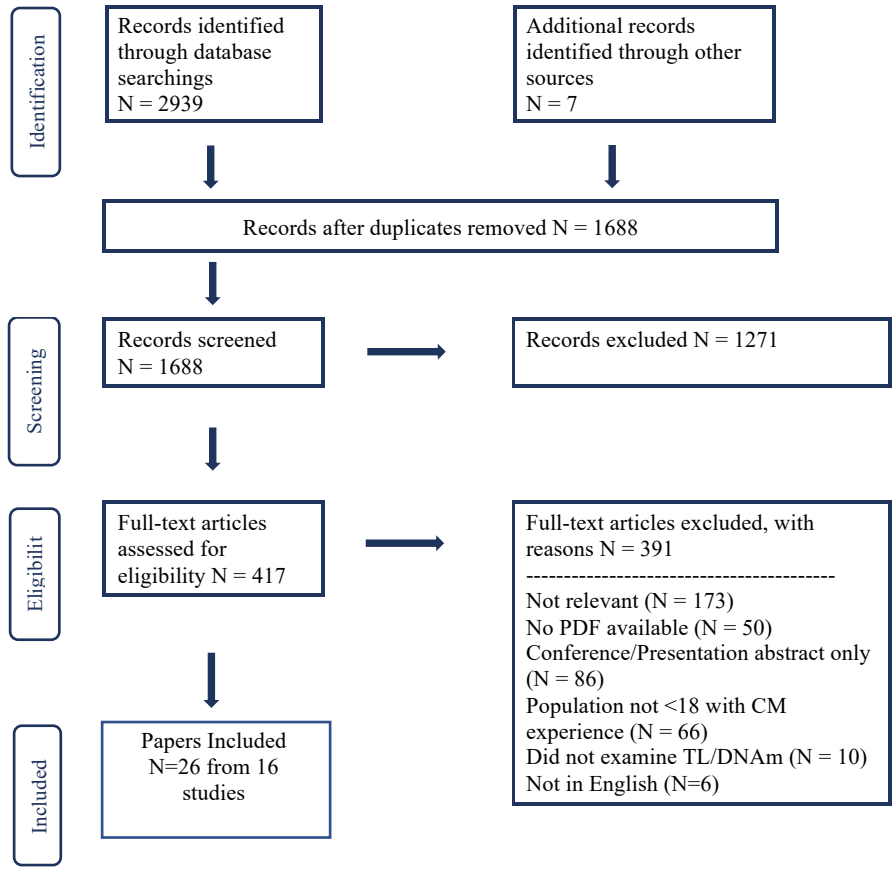

Table 2.

Study Characteristics

Telomere Length									
Study	Sample	Sample size	Age range & mean	Sex distribution	Race/ ethnicity	CM Type & Assessment	Biomarker sample	Variables	Main CM/biomarker findings
(Wade et al., 2020)[a]	Data from the Bucharest Early Intervention Project, longitudinal RCT.	8-10 years: N=195 12-14 years: N=162	8-10 years: M= 8.68 12-14 years: M=13.04	8-10 years: 52.1% male 12-14 years: 51.8% male	69.23% Romanian 19.23% Roma, 11.43% Other, Unknown*	Institutional rearing, neglect/deprivation; assessed via record review	Buccal	Birthweight, sex, IQ, general, internalizing and externalizing psychopathology factors	Percent-time in institutional care at 54 months and 8 years was negatively associated with telomere length at age 8-10 in boys, while percent time in institutional care at baseline and 54 months was negatively associated with telomere length at age 12-14 in girls.
(Drury et al., 2012)[a]	Data from the BEIP; longitudinal RCT. Percent time in institutional care assessed at baseline and 54 months, TL assessed between ages 6-10.	N=100 with valid TL data	6-10 years	59% male	55.1% Romanian 44.9% Romanian, Other, Unknown	Institutional rearing, neglect/deprivation; assessed via record review	Buccal	Percent time in institution, gender, ethnicity, low birthweight, age at buccal swab collection	Percent time in institutional care was negatively associated with TL in middle childhood. Percent institutionalization at baseline but not 54 months was most predictive of shorter TL in girls, while the opposite was true in boys.
(Asok et al., 2013)	Cross sectional study. Participants recruited from ongoing longitudinal RCT examining attachment-based intervention.	N=89	3.9-6.5 years (M=4.94)	53.5%	14.61% white 74.16% African American 5.62% Hispanic 5.62% Bi-racial	High-risk group was recruited through welfare services; some instances of child neglect & IPV in records	Buccal	Child age, child sex, minority status, household income, parental responsiveness	High-risk children evidenced significant telomere shortening compared to low-risk children. This association was moderated by responsive parenting.
(Xavier et al., 2018)	Cross sectional study using data from prospective community school-based study (Brazilian High Risk Cohort).	N=561	6-12 years (M=10.19)	54.9%	not reported	Composite score encompassing CPA, CSA, CEA, neglect; self- and parent-report	Blood	Child age, sex, location, child behaviour and psychopathology	Telomere length was negatively associated with CM scores in boys but not girls.

