



Childhood maltreatment and DNA methylation: A systematic review

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ABSTRACT

DNA methylation (DNAm) – an epigenetic process that regulates gene expression – may represent a mechanism for the biological embedding of early traumatic experiences, including childhood maltreatment. Here, we conducted the first systematic review of human studies linking childhood maltreatment to DNAm. In total, 72 studies were included in the review (2008–2018). The majority of extant studies (i) were based on retrospective data in adults, (ii) employed a candidate gene approach (iii) focused on global maltreatment, (iv) were based on easily accessible peripheral tissues, typically blood; and (v) were cross-sectional. Two-thirds of studies ($n = 48$) also examined maltreatment-related outcomes, such as stress reactivity and psychiatric symptoms. While findings generally support an association between childhood maltreatment and altered patterns of DNAm, factors such as the lack of longitudinal data, low comparability across studies as well as potential genetic and ‘pre-exposure’ environmental confounding currently limit the conclusions that can be drawn. Key challenges are discussed and concrete recommendations for future research are provided to move the field forward.

1. Introduction

Childhood maltreatment is a major neurodevelopmental risk factor that continues to affect up to one in four children worldwide (World Health Organisation, 2016). Acts of maltreatment, including abuse and neglect, represent one of the most toxic forms of childhood adversity, probabilistically increasing the likelihood of maladaptation and psychopathology across the life span (Toth and Cicchetti, 2013). Children who are exposed to maltreatment are more likely to develop a range of social, emotional and behavioral problems, including anxiety, depression, conduct problems and hyperactivity (Cecil et al., 2017; de Oliveira et al., 2018; Keyes et al., 2012; Ouyang et al., 2008). Many of these effects are not confined to childhood but can extend well into the adult years. Indeed, childhood maltreatment is a robust predictor of lifetime psychiatric disorders (Caspi et al., 2014), associating not only with the occurrence of mental health problems per se, but also with an earlier age of onset, higher comorbidity, greater symptom severity and poorer response to treatment (e.g. psychological treatment and pharmacotherapy such as antidepressant use), when such problems do emerge

(Hovens et al., 2012; Nanni et al., 2012; Nemeroff et al., 2003). Maltreatment has also been shown to compromise other important aspects of individual function, including relationship quality, educational attainment, employment prospects and earnings, as well as physical health (Danese et al., 2009). Consequently, childhood maltreatment is recognized as a key target for prevention and intervention efforts (MacMillan et al., 2009; McCrory and Viding, 2015).

An important question for research, clinical practice and public health is to understand how childhood maltreatment can increase risk for negative outcomes even decades after the exposure itself has ceased. Research suggests that, in part, this enduring effect may be indirectly mediated by subsequent factors associated with childhood maltreatment, such as exposure to further adversity and revictimization later in life (e.g., peer victimization, intimate partner violence; Shields and Cicchetti, 2001) as well as harmful behaviors that may develop as a result of maltreatment (e.g., substance use; Lansford et al., 2010). However, growing evidence also points to a more direct pathway, whereby exposure to severe stressors in childhood, such as maltreatment, becomes ‘biologically embedded’, altering development and

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function in a way that engenders latent vulnerability for poor outcomes and reduced resilience (McCrory and Viding, 2015). This is supported, for example, by evidence of numerous biological correlates of childhood maltreatment, including neuroendocrine dysregulation, heightened inflammatory response, alterations in metabolic function, atypical brain structure and function, as well as accelerated cellular ageing (Berens et al., 2017). Despite increasing knowledge of the biological sequelae of maltreatment, however, the molecular mechanisms underlying these associations remain unclear. In recent years, epigenetic processes have emerged as a promising mechanism by which early adverse experiences such as maltreatment may drive biological changes, shaping long-term trajectories of development, health and disease risk.

1.1. Epigenetics: a potential mechanism for the biological embedding of environmental exposures

The ‘epigenome’ refers to a collection of epigenetic processes that regulate *when* (i.e. during the lifespan) and *where* (i.e. in the body) genes are expressed – primarily via chemical modifications to DNA, histone proteins, and chromatin structure (Jaenisch and Bird, 2003). Of these, DNA methylation (DNAm) is currently the most commonly investigated epigenetic mechanism in human research on early life stress and psychiatric risk, as it is easier to quantify and relatively more stable compared to other epigenetic processes (Jones et al., 2018). DNAm refers to the addition of a methyl molecule to specific DNA base pairs, primarily in the context of cytosine-guanine (CpG) dinucleotides. Rather than being randomly distributed across the genome, CpG sites tend to cluster into ‘CpG islands’ that are often located in the proximity of gene promoter regions. These islands are typically unmethylated (i.e. no methyl molecules attached), enabling transcription factors to bind to the DNA sequence and to subsequently activate gene expression. In contrast, when methyl groups attach to CpG islands, they physically impede transcription factors from binding to the DNA sequence, thereby interfering with gene expression. Overall, the association between DNAm and gene expression has been found to vary depending on the gene region examined: whereas higher DNAm levels within gene promoters and enhancers are usually associated with decreased expression of that gene, DNAm levels in the gene body region typically associate with increased gene expression (Jjingto et al., 2012). Importantly, DNAm marks can be mitotically passed on during cell division, which can lead to stable alterations in gene activity and downstream biological processes.

While it is well established that DNAm patterns are under considerable genetic control – as evidenced by both twin and molecular genetic studies (e.g., Boks et al., 2009; Zhang et al., 2010) – it is also increasingly clear that environmental influences play an important role in epigenetic regulation (Feil and Fraga, 2012). As a result, DNAm has been posited as a potential mechanism underlying gene-environment interplay on phenotypic expression. This is supported, for example, by studies showing that individual differences in DNAm patterns are largely explained by the joint effect of genes and environments (GxE or G + E; Czamara et al., 2019; Teh et al., 2014) – a finding also observed specifically in relation to childhood maltreatment (Klengel et al., 2013). Research in animals and humans has found that DNAm patterns associate with a range of environmental factors beginning as early as in utero, with some of the strongest evidence implicating tobacco smoking and nutritional exposures (Breton et al., 2009; Cecil et al., 2016a, 2016b; Richmond et al., 2014b; Rijlaarsdam et al., 2017). These epigenetic ‘signatures’ can persist long after the exposure itself, supporting a role of DNAm in the biological embedding of environmental influences. For example, in an often-cited study, Heijmans et al. (2008) found that even 60 years after the event had taken place, individuals who had been prenatally exposed to famine (i.e. severe undernutrition) showed lower levels of DNAm in the Insulin-Like Growth Factor 2 (*IGF2*) gene – a key regulator of fetal development – and higher disease

risk compared to their unexposed, same-sex sibling. In another study, Richmond et al. (2014b) reported that although some of the DNAm marks associated with prenatal exposure to maternal tobacco smoking were reversible with time, others persisted into adulthood, with potentially long-term consequences for health. Similarly, in adults, DNAm patterns have been found to differentiate between never-smokers, ex-smokers and current smokers, as cessation leads to reversibility of some, but not all, smoking-related DNAm changes, even decades after cessation (Ambatipudi et al., 2016). Of relevance to the maltreatment field, growing evidence suggests that, in addition to these more ‘physical’ exposures, DNAm patterns may also be sensitive to ‘social’ exposures, such as the quality of the early caregiving environment (e.g., Barker et al., 2018).

1.2. Early adversity and DNA methylation: evidence from animal and human studies

The first evidence for the impact of early adversity on the epigenome stemmed from research in animals. In a series of seminal studies based on rodents, Weaver and colleagues (2004; 2005) found that variations in maternal care during the first week of life – as indexed by either high (i.e. nurturing care) or low (i.e. poor/neglectful care) levels of licking and grooming – led to long-term changes in the pup’s epigenetic regulation of a gene crucially implicated in HPA axis function: the Glucocorticoid Receptor gene (*Nr3c1*). In turn, these epigenetic changes stably altered *Nr3c1* expression, resulting in variations in the density of glucocorticoid receptors in the brain as well as inter-individual differences in the pup’s physiological and behavioral responses to future stressors. Remarkably, the authors demonstrated that while epigenetic programming by maternal care could persist into adulthood, it could be reversed by early intervention, such as methionine infusion or cross-fostering (Weaver et al., 2004, 2005). Since these early reports, multiple other studies in animals have supported a link between early adversity and DNAm, reporting higher *Nr3c1* methylation in pups exposed to poor maternal care as well as other stressful experiences, such as maternal separation (see Turecki and Meaney, 2016, for a review).

Together, this research has led to a major interest in the role of DNAm as a potential biological mediator of early adversity on developmental outcomes in humans, with a particular focus on the effects of childhood maltreatment. The first study that sought to translate animal findings into humans was that of McGowan and colleagues (2009), who examined *NR3C1* methylation levels in hippocampal tissue from suicide completers. The authors found that, amongst adults who had committed suicide, those retrospectively identified as having experienced maltreatment during childhood showed higher levels of *NR3C1* methylation in the hippocampus – consistent with the findings from pups exposed to low maternal licking and grooming. As reviewed by Turecki and Meaney (2016), subsequent studies have since replicated associations between childhood maltreatment and elevated *NR3C1* methylation with a high degree of consistency, not only in postmortem brain tissue but also extending findings to peripheral tissues (e.g. saliva, blood) in living individuals. Furthermore, a number of studies have found that higher *NR3C1* methylation also associates with maltreatment-relevant outcomes, including physiological markers of stress response (e.g. cortisol reactivity; van der Knaap et al., 2015), endophenotypes of psychiatric risk (e.g. neural responses to trauma-related intrusive memories; Vukojevic et al., 2014), and clinical outcomes (e.g. diagnosis of borderline personality disorder [BPD], major depression and post-traumatic stress disorder [PTSD]; Dammann et al., 2011; Yehuda et al., 2015) – although null results have also been reported (see Turecki and Meaney, 2016 review).

In summary, studies in humans seem to support animal findings in showing that (i) early psychosocial adversity in the form of childhood maltreatment can influence epigenetic regulation of the *NR3C1* gene; and that (ii) changes in *NR3C1* methylation, in turn, associate with stress-related physiological and psychiatric outcomes. As the field

moves forward, however, there is an increasing appreciation that, if we are to gain a more complete picture of how childhood maltreatment influences epigenetic regulation, we must look beyond the *NR3C1* gene and examine wider changes in DNAm across the genome. This is well-reflected in the rapidly growing number of studies examining the relationship between childhood maltreatment and DNAm across other candidate genes (e.g. *SLC6A4*, *FKBP5*, *BDNF*, *OXTR*), as well as emerging research using hypothesis-free, genome-wide approaches. While studies have already been reviewed concerning (i) associations between other types of stressors and DNAm (e.g. *prenatal stress*: Sosnowski et al., 2018; *chronic adult stress*: Bakusic et al., 2017); or (ii) broadly-defined early life stress and epigenetic changes in specific genes (e.g. *NR3C1*: Turecki and Meaney, 2016; *SLC6A4*: Provenzi et al., 2016), no systematic review exists to date on the topic of childhood maltreatment and DNA methylation.