(Ridout et al., 2019)[d]	Longitudinal cohort study of families with and without documentation of moderate to severe CM in the past 6 months. Measures at baseline and repeated at 6-month follow-up.	N=256	3-5 years (M=51.1 months)	47.6%	54.69% non-Hispanic 44.92% Hispanic 0.39% unknown ethnicity; 39.06% white 16.02% black 22.26% biracial 19.53% other 2.73% unknown	Composite score encompassing CPA, CSA, CEA and neglect; assessed via child welfare record review, interview, parent-report	Saliva	Adversity composite, socioeconomic adversity, contextual stress, beta-hemoglobin, mtDNA copy number	CM was positively associated with telomere length at baseline and follow-up
(Shalev et al., 2013)	Prospective-longitudinal study; from the nationally representative Environmental-Risk Longitudinal Twin cohort study. Violence exposure assessed at age 5,7, and 10, TL assessed at age 5 and 10.	N=236	10 years, mean not provided	50.8%	100% Caucasian	IPV, physical maltreatment; assessed via parent-report	Buccal	Child age, Socioeconomic deprivation, BMI, Children's health, IPV, bullying victimization, CPA, violence exposure composite	CPA but not exposure to IPV was negatively associated with TL.
(Drury et al., 2014)	Cross-sectional cohort study.	N=80	5 to 15 years (M=10.2)	51%	94% African American 6% Other	Exposure to IPV; parent-report	Buccal	Child age and sex, maternal education and age at conception, race, and IPV	Exposure to IPV was negatively associated with TL. When stratified by sex, the association was significant in girls only.
(Humphreys et al., 2016)[a]	Data from the BEIP. Percent time in institutional care assessed at baseline and 54 months, TL assessed 2-4 times between ages 6-15 and summed to create two times.	N=79	Time 1: 6.40-9.13 years (M= 8.07) Time 2: 12.94-15.61 (M=14.41)	48.16%	64.48% Romanian, 35.52% other	Institutional rearing; record review	Buccal	FSIQ; Demographics (sex, ethnicity, initial/final age, mean follow-up length); percent time in institutional care	Children with a history of institutional care had significantly shorter telomeres than children with no institutional exposure.

DNA methylation										
Study	Sample	Sample size	Age range & mean	Sex distribution	Race/ ethnicity	CM type & Assessment	Biomarker sample type(s)	Variables	EWAS or Candidate genes	Main Findings

(Seif et al., 2019)	Cross-sectional study; participants recruited from adolescent psychiatric outpatient clinics in Egypt.	N=90	12-18 years, mean not provided	61.1%	Not provided	CPA, CSA, CEA and neglect, number of types; self-report	Blood	Age, sex, CM characteristics – age of onset, duration, frequency of exposure	Global	Children exposed to more than one type of CM showed hypomethylation compared to no CM or children exposed to one type of CM. Significant associations between DNAm and age of onset, duration, and frequency.
(Kumsta et al., 2016)	Data from the English and Romanian Adoptees study.	N=49	15 years, range not provided	53.06%	Not reported	Institutional care, neglect; record review	Buccal	Institutional care exposure of < or > 6 months duration, cognitive and sociocognitive abilities	Global assessment. DMR in region related to *CYP2E1*	The extended deprivation group showed significant hypermethylation across a region associated with the CYP2E1 gene.
(Naumova et al., 2019)	Cross-sectional of children in orphanages and those raised in biological families.	N=58	8-35 months (M=20.44 months)	65.5%	Ethnically homogenous; Eastern Slavic origin	Institutional care; assessed via record review	Blood + Buccal	Child sex, child age, blood cell-type count, duration in months of institutional care, adaptive behaviour	Global assessment. DMRs associated with *MAPK14, ENO1, RB1, SOCS3, HSPA8*	Institutional care exposure was associated with significant differences in methylation compared to children living with biological families.
(Yang et al., 2013)[c]	Summer research camp for school-aged, low-income children.	N=192	5-14 years (M=10.2)	42%	17% European-American 38% Hispanic 30% African-American 15% biracial	Composite score: CPA, CSA, CEA, neglect, IPV; self- and parent-report and record review	Saliva	Child race, child sex, age, number of CM types, CM severity	Global assessment. DMRs of genes: *CCDC85C, FANK1, FRG1, TMED2,WNT3A SLC29A4, PTPRN, PTPRN2, NRC31*	Significant differences in methylation were found in 2868 CpG sites in the maltreated group compared to the non-maltreated group.
(Weder et al., 2014)[c]	Summer research camp for school-aged, low-income children.	N=190	5-14 years (M=10.2)	42%	17% European-American 38% Hispanic 30% African-American, 15% biracial	Composite score: CPA, CSA, CEA, neglect, and exposure to IPV; self- and parent-report and record review	Saliva	Child age, sex and race, depression, Psychiatric diagnoses, Child behaviour, cortisol levels	Global assessment, DMRs associated with *ID3, TPPP, and GRIN1, BDNF, FKBP5, NR3C1*	Maltreated group evidenced significant differences in DNAm in several genes.

(Cicchetti et al., 2016) [e]	Summer research camp for school-aged, low-income children.	N=548	M=9.41	52.2%	67.7% Black 20.6% White 11.7% Biracial or other race 20.6% Latino	Composite score: CPA, CSA, CEA and neglect; record review	Saliva	Child age and sex, child race, ancestry, genotype, timing of CM	Global assessment. DMRs associated with *NRC31, ALDH2, ANKK1*	CM was significantly associated with hypermethylation on one site for both ALDH2 and ANKK1.
(Tyrka et al., 2015a) [d]	Cross-sectional study of children with substantiated cases of moderate to severe CM within the past six months.	N=184	3-5 years (M=50.8 months)	48.9%	22.28% white non-Hispanic 47.28% Hispanic 15.75% Black 14.67 other races	Composite score: CPA, CSA, CEA and neglect; record review and interview, parent report	Saliva	Child age, sex and race. Socioeconomic adversity, CM type and severity, contextual stress, traumatic life events, adversity composite	*NRC31*	One exon of the *NRC31* gene was significantly hypermethylated in the CM group.
(Parade et al., 2016) [d]	Cross-sectional study of children with substantiated cases of moderate to severe CM within the past six months.	N=171	3-5 years (M=50.3 months)	47.95%	22.81% white, non-Hispanic 47.95% Hispanic 14.62% Black 14.62% other races	Composite score CPA, CSA, CEA and neglect; record review, interview, parent report	Saliva	Child age, sex and race. Socioeconomic adversity, CM type and severity, contextual stress, traumatic life events, adversity composite, behaviour problems	*NRC31*	One exon of the *NRC31* gene was significantly hypermethylated in the CM group.
(Parent et al., 2017) [d]	See (Ridout et al., 2019) Measures at baseline and repeated at 6-month follow-up.	N=260	3-5 years (M=4.2 months)	47.7%	45.6% Hispanic 27.7% white non-Hispanic 16.3% Black 21.9% biracial 2.7% other races	Composite score CPA, CSA, CEA, and neglect; record review, interview, parent-report	Saliva	Child age, sex and race, number of days between baseline and follow up assessment, Socioeconomic adversity, CM type and severity, contextual stress, traumatic life events, adversity composite	*NRC31*	One exon of the *NRC31* gene was significantly hypermethylated in the CM group at baseline.
(Van der Knapp et al., 2014)	Data from the TRAILS prospective, longitudinal, population study of Dutch adolescents.	N=468	14-18 years (M=16.1)	49.6%	Dutch	CPA and CSA; self-report	Blood	Sex, age, smoking, traumatic youth experiences, number of exposures, stressful life events and timing, Perinatal stress	*NRC31*	Single and repeated exposure to CSA and repeated exposure to CPA was associated with hypermethylation at the first location on *NR3C1.* Only single exposure to CSA was associated with hypermethylation at the second location on *NR3C1.*