1.3. The current review

Here, we conducted the first systematic review of research examining the relationship between childhood maltreatment and DNA methylation. Specifically, we collated findings from existing human studies in this area, irrespective of approach (e.g. candidate vs genome-wide), tissue (e.g. brain vs peripheral tissues), age range assessed (e.g. childhood, adolescents, adults), and sample type (e.g. general population, community high-risk samples, psychiatric inpatients). Epigenetic mechanisms other than DNAm (e.g. histone modifications and non-coding RNAs) were excluded, as these remain rarely investigated in human populations due to challenges with sample storage and processing (Jones et al., 2018). Based on the studies identified, we evaluate the current state of the literature, highlight main challenges for the field and propose key recommendations for future research.

2. Methods

Searching and reporting of results followed the general recommendation from the PRISMA 2009 revision (Moher et al., 2009; Peng et al., 2018).

2.1. Inclusion and exclusion criteria

The current systematic review included studies that investigated associations between DNAm and childhood maltreatment. To search for all studies conducted in this area, we included articles published at any time before the 1st of January 2019. Inclusion criteria were as follows: (1) studies must report empirical evidence (reviews were excluded); (2) they must focus on human populations (animal studies were excluded); (3) they must examine childhood maltreatment, such as abuse and/or neglect (studies that examined a global index of adversities or other childhood stressors, such as poverty, institutionalization and parental psychopathology were excluded, unless they also reported specific associations with maltreatment; studies measuring exposure to maltreatment and life stressors after childhood were also excluded); (4) they must measure DNAm levels (other epigenetic mechanisms, such as histone modifications, were excluded); and (5) they must not be solely based on cell-lines. Additionally, studies were excluded if they were conference papers, book chapters, or written in non-English languages. No restrictions were applied regarding: (1) tissue type (e.g. peripheral or central tissue), (2) sample age (e.g., childhood, adulthood), (3) research design (e.g., longitudinal, cross-sectional), (4) approach (e.g. candidate vs genome-wide), and (5) whether or not a phenotypic outcome was also included (e.g., cortisol levels, depression, brain function).

2.2. Search strategy

Four electronic databases (PubMed, Web of Knowledge, Medline,

and EMBASE) were searched for relevant studies written in English. Search terms were applied in MeSH or index terms, as well as text words. Included terms related to either (i) DNA methylation, AND (ii) childhood maltreatment (i.e., child* maltreat*, child* abuse, child* neglect, child* deprivation, child* advers*, child* trauma), NOT (iii) type of manuscript (review, commentary), NOT (iv) animal studies (mice, mouse, animal, rat).

2.3. Study selection & eligibility assessment

Studies were first screened based on the title and abstract. Those that appeared to meet inclusion criteria were then retrieved for full-text screening to assess eligibility. Three independent reviewers screened the articles. Data fields assessed were (1) exposure; (2) phenotype/outcome; (3) sample characteristics (e.g., age, gender, clinical vs. general population); (4) tissue (e.g., saliva, blood); (5) study design (e.g., cross sectional, longitudinal); (6) DNAm time points assessed (1 or more); and (7) approach (candidate, EWAS, global DNAm).

3. Results

3.1. Descriptive summary

Our search yielded 866 records, with 547 remaining after filtering out duplicates (see Fig. 1). Titles and abstracts were then screened, and of these, 459 were excluded for the following reasons: not empirical (e.g. reviews, $n = 165$), conference papers and book chapters ($n = 80$), did not measure DNAm ($n = 8$), did not examine childhood maltreatment ($n = 125$) or examined childhood maltreatment only in combination with other types of adversities (e.g. poverty; $n = 49$), were animal studies ($n = 26$), or written in non-English languages ($n = 6$). 88 studies were retained, and their full-text articles were assessed for eligibility. 16 articles were removed at this stage due to the following reasons: (i) 9 did not report associations specifically with childhood maltreatment, (ii) 1 was a conference paper, (iii) 3 did not focus on DNAm, (iv) 1 was a methods paper, (v) 1 was a preprint on BioRxiv, and (vi) 1 focused on childhood stressors other than maltreatment. A total of 72 original reports were included in the systematic review. 24% ($n = 17$) of included studies were published in 2018 alone, and sample sizes were on average modest ($n = 142$) ranging between $n = 24$ and $n = 1658$ (see Fig. 2). Full details of these studies are provided in Supplementary Table 1.

3.2. Global DNA methylation

A total of three studies examined the relationship between childhood maltreatment and global DNAm levels (i.e. where one score is calculated per individual indexing overall DNAm levels), showing weak evidence of associations. In a mixed sample of psychiatric patients, Murphy et al. (2013) found no association between global DNAm (assessed via 5-mC quantification) and childhood sexual/physical abuse, although global hypermethylation was observed in individuals who had attempted suicide compared to those who did not. Other forms of maltreatment were not assessed. Similarly, Smith et al. (2011) did not identify an association between global DNAm (assessed by averaging all sites on the Illumina27k array) and childhood abuse in a sample of adults with a diagnosis of PTSD vs controls. In a more recent study, Misiak et al. (2015) examined global DNAm levels (via LINE-1 repetitive elements) in patients with first-onset schizophrenia and observed higher DNAm for patients who reported early life trauma vs those without a trauma history. Follow-up analyses indicated that this association was mainly driven by experience of emotional abuse. While all three studies used DNA extracted from whole blood in adults, they differed in a number of characteristics which may explain differences in findings, including the method for quantifying global DNAm, the classification of childhood maltreatment and the psychiatric population examined.

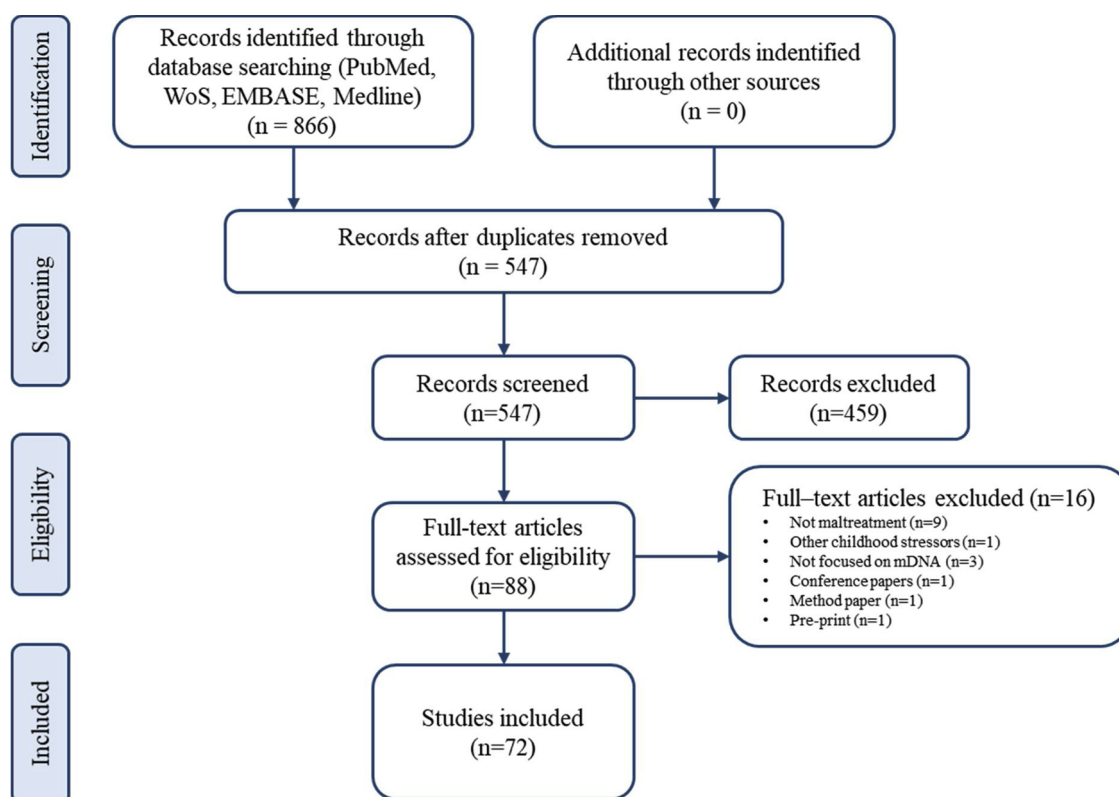


Fig. 1. PRISMA flowchart detailing the filtering steps undertaken to select studies.

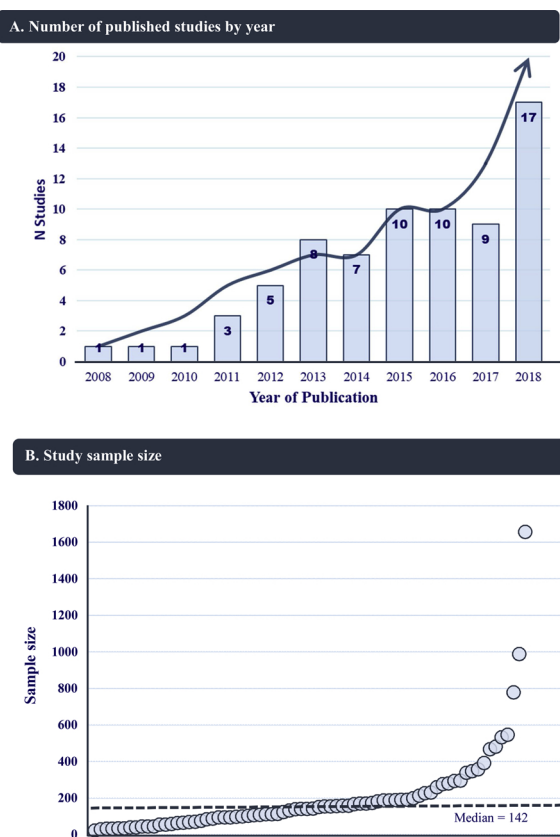


Fig. 2. Publication trend of papers investigating DNAm in maltreated population.

3.3. Epigenetic age

DNAm patterns strongly associate with chronological age, to the extent where methylation-based algorithms have been developed that can predict age with a high degree of accuracy, as well as enabling researchers to use residuals from these models to calculate age ‘acceleration’ (i.e. when epigenetic-predicted age is greater than chronological age, a difference that is hypothesized to reflect advanced biological aging). Three studies used the approach developed by Horvath (2013) to investigate associations between childhood maltreatment and accelerated epigenetic age, with mixed findings. Zannas et al. (2015) found that cumulative lifetime stress, but not childhood maltreatment or current stress alone, predicted accelerated epigenetic aging (based on blood) in an urban, African American cohort of 392 participants, pointing to a potential mechanism through which chronic stress may accelerate biological aging and increase age-related disease risk (e.g. coronary heart disease). A second study by Lawn et al. (2018) found no association between epigenetic age and a range of childhood stressors (e.g. global maltreatment, socioeconomic position, parental psychopathology, illness) amongst adult women from two population-based cohort studies in the UK, the Avon Longitudinal Study of Parents and Children (ALSPAC; n = 989; DNAm drawn from blood at two time points) and the National Survey of Health and Development study (NSHD; n = 773; DNAm from buccal cells at a single time point). A specific association between sexual abuse and accelerated epigenetic age was identified in ALSPAC across the two time points tested, but this could not be ascertained in NSHD as only global maltreatment was measured in that sample. Finally, O’Donnell et al. (2018), did not identify any significant associations between childhood maltreatment and epigenetic age acceleration (measured from blood) in a sample of 188 individuals who were born to mothers either randomly assigned to a control group or a psychosocial intervention program (the authors also conducted epigenome-wide analyses, which are described in the relevant section below).

Table 1
Summary of study characteristics.

Study characteristics (N = 72)	N	%
Design		
Cross-sectional	63	88%
Prospective/longitudinal	9	13%
Developmental period		
Childhood	13	18%
Adolescence	4	6%
73 + Adulthood	48	67%
Postmortem	7	10 %
Replication		
No	68	94%
Yes	4	6%
Maltreatment type examined		
Any/global maltreatment	49	68 %
Abuse only (physical and/or sexual)	23	32%
DNAm approach		
Candidate	51	71%
Hypothesis-free	17	24 %
Global	2	3%
Epigenetic age	2	3%
DNAm tissue		
Peripheral blood	46	64%
Saliva	18	25%
Buccal	5	7%
Brain (postmortem)	7	10 %
Sperm	1	1%
Repeated measures of DNAm		
No	66	92%
Yes	6	8%
Psychiatric and physiological outcomes		
No	22	31%
Yes	50	69%
Most commonly investigated:		
Depression	15	21%
Borderline Personality Disorder	9	13%
Suicide and suicidal ideation	9	13%
Cortisol (e.g. baseline, reactivity)	5	7%
Post-Traumatic Stress Disorder	3	4%
Anxiety	4	6%
Eating disorders	3	4%

N.B. The total number of studies for each characteristic may exceed 72 due to the presence of studies fitting multiple domains.

3.4. Candidate gene studies

As shown in Table 1, 51 studies examined the relationship between childhood maltreatment and DNAm level using a candidate gene approach, whereby specific genes are pre-selected for analysis based on an existing hypothesis. Almost half of all candidate gene studies focused on *NR3C1* (n = 23), followed by *SLC6A4* (n = 8), *FKBP5* (n = 6), *BDNF* (n = 4), *OXTR* (n = 3), *MAOA* (n = 2) and *5-HT3A* (n = 2). Twelve other genes were investigated only once. Below, we focus on genes that have been examined at least twice. Full details of the 51 individual studies are listed in Supplementary Table 1, whereas an overview of findings from the most widely examined candidate genes is provided in Table 2).

3.4.1. HPA axis and neuroendocrine pathway genes

3.4.1.1. Glucocorticoid receptor (*NR3C1*) gene. *NR3C1* encodes the glucocorticoid receptor (GR), to which cortisol and other glucocorticoids bind. As a transcription factor (and modulator of other transcription factors), GRs involved in a wide range of developmental, immune and endocrine processes. In the field of maltreatment, however, it has gained wide attention specifically because of its key role in regulating stress responses within the brain (Turecki and Meaney, 2016). Candidate epigenetic studies on childhood maltreatment have primarily focused on the *NR3C1* gene guided by early evidence from animal experimental models. These have mainly examined the promoter region exon 1_F – the human equivalent of exon 1₇ shown to be differentially methylated in animals exposed to early

Table 2
Summary of study characteristics and results for the most commonly investigated candidate genes.

Gene	N studies (% out of 51)	Main region of interest	Association with child maltreatment		
			Positive	Negative	Null
<i>NR3C1</i>	23 (45 %)	Exon 1 F	17	1	5
<i>SLC6A4</i>	8 (16 %)	Promoter	7	0	1
<i>FKBP5</i>	6 (12 %)	Intron 7 (GRE)	0	4	2
<i>BDNF</i>	4 (8%)	Different regions	2	1	1
<i>OXTR</i>	3 (6%)	Promoter	2	0	1
<i>MAOA</i>	2 (4%)	Different regions	1	1	0
<i>5-HT3A</i>	2 (4%)	Different regions	1	1	0

N.B. GRE: glucocorticoid response element region; Not all studies per category are independent; Classification is based on significance (threshold set by individual studies). The total number of studies for each gene differs from 51 due to the presence of studies examining multiple candidate genes. Also, studies examining genes that were investigated only once are not shown in the table.

adversity – although other regions have also been tested (Shields et al., 2016). For example, Suderman et al. (2012) used a cross-species approach to examine *NR3C1* DNAm levels in postmortem hippocampal tissue of rats exposed to early life stress and humans exposed to maltreatment during childhood. The authors identified a similar pattern of hypermethylation of the *NR3C1* promoter in exposed rats and humans, as well as finding consistent DNAm alterations across the wider *NR3C1* gene locus, thereby supporting a conserved epigenetic mechanism of stress response between species. In line with these findings, 17 of the 23 studies on the *NR3C1* gene (74 %) identified in this review reported increased DNAm in relation to exposure to childhood maltreatment, namely physical, emotional, and sexual abuse or neglect (Bustamante et al., 2016; Cicchetti and Handley, 2017; Farrell et al., 2018; Labonte et al., 2012b; Martin-Blanco et al., 2014; McGowan et al., 2009; Parade et al., 2016; Parent et al., 2017; Peng et al., 2018; Perroud et al., 2011; Radtke et al., 2015; Romens et al., 2015; A. E. Shields et al., 2016; Suderman et al., 2012; Tyrka et al., 2015a, 2012; van der Knaap et al., 2014), whereas one reported decreased methylation levels (Tyrka et al., 2016) and 5 other studies reported null associations (Hecker et al., 2016; Steiger et al., 2013; E. Vangeel et al., 2015, 2018). It is important to note, however, that several of the *NR3C1* studies were based on the same samples and research groups, and thus not all were independent. Furthermore, the criteria for significance varied considerably, with studies differing in the number of CpG sites analyzed and whether multiple testing correction was used. Nevertheless, the overall consistency in findings is notable, with associations between childhood maltreatment and *NR3C1* hypermethylation reported across different types of samples (clinical, community), age groups (children, adolescents and adults), maltreatment measures (self-report, official records) and biological tissues (blood, saliva, brain).

In addition to maltreatment status, hypermethylation of *NR3C1* has also been found to relate to the specific characteristics of maltreatment exposure, with associations more evident for maltreatment that occurs at an earlier age of onset and is more severe and chronic (Cicchetti and Handley, 2017; Perroud et al., 2011). Only one study measured DNAm at multiple time points, showing that the relationship between maltreatment and *NR3C1* methylation can be complex and dynamic over time. Specifically, Parent et al. (2017) examined DNAm within 6 months of documentation of maltreatment and then again after one year. The authors found that although maltreatment status associated with *NR3C1* hypermethylation at baseline, it was also associated with decreased methylation levels over time, emphasizing the importance of longitudinal designs, the need to consider the timing of epigenetic

assessment and the presence of potential moderating environmental factors following maltreatment.

In order to investigate the potential functional consequences of maltreatment-related *NR3C1* methylation changes, a number of studies also included information on downstream biological markers, such as gene expression levels and physiological measures of stress response. Based on postmortem hippocampal samples, two studies found that *NR3C1* hypermethylation associated with reduced gene expression levels amongst maltreatment-exposed individuals, supporting the hypothesis that maltreatment affects regulation of key stress-related genes via DNAm changes (e.g., Labonte et al., 2012a; McGowan et al., 2009). However, none of the studies using peripheral tissues in living individuals measured both *NR3C1* methylation and expression, so that it is unclear whether these functional effects generalize to non-CNS tissue, such as saliva or blood. With regards to physiological markers of HPA axis, findings so far have been mixed, with reports of both positive and negative associations of *NR3C1* methylation with measures of basal cortisol levels and cortisol reactivity (Alexander et al., 2018; Farrell et al., 2018; Tyrka et al., 2012). For example, Farrell et al. (2018) reported that, in a sample of depressed patients, levels of *NR3C1* promoter methylation at one CpG site in exon 1_F positively associated with both severity of emotional abuse and morning cortisol concentrations (indicative of higher basal HPA axis activity), which the authors interpreted as an indication of acquired glucocorticoid receptor resistance. Focusing on cortisol response rather than basal levels, Alexander et al. (2018) found that, amongst healthy individuals exposed to moderate to high levels of maltreatment, DNAm of the *NR3C1* exon 1_F promoter moderated stress response to the Trier Social Stress Test. Specifically, within this group, individuals with high DNAm levels showed 62 % higher cortisol levels following stress exposures compared to those with low DNAm. In contrast, Tyrka et al. (2012) found that increased *NR3C1* promoter methylation associated with an attenuated cortisol response to the dexamethasone/corticotropin releasing hormone (Dex/CRH) test in a sample of 88 healthy adults, although more recently the same research group found a positive association between *NR3C1* promoter methylation and post-Dex/CRH cortisol response in a larger sample of 231 healthy adults (Tyrka et al., 2016). Although these studies are not necessarily in conflict with those examining stress activation – as Dex activity is primarily situated in the pituitary (which lies outside the blood-brain barrier) rather than neurally mediated (Cole et al., 2000) – general inconsistencies in findings mirror those observed in the broader literature on maltreatment and cortisol. Indeed, such research has previously identified heightened as well as blunted cortisol function and reactivity following maltreatment exposure, pointing to the likely complex relationship between maltreatment and indices of HPA axis activity (Bernard et al., 2017).

Finally, 16 (70 %) of studies on this gene have included information on mental health outcomes, in order to clarify the relevance of maltreatment-related *NR3C1* methylation changes for risk of psychopathology. Unlike the consistency of effects observed for maltreatment and *NR3C1* methylation, however, associations with psychopathology have been more mixed. On the one hand, maltreatment-related *NR3C1* hypermethylation has been found to positively associate with symptomatology, such as internalizing symptoms in children (Cicchetti and Handley, 2017; Parade et al., 2016; Tyrka et al., 2015a) and multiple psychiatric outcomes in adulthood, including depression (Peng et al., 2018), aspects of BPD (particularly self-harm; Martin-Blanco et al., 2014), and bulimic syndromes, especially when accompanied by marked impulse-dysregulation and mood instability (Steiger et al., 2013). On the other hand, other studies based on clinical populations with elevated rates of maltreatment exposure have identified negative associations between *NR3C1* methylation and psychopathological outcomes. For instance, in two independent samples of female patients with chronic fatigue syndrome (CFS) versus controls, Vangeel and colleagues (2015; 2018) found evidence of hypomethylation with emotional abuse only amongst CFS patients, however, no significant

association was found between DNAm levels and trauma history after multiple testing correction.