(Romens et al., 2015)	Cross-sectional study.	N=56	11-14 years (M=12.11)	53.6%	66% White and non-Hispanic, 30.3% Black, 3.57% White and Hispanic	CPA; record review	Blood	Child age, child sex, child race, SES	*NRC31*	A positive association between physical maltreatment experience and methylation of one exon of *NRC31* was found.
(Cicchetti & Handley, 2017)[e]	Summer research camp for school-aged, low-income children.	N=534	M=9.41 years	51.5%	61.2% Black 9.9% White 20.6% Latino 8.2% biracial or other race	CPA, CSA, CEA, and neglect and composite score; record review	Saliva	Child age, sex and race; timing of CM, Emotional lability-negativity; internalizing and externalizing behaviour problems	*NR3C1*	A positive association between CPA and methylation of one exon of *NRC31* was found. Early onset of and exposure to more CM subtypes was associated with hypermethylation of *NR3C1.*
(Hecker et al., 2016)	Part of a larger research project, cross-sectional investigation of Tanzanian school children.	N=60	9-15 years (M=11.50)	47%	Tanzanian	CPA and CEA; composite – scores used to separate high- and low-exposure groups; structured interviews	Saliva + blood	Group assignment, child sex, mental health	*NRC31, POMC, CRH, & AVP*	The high-exposure group evidenced significant differential methylation in the *POMC* gene. No clear differences in methylation were found for the remaining genes.
(Tyrka et al., 2015b)[d]	Comparison study of children with substantiated cases of moderate to severe CM within the past six months.	N=174	3-5 years (M=49.87 months)	48.2%	21.8% White non-Hispanic 46.5% Hispanic, 16.7% Black 14.9% other races	Composite score: CPA, CSA, CEA and neglect; record review, interview, parent-report	Saliva	Child age, sex and race. Socioeconomic adversity, CM type and severity, genotype, contextual stress, traumatic life events, adversity composite	*FKBP5* and *NRC31*	Children exposed to CM evidenced significant hypomethylation at two sites of the *FKBP5* gene.
(Non et al., 2016)[a]	BEIP, longitudinal RCT. Percent time in care was measured at multiple times.	N=117	M=12.5 years	51%	63% Romanian 27% Roma 9% other	Institutional care; record review	Buccal	Age at swab collection, child sex, ethnicity, Tanner pubertal stage, percent time in institutional care	*FKBP5* and *SLC6A4*	In both genes, percent time in institutional care was negatively associated with DNAm at specific sites.
(Fujisawa et al., 2019)	Comparison study of children with and without child welfare documentation.	N=85	6-20 years (M=12.9)	64.71%	Japanese	Institutional care sample; self-report	Saliva	Child age and sex, CM type, number of types, CM duration, years since CM, psychiatric symptoms, IQ	*OXTR*	At two CpG sites on the *OXTR* gene, the CM group showed hypermethylation compared to controls.