Overall, only three of these studies explicitly tested the joint effects of maltreatment and *NR3C1* methylation on psychiatric outcomes, either via moderation or mediation analyses. Radtke et al. (2015) reported a significant interaction effect between childhood maltreatment and *NR3C1* methylation in predicting risk of psychopathology. With regards to mediation, Peng et al. (2018) found that together with *BDNF* – another candidate gene – *NR3C1* methylation levels mediated close to 20 % of the association between childhood maltreatment and self-reported depression scores in adults. In contrast, Cicchetti et al. (2017) found no evidence of mediation of *NR3C1* methylation on the association between maltreatment exposure to psychopathology in children, despite the individual paths being significant.

3.4.1.2. FKBP5 binding protein 5 (FKBP5) gene. The *FKBP5* gene, located on the short arm of chromosome 6 (6p21.31), is a co-chaperone of *NR3C1* that regulates its sensitivity and response to stressors. *FKBP5* and *NR3C1* operate together in a complex, negative feedback loop. On the one hand, when *FKBP5* binds to the glucocorticoid receptor (GR) complex, it reduces the ability of GR to bind to cortisol, resulting in decreased GR signaling and translocation to the nucleus. On the other hand, the *FKBP5* gene contains glucocorticoid response elements (GRE) in intron 7, which enable GR binding to in turn regulate its expression (Zannas et al., 2016). Higher activation of *FKBP5* has been found to associate with increased stress-sensitivity and psychiatric risk (Wiechmann et al., 2019). Furthermore, genetic polymorphisms in *FKBP5* have been shown to interact with environmental exposures, including maltreatment, to predict psychiatric outcomes in both human and animal studies (Zannas et al., 2016).

Six epigenetic studies on childhood maltreatment focused on *FKBP5*. Four of these studies consistently reported decreased *FKBP5* methylation in the intron 7 region (coinciding with a glucocorticoid response element) in children and adults exposed to childhood maltreatment (Klengel et al., 2013; Parade et al., 2017; Tozzi et al., 2018; Tyrka et al., 2015b), although two studies in adults found no significant association (Bustamante et al., 2018; Farrell et al., 2018). Four of the six studies also measured *FKBP5* genotype to test genetic moderation: two reported significant hypomethylation amongst exposed individuals carrying the risk allele (Klengel et al., 2013; Tozzi et al., 2018), whereas the other two found no significant interaction effects (Tyrka et al., 2015a, 2015b; Parade et al., 2017). As with the *NR3C1* gene, only one study assessed the levels of *FKBP5* at multiple time points and found that while maltreatment status was associated with hypomethylation at baseline, it did not associate with changes in DNAm a decade later, when participants had entered early adulthood (Parade et al., 2017). However, the presence of additional adversities was found to influence this relationship: children who were exposed to maltreatment and other types of adversities showed persistently low DNAm levels across time.

3.4.1.3. Oxytocin receptor (OXTR) gene. Oxytocin is a hormone and neuropeptide that has been implicated in a range of social behaviors (e.g. empathy, bonding, attachment) and in modulating the individual's sensitivity to the social environment (Bartz et al., 2011). The Oxytocin Receptor (*OXTR*) gene, located on chromosome 3, is expressed both in the brain and within peripheral organs, where it synthesizes oxytocin receptors. Genetic and epigenetic variation in *OXTR* has been found to associate with individual differences in prosociality and maladaptive behaviors linked to early adversity (Maud et al., 2018). Based on our review, we identified three studies examining the relationship between *OXTR* methylation and childhood maltreatment, with weak evidence for an association (Gouin et al., 2017; Kogan et al., 2018; Smearman et al., 2016). In a sample of 393 African American adults, Smearman et al. (2016) found that childhood abuse associated with increased *OXTR* methylation in 4 out of 18 CpG sites investigated, but this

association did not survive multiple testing correction. Variation in *OXTR* did, however, associate with nearby single nucleotide polymorphisms, supporting genetic influences. Furthermore, while *OXTR* methylation did not mediate the link between childhood maltreatment and psychopathology (indexed by depression and anxiety symptoms), maltreatment status significantly interacted with DNAm to predict psychopathology. The second study, by Gouin et al. (2017), compared DNAm from a small sample of adults who were followed longitudinally from childhood, and who were either exposed to maltreatment ($n = 24$) or not ($n = 22$). The authors reported that the exposed group showed higher levels of *OXTR* methylation in a CpG site in the promoter region, although this difference did not survive multiple correction. Stratified analyses by gender revealed stronger associations between maltreatment exposure and *OXTR* methylation in females compared to males. Finally, Kogan et al. (2018), followed a cohort of 358 young African American men over three time points spanning late adolescence into early adulthood. Despite the lack of evidence for the hypothesized association between childhood trauma and *OXTR* methylation, they identified a distal influence of maltreatment that carries forward through more proximal social ties. Specifically, childhood trauma was associated with changes in prosocial ties between the first and second time point, which in turn predicted *OXTR* methylation.

3.4.2. Neurodevelopmental and neurotransmitter pathway genes

3.4.2.1. Serotonin transporter (*SLC6A4*) gene. Serotonin is a major neurotransmitter in the central nervous system, modulating a range of important functions, including mood regulation, affective processing learning and memory. Most epigenetic studies in this area have focused on the promoter region of *SLC6A4* (also known as *5-HTT* and *SERT*), a 14 exon gene located on chromosome 17, encoding the serotonin transporter. The selection of this gene has been heavily influenced by experimental and human studies reporting disrupted serotonin function in response to early life stress, as well as evidence from the gene-environment interaction literature, which showed that presence of the *SLC6A4* risk allele significantly moderates the effect of childhood maltreatment risk for depression and other psychiatric disorders – although these findings have been recently disputed (Border et al., 2019). Overall, the identified studies suggest a pattern of *SLC6A4* promoter *hypermethylation* in individuals exposed to childhood maltreatment. However, it is important to note that several of these studies are based on the same Iowa Adoptee Study, reporting hypermethylation amongst individuals exposed to sexual abuse (Beach et al., 2013, 2010, 2011; Vijayendran et al., 2012), or a combination of sexual and physical abuse (Beach et al., 2010). Specifically, this set of studies found that the association between sexual abuse and *SLC6A4* methylation was specific to females, possibly due to the much higher levels of exposure reported by females than males. Other types of abuse did not associate with *SLC6A4* methylation when examined separately from sexual abuse. The sample in the 2010 report was smaller with participants selected at random for inclusion in that study. Later reports examined a larger sample reflecting all available participants.

Drawing on this cohort, Beach et al. (2013) further observed that (i) sexual abuse and genetic load (indexed via parental psychopathology) exerted main effects on depressive and antisocial symptomatology of study participants; (ii) these two factors interacted to predict *SLC6A4* methylation levels – supporting DNAm as a potential mechanism underlying previously reported gene-environment interactions; and (iii) *SLC6A4* methylation mediated the effect of sexual abuse on antisocial psychopathology.

Reports from the other four studies are generally consistent with the above. Two support the finding of elevated DNAm in individuals exposed to childhood maltreatment (Booij et al., 2015; Kang et al., 2013). Specifically, Kang and colleagues found that physical abuse and sexual abuse were both significantly associated with higher methylation of the

SLC6A4 promoter region, measured as the average methylation level across 81 CpG sites, with physical abuse showing stronger associations. Similarly, Booij and colleagues found that global maltreatment associated with hypermethylation of the *SLC6A4* promoter, and that these effects were mainly driven by physical abuse. One other study in twins found nominal associations between childhood maltreatment and increased *SLC6A4* methylation, but these did not survive multiple correction. In contrast, the last study investigated a broad range of adversities in 133 Caucasian participants including childhood maltreatment and concluded that there was no significant effect of stress on the mean *SLC6A4* methylation levels (Wankerl et al., 2014).

3.4.2.2. Serotonin 3A receptor gene (*HTR3A*). Aside from the serotonin transporter, two studies examined the *HTR3A* serotonin receptor gene. *HTR3A* encodes a ligand-gated ion channel which, in humans and animals, has been shown to be involved, amongst other processes, in neural circuit formation, the regulation of amygdala excitability and fear extinction. Furthermore, in humans, genetic variations in the *HTR3A* have been shown to interact with early-life adversity to regulate serotonergic activity (Jang et al., 2015). In a sample of 346 patients with psychiatric diagnoses of ADHD, BPD and bipolar disorder, Perroud et al. (2016) found that childhood maltreatment, especially physical abuse, was associated with higher severity of all three disorders (indexed by number of mood episodes, history of suicide attempts, and previous hospitalization), and that this effect was mediated by higher DNAm at two CpG sites in *HTR3A* (drawn from blood). In contrast, Schechter et al. (2017) found a negative association between exposure to childhood maltreatment and *HTR3A* promoter methylation, extracted from saliva, in a sample of 35 women who were either diagnosed with PTSD vs controls. The authors interpreted these discrepant findings as reflecting differences underlying the pathophysiology of PTSD versus the mood disorders examined by Perroud et al.

3.4.2.3. Monoamine oxidase A (*MAOA*). codes for a mitochondrial enzyme involved in metabolizing neurotransmitters, chief amongst them serotonin and dopamine. The gene contains a functional VNTR polymorphism in the promoter region that has been widely examined in relation to psychopathology, including risk for antisocial behavior, aggression and depression – disorders for which childhood maltreatment has been established as part of multifactorial etiologies. Checknita et al. (2018) found evidence for an association between sexual abuse and hypermethylation of the *MAOA* first exon region in a female sample (without such a pattern occurring in relation to physical abuse). These DNAm levels were also found to mediate the relationship between sexual abuse and depression (but not other psychopathologies). In contrast, the study in twins by Peng et al. (2018) did not identify any independent association between childhood maltreatment and *MAOA* methylation, although joint effects were observed together with the four other candidate genes under investigation (*NR3C1*, *BDNF*, *SLC6A4* and *MAOB*).

3.4.2.4. Brain-derived neurotrophic factor (*BDNF*) gene. *BDNF* has recurrently been implicated in neuronal cell proliferation and survival, and synaptic activity. *BDNF* has also been found to modulate risk for various psychopathologies, by acting on biological mechanisms such as neuroplasticity, inflammation or hypothalamic–pituitary–adrenal (HPA) axis functionality, all processes that are sensitive to stress exposure (Miskolczi et al., 2019). The neurotrophin has also been linked with food-related energy homeostasis and the risk for eating disorders. Thaler et al. (2014) found increased DNAm at specific CpG sites in individuals with Bulimia nervosa as compared to controls. This association was stronger in individuals who had been exposed to child abuse and/or individuals with BPD psychopathology. Perroud et al. (2013) examined *BDNF* promoter methylation in a psychotherapy intervention study for patients with BPD. DNA methylation at CpG sites located in exons I and IV

was significantly higher in patients than controls, and childhood abuse severity predicted higher levels of *BDNF* methylation pre-therapy. Therapy non-responders showed a significant *BDNF* methylation increase post-therapy, whereas responders were characterized by a decrease. Clinically, DNAm changes were associated with changes in depression, hopelessness and impulsivity scores. In a separate study based on patients with depression, Wang et al. (2018) found that maltreatment history associated with lower *BDNF* DNAm levels. In addition, *BDNF* hypomethylation and its interaction with stressful life event scores were linked impaired antidepressant (escitalopram) treatment response. Specifically, those patients who did not show remission/response to antidepressants had significantly lower DNAm than those who did, at baseline. The same was found for those patients with less symptom improvement, who showed lower DNAm at follow-up than those with more improvement. In those responding to antidepressant use, there was a significant increase in DNAm from baseline to follow-up. No significant relationships to childhood trauma for the follow-up component could be detected, but at baseline, increased childhood trauma was associated with decreased DNAm. The last study, by Peng et al. (2018), found that global childhood maltreatment associated with hypermethylation at three *BDNF* CpG sites, although associations did not survive multiple correction.

3.7. Hypothesis-free studies

In total, 17 (24 %) studies used a hypothesis-free approach (Supplementary Table 1). Of these, the majority (n = 14) ran epigenome-wide association (EWAS) analyses, where all CpG sites on the array are tested individually for associations with maltreatment to identify differentially methylated positions (DMPs). The remaining three studies used data reduction strategies to decrease the dimensionality of the data, including principal component analysis, gene-set analysis, and epigenome-wide regional analysis to identify differentially methylated regions (DMRs; i.e. sets of adjacent or physically proximal CpGs significantly associated with maltreatment exposure). Here, we first discuss research based on children and adolescents, before turning to adults and postmortem studies.

3.7.1. Child studies

Four studies examined child samples, all of which classified presence of (any) maltreatment based on official records and used the Illumina 450k array based on DNA extracted from saliva (Supplementary Table 1). Yang et al. (2013) investigated epigenetic correlates of maltreatment in a sample of 192 children, who were either removed from their parents due to substantiated cases of abuse and/or neglect over the prior 6 months (n = 96) vs demographically-matched controls (n = 96; age range = 5–14yrs). Neglect was the most common type of maltreatment recorded, although most children were exposed to multiple forms of adversity. The authors reported that over 2800 sites were differentially methylated between groups after genome-wide correction. These DNAm sites mapped onto genes that were significantly enriched for a range of biological processes of relevance to physical health problems e.g. cancer, although the study did not explicitly relate DNAm differences to health outcomes. Using the same sample of children, Weder et al. (2014) further investigated the relationship between DNAm and depression risk in maltreated children. The authors found that three DNAm sites associated with depression scores in the entire sample after genome-wide correction, which mapped onto genes implicated in stress response and neural plasticity (*ID3*, *TPPP*, *GRIN1*). The site in *ID3* also associated with diurnal cortisol secretion. Overall, these three sites were nominally associated with maltreatment exposure, as were sites in *BDNF*, *FKBP5* and *NR3C1*, based on a candidate gene follow-up analysis. In a separate sample, Cicchetti, Hetzel, Rogosch, Handley, and Toth (2016) examined epigenetic correlates of maltreatment in 548 low-income children (mean age = 9yrs) who attended a research summer camp program, half of whom were identified as having experienced maltreatment based on

official records. As with the above studies, neglect was the most prevalent form of maltreatment in this sample, and the majority of maltreated children experienced multiple forms of abuse/neglect. The EWAS results indicated that over 1800 sites were differentially methylated between groups after genome-wide correction. Consistent with Yang et al. (2013), maltreated children generally showed elevated DNAm levels at low and medium methylation sites, and reduced DNAm levels at high methylation sites. Also in line with Yang et al., gene ontology analyses indicated enrichment for disease-relevant processes as well as mental health-related terms. Specific associations between DNAm in top sites and maltreatment varied by gender, ethnicity and developmental timing of exposure. Of note, sites on the X and Y chromosomes were removed from the analysis but sex was not controlled for in the epigenome-wide analysis, although it has been found to associate with DNAm levels in autosomes (Suderman et al., 2017). Furthermore, none of the above studies controlled for cell-type composition in saliva, which typically includes a mixture of buccal epithelial cells and white blood cells – as such, it is unclear to what extent these patterns may simply be reflecting immune cell composition. The last study, by Kaufman et al. (2018), did not examine associations between maltreatment exposure and DNAm directly, but rather modelled their interaction in predicting child BMI amongst 234 children (mean age = 11yrs, 52 % maltreated), split into a discovery (n = 160; Illumina 450k array) and replication (n = 74, Illumina EPIC array) sample. Ten CpG sites were found to interact with childhood maltreatment exposure at a genome-wide level in the discovery sample, including sites previously implicated in obesity-risk. These findings however did not replicate based on the EPIC array in the smaller replication sample, although one CpG site located in the *GALE* gene showed a trend level interaction. One CpG site in the *PCK2* gene, which associated with BMI across both samples, was found to mediate the effects of childhood maltreatment on obesity risk.

3.7.2. Adolescent studies

Two of the identified studies focused on adolescent samples, both using an EWAS approach. The first, from our group, sought to characterize the DNAm ‘signatures’ of five forms of childhood maltreatment in a high-risk sample of inner-city youth (buccal cells; n = 124; age range = 16–24), 68 % of whom reported experiencing maltreatment while growing up (based on the CTQ; Cecil et al., 2016a, 2016b). We found that physical exposures (i.e. physical abuse and neglect, sexual abuse) showed the strongest genome-wide associations, implicating multiple genes previously associated with psychiatric and neurodegenerative disorders (e.g. *GABBR1*, *GRIN2D*, *CACNA2D4*, *PSEN2*). Based on gene ontology analyses, we found that although maltreatment types showed *unique* DNAm patterns enriched for *specific* biological processes (e.g. physical abuse with cardiovascular function, fear processing and wound healing vs. physical neglect with nutrient metabolism), they also shared a ‘common’ epigenetic signature enriched for biological processes related to regulation of nervous system development and organismal growth. The second study, by Marzi et al. (2018), examined associations between different types of victimization and DNAm in a large population-based study of twins E-risk; n = 1658; age at blood draw = 18yrs. Overall, the authors found weak and mixed evidence for a relationship between peripheral DNAm and victimization. Using prospective measures of maltreatment, 48 genome-wide significant associations were identified across individual types of maltreatment, 39 of which were related to sexual abuse. When using retrospective measures of maltreatment CTQ – which had only fair agreement with the prospective measures – another 48 loci were identified, 22 of which related to sexual abuse. However, these sites did not overlap with those identified prospectively, and were not replicated in an independent sample of 818 adults who completed the CTQ within the Dunedin Longitudinal Study age 38yrs after multiple testing correction. To complement the genome-wide analyses, the authors also followed up specific stress-related candidate genes *NR3C1*, *FKBP5*,

BDNF, *AVP*, *CRHR1*, *SLC6A4*), but identified only two associations with childhood maltreatment after gene-based correction (one locus in *BDNF* and one in *FKBP5*).

3.7.3. Adult studies

Most epigenome-wide studies ($n = 11$; 65 %) examined associations between maltreatment and DNAm in adults (Supplementary Table 1). Here we first summarize studies in living individuals based on high-risk ($n = 6$) and general population ($n = 3$) samples – which mainly measured DNAm in blood – followed by post-mortem studies in brain tissue ($n = 2$). In a psychiatric sample of patients with BPD and comorbid MDD, Prados et al. (2015) employed a machine learning approach to predict maltreatment exposure based on DNAm patterns (blood) and found that prediction performance was most optimal for global maltreatment (defined as total number of maltreatment types reported in the CTQ), as opposed to any individual form of maltreatment alone. The most predictive DNAm site (validated with pyrosequencing) was located in the vicinity of MicroRNA 137 (*MIR-137*), which is involved in the regulation of neuronal and HPA-related genes, including *NR3C1*. Focusing exclusively on the CTQ abuse subscales, Mehta et al. (2013) examined differential transcriptomic and epigenetic patterns between individuals in three different groups (i) patients with PTSD and exposure to abuse ($n = 32$), (ii) patients with PTSD and no exposure to abuse ($n = 29$), and (iii) control individuals without PTSD who were exposed to trauma, but not abuse ($n = 108$). Patterns that were found to associate with abuse exposure did not overlap with those identified for PTSD. The majority of differentially expressed transcripts related to child abuse also showed differential DNAm at coinciding CpGs sites. In contrast to Mehta et al., another study comparing groups of individuals following a similar operationalization (i.e. PTSD + abuse, PTSD-abuse, control + abuse, control-abuse; $n = 110$) found no significant associations between DNAm and abuse history, although associations with PTSD were identified (Smith et al., 2011). Marinova et al. (2017) compared DNAm patterns (buccal cells) between Swiss elderly individuals who had experienced severe adversity and forced labor in childhood ($n = 30$) vs demographically-matched controls ($n = 15$). Former child laborers reported significantly greater rates of global maltreatment and differed from controls across 71 DNAm sites, which were enriched for processes related to neural and organismal development (consistent with enriched terms from our study; Cecil et al., 2016a, 2016b). In addition to examining epigenetic age acceleration (as described in the section above), O'Donnell et al. (2018) carried out hypothesis-free analyses in their sample of 188 adult offspring of women randomized to a nurse visitation program, by performing genome-wide principal component analysis on CpG sites showing high variability in DNAm levels (defined as having a range ≥ 10 %). The authors extracted the first 10 principal components, two of which were found to associate with maltreatment and relate to transcriptional processes. Associations were partially explained by smoking but not by polygenic risk scores for psychiatric disorders. One other study also used principal components analyses to reduce the dimensionality of epigenome-wide DNAm data. Based on sperm DNAm from three groups of men exposed to varying degrees of child abuse ($n = 34$), Roberts et al. (2019) found that abuse explained over 6% of variance in one of the top 9 principal components. The authors also identified a number of DMRs associated with child abuse, with three specific sites found to be most useful in classifying exposure levels based on machine learning. Generally, associations between abuse and DNAm were not explained by factors such as psychiatric disorders, lifetime trauma exposure, smoking and BMI.