(Marini et al., 2020)[b]	Data from the ALSPAC.	N=973	7-year timepoint	49.8%	97.2% white	CPA, CSA and CEA; parent-report	Blood	Adversity exposure, child race/ethnicity, number of births in the pregnancy, number of previous pregnancies, maternal marital status, highest level of maternal education, age, smoking during pregnancy, child birthweight, parental home ownership, parent job status	Horvath and Hannum epigenetic clocks	Exposure to CSA or CPA in the sensitive period at 3.5 years was associated with epigenetic age acceleration using Hannum's epigenetic clock model. When stratified for sex, any type of abuse exposure was associated with increased epigenetic age in girls.
(Tang et al., 2020)[b]	Data from the ALSPAC.	N=974	Range not provided, M=17.13	48.7%	97.2% white**	CPA, CSA, CEA, neglect and exposure to IPV; parent-report	Blood	Other ACEs including bullying, parent mental health problems, parent convicted, parental separation, household substance abuse; ACE count, age, morning plasma cortisol	Horvath epigenetic clock	Exposure to CEA or CPA before age 14 was associated with Horvath accelerated aging at age 17 in maltreated girls but not in boys.

[a]Data from the Bucharest Early Intervention Project; [b]Data from the Avon Longitudinal Study of Parents and Children (ALSPAC); [c]Data from (Kaufman et al., 2006); [d]Data from same group of participants (Tyrka, 2015a); [e]Data from same group of participants (Cicchetti et al., 2016); *sample details not reported in article, pulled from detailed description of original sample (Zeanah et al., 2013), **sample details not reported in the article, pulled from supplementary detailed description of ALSPAC participants (Marini et al., 2020).

Note: CPA: child physical abuse; CSA: child sexual abuse; CEA: child emotional abuse; IPV: intimate partner violence; BEIP: Bucharest Early Intervention Project; DMR: differentially methylated regions; ACEs: Adverse Childhood Experiences; FSIQ: Full Scale Intelligence Quotient; SES: Socioeconomic status; BMI: Body Mass Index

Discussion

Taken together, findings from this systematic review broadly indicate that CM is associated with changes to both TL and DNAm in children and youth, however, specific associations were varied. Sex, race, and CM timing were among characteristics that were differently associated with biomarker changes. In assessing the sample characteristics and methodology, we can point to several aspects that may influence study findings, lending heterogeneity to the overall findings.

Included studies featured a variety of CM definitions that included one or more subtypes. Within studies that examined multiple CM types, CM types often co-occurred. The distribution of CM types experienced was not always comparable between studies, even if both studies assessed similar subtypes overall. An additional consideration is that CM measurement tools were not standardized across inclusions. While some investigations used self- and parent report measures, others examined child welfare documentation to assess CM. Because CM is often perpetrated by family members, it is possible that parent-report measures may provide biased accounts. Similarly, self-report measures may not provide accurate reports for several reasons, including lack of detailed memory and hesitancy to divulge information (McKinney et al., 2009). On the other hand, not all maltreated children become involved with child welfare services, thus reliance on substantiated cases may disproportionately sample certain types or severity of CM (McKinney et al., 2009).

Between studies, factors such as BMI were inconsistently reported, despite being demonstrably associated with epigenetic changes (Simpkin et al., 2016). Similarly, few studies reported on pre-existing physical and psychological diagnoses. Assessment of other adversities and contextual stressors differed significantly between investigations, both of which could

influence associations. Race and ethnicity were differently associated with biomarker changes in some studies and were inconsistently evaluated, although an assessment of one or the other was present in most studies. Inconsistencies in reporting race/ethnicity raises the complex, but important consideration of how researchers can better assess race moving forward; and particularly where lived experience, socioeconomic status and stress that may be conferred by systemic barriers, discrimination and treatment may exert effects on findings, rather than treating race exclusively as a biological factor.