Three studies in adults were based on general population samples. Based on 40 males from the 1958 National Child Development Study (NCDS), Suderman et al. (2012) found widespread differences in promoter DNAm (blood) between those who reported childhood abuse vs controls, with significant enrichment for genes involved in transcriptional regulation and development. Interestingly, these patterns did not

overlap with those associated with low socioeconomic position. The top hit for childhood abuse (in the *PM20D1* gene involved in mitochondrial function) was validated with pyrosequencing and replicated in an additional sample. The second study examined epigenome-wide correlates (buccal cells; Illumina 450k) of cortisol reactivity in 85 healthy adults from the general population, and then associated these to global CTQ maltreatment scores (Houtepen et al., 2016). Although no associations survived genome-wide correction, the top site (cg27512205, annotated to *KITLG*, a gene involved in a range of cellular developmental processes) was replicated in two independent samples (buccal cells; blood), showed concordance with DNAm levels in the brain, and partially mediated the association between childhood maltreatment and blunted cortisol reactivity. Of note, a replication attempt by Wrigglesworth et al. (2018), focusing on *KITLG* as a candidate gene in an elderly population provided further evidence for a link between *KITLG* methylation and cortisol under a stress condition, although no association was identified with childhood trauma. A more recent study by Houtepen et al. (2018) sought to examine epigenome-wide patterns associated with adverse childhood experiences, including maltreatment, in women from two large population-based samples (ALSPAC: $n = 780$, blood; NSHD: $n = 552$, buccal cells). Consistent with the study by Marzi et al. (2018) in adolescents, the authors identified weak and mixed evidence for maltreatment-related DNAm effects: DMRs associated with maltreatment within each sample were not replicated, and regression coefficients for the top 1000 CpGs for each analyses were only weakly correlated between cohorts. Of note, however, DMRs associated with individual maltreatment types in ALSPAC, such as sexual abuse, could not be tested in NSHD, as only data on global maltreatment was available in that sample. Instead, the authors examined sites previously reported to associate with sexual abuse within a different population sample, the Dunedin study, and found that these were indeed also enriched for sexual abuse in ALSPAC.

Finally, two studies investigated epigenome-wide DNAm patterns in brain tissue. Based on post-mortem hippocampal tissue from individuals who had committed suicide ($n = 41$, of whom 25 with history of childhood abuse based on psychological autopsies) vs matched controls, Labonte et al. (2012a) reported group differences across 307 gene promoters, most of which were hypermethylated in abused individuals. Follow-up analyses in a subset of individuals confirmed that these patterns of hypermethylation typically associated to decreased gene expression. A recent study by Lutz et al. (2017) also examined brain tissue in order to investigate epigenetic, transcriptomic and morphological correlates of childhood abuse in the anterior cingulate cortex – a brain region previously found to be altered in maltreated individuals based on neuroimaging data. The study comprised of 47 suicide completers with major depressive disorder (27 of whom also experienced childhood abuse) vs 26 matched controls. Drawing on a highly comprehensive set of analyses, the authors found that individuals who had experienced childhood abuse differed from controls across 115 DNAm regions, which were enriched for oligodendrocyte and myelin-related genes. These epigenetic changes were indeed found to occur specifically in oligodendrocyte (as opposed to neuronal) populations, based on cell sorting, and were linked to altered gene expression. In turn, changes in gene expression were found to correlate highly with those observed in the brain of adult rats exposed to low maternal care early in life. Furthermore, the identified epigenetic and transcriptomic alterations were supported by evidence of reduced white (but not grey) matter density and decreased myelin axonal thickness in the child abuse group only. The same patterns were not observed in the depressed-only group, suggesting that they are not explained by psychopathology.

4. Discussion

In this systematic review, we summarize evidence from a decade (2008–2018) of human research investigating the relationship between

childhood maltreatment and DNA methylation. We identified 72 empirical studies, a fourth of which were published in 2018 alone. While the majority of studies supported an association between childhood maltreatment and DNAm patterns – with the strongest evidence implicating the *NR3C1* gene – the current evidence base is far from consistent, with the strength and direction of associations varying widely across studies. Inconsistencies are likely to stem in large part from marked differences in methodology and sample characteristics, which currently limit the comparability of findings and possibilities for pooling estimates in meta-analyses. Going forward, it will be necessary to push towards more open, collaborative and reproducible science (e.g. via consortium initiatives), in order to increase statistical power, detect more robust associations and reduce false positives. Although particularly challenging in the context of maltreatment research, the use of longitudinal and genetically-sensitive designs that can account for a range of environmental and genetic confounders will also mark an important step for better delineating the relationship between (different forms of) childhood maltreatment and DNAm. In this section, we first begin by summarizing similarities and differences between the identified studies, before outlining key challenges for the field and setting out twelve specific recommendations for future research.

4.1. Summary of study characteristics and findings

4.1.1. Study characteristics

In this review we identified a total of 72 empirical studies, the majority of which (i) employed a candidate gene (i.e. hypothesis-driven) approach, (ii) examined DNAm from readily-accessible peripheral tissues (e.g. blood, saliva), (iii) used a cross-sectional design with variables measured at a single time point, (iv) assessed maltreatment history via self-report, and (v) involved adults, typically in the context of psychiatric samples. While most studies focused on the effect of overall maltreatment, either classified categorically (e.g. any vs no exposure; different thresholds or counts of exposure) or continuously (e.g. via composite or total scores), some examined associations with specific forms of maltreatment. Of these, physical and sexual abuse were by far the most assessed. In contrast, neglect was examined the least, even though it emerged as the most prevalent form of maltreatment within studies that classified exposure based on substantiated cases from official records (e.g., Cicchetti et al., 2016; Yang et al., 2013). Furthermore, little attention was paid to emotional abuse, despite growing evidence that this form of maltreatment shows the strongest independent associations with poor mental health across symptom domains, raters and gender (Cecil et al., 2017; de Oliveira et al., 2018). Over two-thirds of studies collected additional information on physiological, behavioral and/or psychiatric measures, in order to examine whether maltreatment-DNAm associations explain individual differences in stress response and psychiatric risk. These studies focused primarily on outcomes known to strongly associate with maltreatment exposure, such as depression, BPD, suicidality, post-traumatic stress and anxiety as well as alterations in HPA axis functioning. Overall, very few studies included an independent replication sample or meta-analyzed estimates from different samples. Furthermore, few studies utilized a prospective design, measured DNAm at repeated time points or assessed the functional effect of maltreatment-related DNAm changes at different biological levels (e.g. on gene expression or brain function).

Based on the available data, it is not yet possible to conclusively assess how exposure to maltreatment – or its subtypes – associates with DNAm in different genes, and how these associations may vary across important factors such as timing and chronicity of exposure, age, sex, tissue type and presence of psychiatric symptoms or other health problems. A main barrier for this is the high heterogeneity among studies, both in terms of sample characteristics as well as methodology (e.g. way of measuring DNAm, type of analysis performed, choice of covariates, significance threshold used, etc.), which currently limits comparability

of findings. Another issue concerns reporting practices, as key statistics needed for performing meta-analyses, such as standard errors, are often not provided. Furthermore, in the case of EWAS studies, in which hundreds of thousands of different sites across the genome are tested, only associations that meet a certain threshold of significance are usually presented (e.g. genome-wide Bonferroni threshold), whereas full results are seldom provided (e.g. as supplementary material or in a public repository). As such, it is not currently possible to establish how convergent the epigenome-wide ‘signatures’ of maltreatment may be across these hypothesis-free studies. Bearing these limitations in mind, we highlight here some interesting similarities and differences in findings that emerged while reviewing the studies, which warrant further investigation.

4.1.2. Similarities in findings

A number of candidate genes were investigated by multiple studies and, promisingly, showed a consistent direction of associations. The glucocorticoid receptor gene *NR3C1* was by far the most extensively examined, with 17 (74 %) out of 23 studies reporting a *positive* association between childhood maltreatment and levels of *NR3C1* methylation (typically in the Exon 1 F region). Of the five studies that reported null results, two showed a positive direction consistent with the above, while the other three studies did not include any effect size estimates, so that it was not possible to establish the direction of associations. The last study reported a *negative* association between childhood maltreatment and adult *NR3C1* DNAm, which the authors interpreted as reflective of the complexity of epigenetic regulation of this gene in response to maltreatment and other stressors over time. Overall, the consistency in findings is notable, as positive associations were identified in studies using different samples (e.g. psychiatric, community), age ranges (children, adolescents, adults), maltreatment measures (e.g. self-reported vs official records), and importantly, tissue types (saliva, blood and postmortem brain [primarily hippocampus]). Furthermore, the findings are consistent with previous animal experimental work demonstrating the impact of early adversity (quantified as low maternal licking and grooming) on increased *NR3C1* methylation, with downstream effects on gene expression levels, glucocorticoid receptor density in the brain as well as physiological and behavioral responses to future stressors (Turecki and Meaney, 2016).

Another gene that showed promising findings is *SLC6A4*, coding for the serotonin transporter. This was the second most commonly investigated gene after *NR3C1*, examined by eight studies, all of which were based on adults, focused on childhood abuse (sexual and/or physical) and measured DNAm from blood. Seven (87.5 %) of the studies reported a positive association between childhood maltreatment exposure and higher *SLC6A4* promoter methylation. The remaining study also identified a positive association, but this did not survive multiple testing correction. Together, these findings add to the body of literature from existing genetic, neurophysiological and animal research implicating serotonin neurotransmission in stress response and susceptibility to psychiatric risk following maltreatment exposure. It is important to note, however, that in both the case of *NR3C1* and *SLC6A4*, reported effect sizes were generally small, replication attempts were low and the functional relevance of the identified DNAm changes was not tested. As such, it is unclear to what extent reported alterations impact gene expression levels and downstream biological function in humans. This is particularly problematic in cases where studies utilized an average DNAm score across many CpG sites spanning a wide gene region, as the relationship between DNAm levels at these CpG sites and gene expression may have varied depending on location, thereby raising questions about the biological significance of such an approach. More broadly, concerns have been raised in recent years regarding the use of a candidate gene approach, particularly from the field of psychiatric genetics, as it is most commonly employed by single studies with low samples sizes and is more susceptible to publication biases, false positives and winner's curse (Border et al., 2019). Such concerns

underscore the importance of moving towards transparent reporting and collaborative science, which will be needed if we are to facilitate meta-analyses, and thus enable us to systematically evaluate the robustness of these findings.

As with other complex exposures and outcomes, it is unlikely that epigenetic changes associated with maltreatment are restricted to single genes in isolation. Rather, such exposures are likely to involve changes in wider gene networks and pathways. Preliminary support for this emerged from the results of pathway analyses across several EWAS studies. Specifically, a number of these hypothesis-free studies found that maltreatment-related DNAm changes were more likely to occur in genes involved in neural (e.g. ‘axon guidance’), developmental (e.g. ‘multicellular organismal development’) and cardiovascular processes (‘cardiac muscle hypertrophy’; Cecil et al., 2016a,2016b; Cicchetti et al., 2016; Marinova et al., 2017) – consistent with the broader literature on maltreatment, pointing to the negative effect of abuse and neglect on these biological systems. Despite these promising findings, it is not currently possible to systematically compare the results of site- and pathway-level analyses from EWAS studies, as genome-wide output is not typically provided. With regards to other findings, there was, in general, weak evidence for an association of childhood maltreatment with global DNAm, accelerated epigenetic ageing, and epigenetic variation in other candidate genes, although data is not sufficient at present to evaluate this comprehensively.

4.1.3. Differences in findings

Two key discrepancies in findings stand out from our review. The first concerns differences in findings *within* EWAS studies. Although most EWAS studies found genome-wide significant associations between childhood maltreatment and DNAm, they differed widely from one another in the number of associations reported (ranging from 1 to 2868) and the specific DNAm sites identified. As mentioned above, these differences are likely to reflect heterogeneity in sample characteristics (e.g. sample size, which influences statistical power) and methodology (e.g. choice of covariates). For example, some studies did not correct for key factors known to influence DNAm levels, such as sex and cell-type, potentially leading to inflated results. A major source of difference, however, related specifically to the use of high-risk vs population-based samples. While studies from high-risk samples (e.g. children with substantiated histories of maltreatment, socially disadvantaged youth, psychiatric inpatients) typically identified genome-wide associations between childhood maltreatment and DNAm, studies using population-based samples did not – or were not able to replicate the identified associations. A number of factors may explain these differences. On the one hand, population-based studies might have been more robust to false positives, as they were on average considerably larger than high-risk studies, and typically featured an independent replication sample. On the other, studies from the general population may have been less able to detect effects due to lower base rates of maltreatment exposure in the sample (i.e. more severe cases may be under-represented due to lower enrolment, higher attrition and loss to follow-up). Furthermore, maltreatment measures may be less comprehensive and precise in population-based studies (e.g. single items, binary classification of exposure) compared to those utilized in high-risk studies, leading to more measurement error – an issue that also posed challenges to the field of genome-wide association studies (GWAS) prior to the rise of EWAS studies. Greater harmonization of measures and methodology across high-risk and population-based study could facilitate collaborative efforts to trace epigenetic correlates of maltreatment across the full range of exposure.

The second discrepancy concerns differences in findings *between* candidate gene and EWAS studies. Specifically, genes selected for candidate gene studies did not converge with those identified using hypothesis-free, epigenome-wide approaches. This is well illustrated in the case of the *NR3C1* gene, which, despite being the most commonly investigated candidate gene in the field (with associations having been

reported across tissues and species), was not identified by any of the EWAS studies employing an epigenome-wide threshold of significance. This could indicate that most candidate gene findings are likely to be false positives, as previously suggested in the field of psychiatric genetics (e.g. Border et al., 2019). However, it is also possible that associations between maltreatment and DNAm in candidate genes, such as *NR3C1*, are in fact robust, but not strong enough to withstand genome-wide thresholds of significance. To evaluate these scenarios, it will be useful in future for EWAS studies to additionally report findings for candidate genes (either as a follow-up analysis, as done in e.g. Marzi et al., 2018, or ideally by providing the full EWAS results), which will make it possible to compare estimates with candidate gene studies as well the direction and strength of significance.

4.2. Current challenges and recommendations for future research

Below, we present three key challenges for current research, and propose a set of concrete recommendations for moving the field forward.

4.2.1. Challenge 1: overcoming study heterogeneity to identify more robust and replicable associations

As discussed above, one of the key challenges for the field concerns the large heterogeneity between studies, which often feature different target genes, ways of operationalizing maltreatment, sample characteristics and analytical pipelines. Such differences limit comparability between studies – a necessary step for assessing more systematically the evidence base and identifying robust associations. In order to address this challenge, it will be important to define common practices that facilitate data harmonization across studies. Four specific recommendations for addressing this challenge are provided below:

4.2.1.1. Use dimensional, multi-type measures of maltreatment. Studies should consider how best to operationalize maltreatment, preferentially using continuous measures that help to capture the full range of exposure and maximize statistical power. Where possible, studies should not examine only global scores of maltreatment, but also specific types of abuse and neglect (e.g. Cecil et al., 2016a,2016b). This will make it possible to better establish whether different types of maltreatment are associated with shared vs unique epigenetic ‘signatures’ – although such efforts are undoubtedly complicated by the high rate of co-occurrence between maltreatment types, which would need to be accounted for in analyses (see *Challenge 2* below for more details). Furthermore, collecting information on maltreatment characteristics (e.g. age of onset, chronicity, recency) will enable in future to test to what extent these factors moderate observed associations between maltreatment exposure and DNAm. With regards to the measures themselves, it is currently difficult to establish what should be preferred, as different sources (e.g. parent, self, official records) have different advantages and disadvantages. Interestingly, one longitudinal study reviewed here found that DNAm patterns associated with maltreatment differed when using prospective, independent reports of maltreatment in childhood compared to self-reported retrospective measures of the same participants in adulthood (Marzi et al., 2018). Ideally, future studies should use multi-rater assessments of maltreatment to enable more systematic comparison of DNAm patterns across reporting methods.

4.2.1.2. Adjust for appropriate covariates. As already done in other areas of research such as population epigenomics (Felix and Cecil, 2019), studies should always aim to report first findings from a baseline model that adjusts for core covariates that are standard in the field (thus facilitating comparability across studies), including technical covariates (e.g. batch effects), sex, age, cell-type composition, and population stratification for ethnically mixed samples. Depending on the research question and study design, researchers may then choose to rerun

analyses with an additional set of covariates that may relate to both maltreatment exposure and DNAm for sensitivity purposes, such as socio-economic status, tobacco smoking, and body mass index.

4.2.1.3. Make reporting more transparent. We encourage researchers to adopt a more transparent and reproducible way of reporting results, which will facilitate future re-use of summary data for the purposes of replication, meta-analyses and secondary data analysis. Such practices should include (i) providing annotated scripts (e.g. from R, Stata or SPSS) of the analyses performed; (ii) making all results fully available (e.g. EWAS genome-wide summary statistics included as supplementary material or uploaded on a public repository); (iii) avoiding an over-reliance on p-values and significance levels, by also reporting effect sizes and confidence intervals that can aid interpretation of findings; and (iv) reporting key statistics needed for meta-analyses, such as the direction and strength of effects, as well as standard errors.

4.2.1.4. Push towards collaborative science. Finally, as a growing field we must embrace collaborative science in order to increase our power to detect what are likely to be subtle associations between maltreatment and DNAm, maximize our ability to identify robust effects and weed out false positives. These issues are particularly pertinent to research on maltreatment, as studies tend to be understandably small, especially when based on high-risk samples. Coordinated efforts, such as the establishment of consortia, will mark a crucial step in achieving this goal by connecting different research groups around the world, facilitating knowledge exchange and multi-disciplinary collaboration as well as establishing best practices for data harmonization and common analytical pipelines. Ultimately, this will enable the pooling of resources to perform better powered, multi-sample studies. Such efforts have already proven to be successful in other areas of epigenetic research, with active consortia currently dedicated for example to understanding the genetic basis of DNAm (e.g. GoDMC; <http://www.godmc.org.uk>); examining prenatal influences on child DNAm (e.g. PACE; <https://www.niehs.nih.gov/research/atniehs/labs/epi/pi/genetics/pace/index.cfm>); and identifying epigenetic markers of cardiovascular and ageing-related disease (e.g. CHARGE; <http://www.chargeconsortium.com/>).

4.2.2. Challenge 2: tackling the issues of directionality and confounding to characterize maltreatment effects with greater precision

The vast majority of studies reviewed here were based on cross-sectional data, with maltreatment exposure and DNAm measured at the same time point. Such a design reflects often inherent difficulties in carrying out research on maltreatment – especially in children and high-risk samples – including ethical constraints and difficulties in following maltreated individuals longitudinally. Yet, it also poses two major challenges: *directionality* and *confounding*. Based on cross-sectional data alone, it is not possible to establish whether observed DNAm alterations precede, follow, or simply correlate with maltreatment exposure. For example, maltreatment-associated DNAm patterns may reflect in part other preceding or concurrent environmental exposures that are not accounted for in analyses. Indeed, maltreatment is known to correlate with a range of prenatal adversities (e.g., maternal smoking, substance use, psychopathology, exposure to stressful life events and intimate partner violence; Austin et al., 2018), some of which have already been linked to child DNAm patterns (e.g., Joubert et al., 2016; Laufer et al., 2017). Even after birth, maltreated children have been found to be more likely to experience additional adversities (e.g. low SES, community violence exposure; Cecil et al., 2014) compared to their non-maltreated peers, and to experience re-victimization in different social contexts (e.g. bullying; Benedini et al., 2016) – exposures which may partly explain or compound associations between maltreatment and DNAm. Furthermore, it is well-established that different types of maltreatment co-occur, with multi-type exposure being the norm rather than the exception for maltreated children (Cecil et al.,

2017; Herrenkohl and Herrenkohl, 2009). This, critically, is not explicitly modelled in epigenetic studies of maltreatment, where individual types of abuse and neglect are examined in isolation, without accounting for their co-occurrence. In summary, based on cross-sectional data, it is currently difficult to establish to what extent the identified DNAm patterns may be due to unmeasured environmental exposures, including prenatal factors, postnatal adversities or other co-occurring types of maltreatment.

Besides the environment, genetic variation is likely to be another important unmeasured influence. Although much of the interest in epigenetics stems from its potential sensitivity to the environment, it is also largely regulated by DNA sequence variation (Gaunt et al., 2016). Indeed, many epigenetic processes that are essential for healthy development and function, such as cell-type differentiation, genomic imprinting and X-chromosome inactivation, are highly conserved and influenced by genetic factors. In the context of maltreatment, genetic variation may affect observed associations in two main ways. First, as a *confounder*. In this scenario, genetic factors could associate both with a child's risk for maltreatment as well as his or her DNAm patterns, creating a spurious association between the two. Indeed, we already know from other research that adversities such as stressful life events (Clarke et al., 2018) and bullying (Schoeler et al., 2018) are sensitive to genetic influences. In other words, rather than being randomly distributed across the population, social exposures may be more likely to occur to certain individuals, partly because of their genetic background. Thus, what can appear to be purely environmental exposures may actually contain a 'genetic' component. In the case of maltreatment, it is possible for example that certain parental genetic characteristics that increase risk of maltreatment (e.g. those influencing behavioral inhibition, emotional regulation, and psychiatric risk) get passed on to children, and that these same characteristics associate with DNAm patterns. This would result in an observed correlation between maltreatment and DNAm, which is genetically – as opposed to environmentally – explained.