Differences in recency of CM exposure, temporal proximity of exposure to biomeasure, and age at sample collection may have also affected findings. Frenck and colleagues report a rapid period of telomere shortening as part of normal development up to age 3, followed by a period of relative stability until adulthood (Frenck et al., 1998). Considering the age ranges of studies that found positive associations between CM and TL, a deeper understanding of potential lengthening or maintenance mechanisms during this stable period is needed (Ridout et al., 2019). Although we limited our inclusions to populations under 18, investigating a narrower age range might also provide more consistent findings, as oversampling of some age groups within wide age ranges could skew results. Overall, DNAm findings were more consistent, which may in part be explained by the larger pool of research available.

Resilience

While many differences in findings may be explained by one or more of the aforementioned factors, we must also consider aspects of biology and environment that may confer resilience to biological embedding. Clarifying protective factors is a critical component of breaking the cycle of CM. Bronfenbrenner's bioecological model of child development provides a nested framework for understanding the impact of various factors on development

(Bronfenbrenner & Morris, 2006). Below we outline resilience factors that may influence the impact of CM exposure on biomarkers at various levels of the model.

At the center of the model is the individual. Individual characteristics that emerged as potential resilience factors in the association between CM exposure and biomarker changes include sex, age, and acute stress. Multiple studies indicated effects in one sex but not the other, and/or in both sexes but at different time points with varying results (see study specific findings above and in Table 2). It is possible that different CM types interact with sex to exert differing effects on biomarkers. It is similarly conceivable that biological differences modulate effects of CM on biomarker processes. Marini et al. (2020) found that girls were, on average, epigenetically older than boys. Conversely, estrogen has been linked with increased telomerase activity, and there is evidence to suggest that women have longer telomeres than men (Kyo et al., 1999; Ridout et al., 2019). This may confer resilience to TL changes in girls who have begun to go through puberty. Further investigation into potential sex-related resilience effects, at various developmental timepoints is needed. Lastly, acute stress has been linked to an upregulation in telomerase activity which acts to lengthen telomeres, potentially mitigating shortening effects (Ridout et al., 2019). This reflects the three-hit concept of vulnerability and resilience that suggests genetic predisposition interacts with early environment to produce phenotypes in which in turn react with later life environments to confer vulnerability or resilience to negative outcomes (Daskalakis et al., 2013)

Microsystem effects on child development refer to aspects outside the individual that they interact with directly, including family, friends, school, and home (Bronfenbrenner & Morris, 2006). Asok et al. (2013), found significant moderating effects of responsive parenting on telomere shortening. Preliminary evidence from a randomized control trial examining an

evidence-based intervention program geared toward improving parent-child relationships, biological, and behavioural outcomes in families involved within Child Protective Services, supports the importance of parental responsiveness. Participants randomized to the intervention, showed differential methylation in whole-genome DNAm compared to the control group post-intervention (Hoye et al., 2020). Together, these findings support sensitive parenting and supportive parent-child relationships as a protective factor.

Microsystem aspects interact to make up the mesosystem (Bronfenbrenner & Morris, 2006). At this level, effects might include interactions between parents and social workers or parents and siblings. Although no clear mesosystemic resilience effects emerged, based on the complexity of biological and environmental interactions in child development, it is likely that positive influences exist at this level. Exosystemic factors include aspects such as media, community services, parents' workplace, and child's school (Bronfenbrenner & Morris, 2006). We found two potential resilience factors at this level. First, using data from the ALSPAC, Marini et al. (2020) noted less accelerated epigenetic aging in children of married mothers, with more education and from higher SES. This finding was supported by evidence of a buffering effect of maternal education on TL in maltreated boys (Asok et al., 2013). Second, Ridout et al. (2019) investigated the possibility that TL could be impacted by utilization of social or psychological services. They included instances of outpatient mental health treatment, home based services, and services provided by the local school department in their assessment. Although not significantly, there was evidence that TL was positively associated with service utilization (Ridout et al., 2019).

Cultural values and norms make up the macrosystem level (Bronfenbrenner & Morris, 2006). Seif and colleagues (2013) proposed that some forms of physical and emotional abuse

may be viewed as "disciplinary" rather than abusive, and thus socially acceptable, garnering a different, potentially less sensitive, response than experience of another CM type such as sexual abuse. However, consistently in both biomarkers, physical abuse was associated with biological changes, even when considered the cultural norm (Hecker et al., 2016). While this is not a resilience factor, it is a cultural factor that may be increasing risk of negative outcomes. Additionally, Marini et al. (2020) found that children exposed to any adversity type were more likely to be non-white and from low socioeconomic backgrounds. Though we are only discussing resilience factors here, a focus on how we respond to different CM types and increasing support for children experiencing all types of CM may promote positive outcomes and reduce impact long term. Any work toward changing outcomes for maltreated children should examine and address the systemic challenges embedded in our cultural values and norms that make some children more likely to be maltreated in the first place.

Conclusion

Epel and Lithgow (2014) explain the importance of a common knowledge base and taxonomy in understanding the ways in which stress biology influences accelerated aging mechanisms. While we can reasonably conclude that CM is associated with changes in both biomarkers, it seems clear that a similar need is present here in order to elucidate the features of these associations. CM is implicated in significant negative downstream physical and mental health effects (Butchart et al., 2006). A clearer understanding of CM-biomarker associations may provide valuable insight into potential connecting mechanisms. Given that effects of CM were most significant when CM types were assessed cumulatively (Shalev et al., 2013), it follows that efforts to minimize even some CM exposure may have positive downstream effects on child outcomes. Further exploration of factors that may confer resilience and a deeper understanding

of when they are most effective and for whom they are most impactful, would support existing and future intervention development and more positive outcomes for children and families.

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Appendix

Appendix A: Gene Functions and Associations

Gene	Functions and Associations
NR3C1	Encodes glucocorticoid receptor protein that binds the stress hormone cortisol. Regulatory role in stress response via the hypothalamic-pituitary-adrenal axis. Also involved in regulating inflammation, development, metabolism, and immune function (Tyrka et al., 2015a; Weizmann Institute of Science (WIS), n.d.).
FKBP5	Modulates stress response via glucocorticoid receptor activity. Associated with mood disorders. Regulatory role in inflammation and immune function (Tyrka et al., 2015b; O'Leary et al., 2013).
CYP2E1	Involved in fatty acid metabolism (Kumsta et al., 2016; WIS, n.d.).
MAPK14	Immune function: Encoded protein integrates cellular signals. Involved in proliferation, differentiation, and transcription (Naumova et al., 2019; WIS, n.d.)
GNB1	Immune function: Encodes G-proteins important for cellular signaling (Naumova et al., 2019; WIS, n.d.).
ENO1	Immune function: Encodes protein involved in glucose metabolism and may act as a tumor suppressor (Naumova et al., 2019; WIS, n.d.).
RB1	Immune function: Encodes tumor-suppressor protein. Involved in regulation of apoptosis, DNA replication, and differentiation (Naumova et al., 2019; WIS, n.d.).
SOCS3	Immune function and allergic response (Naumova et al., 2019; WIS, n.d.).
HSPA8	Immune function, cancer, neurodegenerative diseases and aging. Involved in signaling, apoptosis, cell growth and differentiation. (Naumova et al., 2019; Mayer et al., 2005)
CCDC85C	Encodes protein that may be involved in cell-cell adhesion and cortical/neural development (Yang et al., 2013; WIS, n.d.)
FANK1	May be involved in regulation of apoptosis (Yang et al., 2013; WIS, n.d.).
FRG1	Associated with aspects of muscle function (Yang et al., 2013; WIS, n.d.).
TMED2	Encodes protein that may be involved in aspects of cellular transport (Yang et al., 2013; WIS, n.d.).
WNT3A	Associated with embryonic and neural development. Plays a role in cell differentiation, proliferation and tumour growth (Yang et al., 2013; WIS, n.d.).
PTPRN	Regulates numerous cellular processes such as growth, differentiation and division (Yang et al., 2013; WIS, n.d.).
SLC29A4	Encodes protein involved in controlling length and intensity of monoamine (serotonin, dopamine, norepinephrine) signaling (Yang et al., 2013; WIS, n.d.).
PTPRN2	Involved in normative accumulation and secretion of monoamines in the brain, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in the pituitary gland of females, and insulin in pancreatic islet cells (Yang et al., 2013; WIS, n.d.).
ID3	Involved in stress response. Encodes a protein that inhibits DNA binding, thus regulating transcription. Involved in various cellular processes such as growth, senescence, differentiation, apoptosis, and blood vessel formation (Weder et al., 2014; WIS, n.d.).
TPPP	Encodes tubulin polymerization promoting protein. Associated with schizophrenia 13, sudden infant death syndrome, and differentiation of glial cells (Weder et al., 2014; WIS, n.d.).
GRIN1	Encodes protein building block of receptors in the glutamate receptor family. Plays an important role in synaptic plasticity (Weder et al., 2014; WIS, n.d.).

BDNF	Encodes protein that is implicated in neuronal health and differentiation. Active in brain areas associated with learning, memory, and cognition (Weder et al., 2014; WIS, n.d.).
ALDH1, ALDH2	Involved in alcohol metabolism; associated with dopamine DRD2 gene which plays an important role in a number of processes including movement, cognition, mood and motivation (Cicchetti et al., 2016; WIS, n.d.)
POMC	HPA axis gene. Encodes proteins that are precursors to several peptide hormones involved in appetite regulation, sexual behavior, movement of melanin, emotion, attachment, motivation, stress, pain, control of food intake, and glucocorticoid secretion (Hecker et al., 2016; WIS, n.d.)
CRH	HPA axis gene. Encodes protein that is processed to produce hormone implicated in stress response (Hecker et al., 2016; WIS, n.d.).
AVP	HPA axis gene. Encodes protein that acts on kidney to regulate extracellular fluid volume, and on blood vessels as a factor in vasoconstriction (Hecker et al., 2016; WIS, n.d.).
SLC6A4	Stress-related. Involved in serotonin transport. (Non et al., 2016; WIS, n.d.)
OXTR	Encodes oxytocin receptors. Implicated in social interactions, bonding, maternal behaviour, stress and anxiety. (Fujisawa et al., 2019; Feldman et al., 2010)

Appendix B: Socioeconomic Status Measures

Article	Socioeconomic Status Measure(s)
Asok et al., 2013	Income
Drury et al., 2014	Maternal education, household monthly income
Marini et al., 2020	Parent job status, maternal education, and home ownership.
Ridout et al., 2019	Qualification for public assistance; composite score encompassing parental education ≤ high school degree, parental unemployment, and single parenthood
Shalev et al., 2013	Composite score encompassing household income, parents' highest education and parents' highest occupational grade
Cicchetti et al., 2016; Cicchetti and Handley, 2017	Low-income sample
Naumova et al., 2019	Institutional Care Group - 75% of children in institutional care were abandoned due to socio-economic challenged; Biological families group – socioeconomic status characterized as low or low-middle income
Parade et al., 2016	Parental education, parental employment status, qualification for public assistance
Parent et al., 2017	Parental education, parental employment status, single parent status, qualification for public assistance
Tyrka et al., 2015a & 2015b	Qualification for public assistance; composite score encompassing parental education ≤ high school degree, parental unemployment, and single parenthood
Romens et al., 2015	Hollingshead Four-Factor Index of Socioeconomic Status - composite score of parents' marital status, employment status, educational attainment, and occupational prestige (Hollingshead, 1975)
Tang et al., 2020	Household's highest socioeconomic class, mother's highest education qualification
Van der Knapp et al., 2014	Controlled for between the two groups

The remaining papers did not report on SES characteristics. Some parent studies collected additional SES information. In the case of articles using data from larger studies, they are only included here if they described SES information in their report.

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Note: if listed in primary reference list, appendix references not duplicated here