The second way that genetics could influence maltreatment-DNAm associations is as a *moderator*. Previous research has shown that genes and the environment not only correlate with one another, but can also interact. In other words, genes can influence how sensitive we are to our environment, and in turn, our environment can influence the degree to which our genes are expressed. In one of the first examples of such gene-environment interactions (GxE), Caspi et al. (2002) found that, amongst children with a history of maltreatment, those who carried the 'low-activity' allele of the monoamine oxidase A (MAOA) gene – conferring a reduced ability to break down neurotransmitters, such as dopamine and serotonin – showed the most severe levels of antisocial behavior compared to children who carried the 'high-activity' genotype. Since this early report of GxE, a large body of research has provided empirical support for the idea that an individual's genetic makeup can moderate his or her response to external events, partially explaining why people exposed to the same stressor can react in very different ways (Manuck and McCaffery, 2014). These findings have recently been extended to epigenetics, showing that gene-environment interactions largely account for individual variation in DNAm patterns (Czamara et al., 2019; Teh et al., 2014). As such, associations between maltreatment and DNAm may vary as a function of an individual's genetic makeup, which may help to explain some of the inconsistencies in findings reported in this review.

Finally, observed associations may reflect the *sequelae* of maltreatment, rather than maltreatment exposure per se – particularly in studies based on adults where the time elapsed since the exposure is greater. For example, maltreatment engenders risk for a wide range of negative outcomes, including psychopathology, substance use, risky behaviors and cardiometabolic disorders (e.g., Green et al., 2010; M. O. Murphy et al., 2017). In turn, these outcomes have been associated with alterations in DNA (Cecil et al., 2015; Costantino et al., 2018; Torsten Klengel and Binder, 2015). This problem is well-exemplified in several

studies reviewed here, whereby the strongest DNAm sites associated with maltreatment were found to be located in the *AHRR* gene – a known epigenetic biomarker for tobacco smoking – as well as other genes robustly associated with smoking (e.g., Marzi et al., 2018; O'Donnell et al., 2018). While these findings suggest confounding by smoking status, they also point to a potentially important mechanism through which maltreatment exposure may affect biological function and downstream health outcomes. Besides the sequelae of maltreatment, DNAm patterns may also be affected by the use of medication to treat these sequelae (Lötsch et al., 2013), a variable that is seldom controlled for in epigenetic studies on adversity.

To address the issues of directionality and confounding illustrated in this section, we list here five strategies that can be leveraged to measure maltreatment effects with greater precision. We acknowledge that some of these may be particularly difficult to implement in the context of maltreatment research, and provide tools and resources that we hope will facilitate these efforts.

4.2.2.1. Utilize prospective, longitudinal designs. Prospective designs, whereby children are followed across development (ideally from pregnancy onward), can help to disentangle the direction of effects between maltreatment exposure and DNAm, as these variables are collected in temporal order. When measured repeatedly, it additionally becomes possible to examine how environmental adversities and DNAm interrelate over time, and whether sensitive windows of development exist whereby the effects of adversity on DNAm are strongest. Furthermore, such studies enable to correct for the influence of preceding exposures, for example by adjusting for prenatal environmental factors or DNAm patterns at birth, which can be used as a proxy for 'baseline' (i.e. pre-maltreatment) methylation levels. A number of large prospective birth-cohorts already exist that have followed children from pregnancy, have collected data on maltreatment, and feature repeated measures of DNAm and other adversities over time. These include the Generation R Study (Kooijman et al., 2016) and the Avon Longitudinal Study of Parents and Children (Boyd et al., 2019), both of which have open policies for collaboration. Procedures for data request can be found in the following links (<https://generationr.nl/researchers/collaboration/>; <http://www.bristol.ac.uk/alspac/researchers/access/>).

4.2.2.2. Measure correlated exposures. Researchers are encouraged to measure concurrent exposures correlated with maltreatment, including socio-economic status and violence exposure across multiple settings, spanning peers, partners and the wider community. This will enable to test to what extent maltreatment-associated DNAm may be explained by co-occurring exposures. In addition, researchers must ensure that all main forms of maltreatment are measured (e.g. emotional, sexual and physical abuse; emotional and physical neglect; exposure to intimate partner violence), ideally using continuous assessments, so as to characterize the shared vs unique 'signatures' of maltreatment types. This is a valuable question to clarify, as there is mounting evidence that 'threatening' forms of maltreatment (e.g. abuse) may trigger different biological, neural and behavioral adaptations compared to forms of maltreatment marked by 'deprivation' (e.g. neglect; McLaughlin et al., 2014). Against this backdrop, it is important to note that the use of more detailed, comprehensive phenotyping brings about its own set of challenges, as it complicates efforts to carry out large, well-powered studies. Indeed, large samples – such as those from population-based cohorts – typically feature less detailed measures of maltreatment and co-occurring exposures compared to smaller, more selected samples. One approach to balance adequate statistical power with detailed phenotyping is the aforementioned call for consortia focused around epigenetics and maltreatment, although in order to be successful, this endeavor will require careful harmonization of data.

4.2.2.3. Account for genetic influences. A key priority for future research

will be to better account for genetic influences, in order to minimize confounding and to more accurately capture environmental effects on the epigenome. This is imperative given recent evidence that most inter-individual variation in DNAm is explained by genetic main effects and gene-environment interactions, as opposed to 'purely' environmental effects (Czamara et al., 2019; Teh et al., 2014). Consequently, it is currently unclear to what extent conducting DNAm studies on maltreatment in the absence of genotypic information provides meaningful information, as associations may vary depending on genetic background. Accounting for genetic influences can be done in several ways. One is to use genetically-sensitive designs, such as twin studies (e.g. the E-Risk study; Fisher et al., 2015), which enable to 'isolate' environmental influences on DNAm while controlling for genetic similarity between twins. Another is to actually measure DNA sequence variation, making it possible to directly control for genetic influences in the analyses, as well as to test for potential genetic moderation (e.g. either at the SNP-level if sample sizes allow, or by using polygenic scores). More broadly, the use of genetic data on methylation quantitative trait loci (mQTLs; i.e. SNPs that influence DNAm levels either proximally *in cis* or distally in *trans*; Gaunt et al., 2016) opens new and exciting opportunities within the field of environmental epigenetics, as a way of integrating genetic and environmental influences on the epigenome. Such an approach, however, will necessitate the development of innovative bioinformatics tools as well as simulation work to inform sample sizes that are needed to reach adequate statistical power for modelling GxE effects. Finally, in cases where DNA data is not available, it is possible to indirectly examine genetic influences via the use of publicly accessible online resources. Two of these are the ARIES mQTLdb resource (<http://www.mqtladb.org/>; Gaunt et al., 2016), based on 1000 mother-child pairs with genetic and longitudinal epigenetic data from the ALSPAC cohort, and GoDMC (<http://www.godmc.org.uk/>; Bonder et al., 2017), a consortium effort to uncover the genetic basis of DNAm using pooled data from over twenty research groups. Both resources allow researchers to check whether their DNAm sites of interest are known to be influenced by common genetic polymorphisms (available for CpG sites covered by the Illumina 450k array). Researchers can also access heritability estimates from twin studies to look up the proportion of variance of a particular CpG that is explained by additive genetic influences vs shared and non-shared environmental influences (<https://www.epigenomicslab.com/online-data-resources/>; Hannon et al., 2018). While online resources cannot clarify what specific role genetic variation plays in the relationship between maltreatment and DNAm, they can provide useful insights into the extent to which genetic influences may be involved.

4.2.2.4. Build integrative models. Studies that measure maltreatment, DNAm and individual outcomes (e.g. psychiatric disorders), should routinely aim to integrate this information within a single model, in order to test (i) unique associations between these variables, and, importantly (ii) the extent to which DNAm may mediate maltreatment effects on individual outcomes. While a number of the studies reviewed here did formally test for mediation, analyses were based on cross-sectional data. In future, mediation models should be performed on longitudinal data that follows the hypothesized temporal order of associations (i.e. maltreatment → DNAm → outcomes), minimizing issues of reverse causality. Please refer to the review by Barker et al. (2018) for empirical examples of longitudinal integrative models featuring environmental exposures, DNAm data, and individual outcomes.

4.2.2.5. Apply causal inference methods. Although longitudinal data can help to clarify the direction of effects between maltreatment and DNAm, it is still based on a correlational design, which is vulnerable to confounding and precludes inferences regarding causality. In future, it will become increasingly important to test the extent to which

maltreatment exposure *causes* changes in DNAm, and whether such changes in turn *cause* downstream negative health outcomes. While certain methods that have been proposed for strengthening causal inference (e.g. Mendelian randomization) may be particularly challenging to apply in the case of maltreatment, as they require the use of genetic proxies for the exposure under consideration, other methods may be more feasible. Examples include the use of negative controls, quasi-experimental designs and non-genetic instrumental variables, as well as cross-cohort and cross-species comparisons (see Richmond et al., 2014a, 2014b for an overview). Ideally, research should be based on a combination of these strategies to allow for triangulation of results, as each method has its own strengths and limitations.

4.2.3. Challenge 3: improving our understanding of the epigenome

The last challenge we want to highlight is more biological, in that we still know very little about the epigenome itself. First, even though commonly available array technologies, such as the Illumina 450k array (or the more recent Illumina EPIC array), allow us to measure DNAm levels across hundreds of thousands of CpG sites, they only cover 2–4 % of CpG sites across the genome. Second, this data is typically analyzed in ‘two-dimensional’ space (one value per CpG site along the genome), which does not adequately reflect the three-dimensional organization of DNA, whereby certain regions seemingly distant from one another (e.g. on different parts of a chromosome) may actually physically interact with one another. Third, DNAm is only one of several epigenetic mechanisms that work in concert, including histone modifications and non-coding regulatory RNAs. As such, we are only able at present to capture a small snapshot of a much bigger picture that may be relevant for understanding the epigenetic embedding of maltreatment exposure. To complicate matters, DNAm is dynamic, varying across factors such as age, sex, tissue and cell-type. This makes it challenging to characterize how an exposure like maltreatment may impact epigenetic patterns at a given point in time, or in a given type of cell within the body. It also means that DNAm patterns found to associate with maltreatment using easily accessible peripheral tissues, such as saliva or blood, may not reflect what is happening in the brain – likely the most relevant organ for the study of maltreatment and its sequelae (e.g. psychiatric disorders). This is especially relevant in the case of DNA hydroxymethylation – a more recently discovered epigenetic process that plays a role in numerous brain functions, including neurodevelopment (Spiers et al., 2017), and has been linked to adversity in experimental studies (Massart et al., 2014). Because DNA hydroxymethylation is enriched in brain tissue, but not in peripheral tissues, and because widely used methods for epigenotyping are unable to distinguish it from DNA methylation, it is currently a particularly difficult epigenetic marker to study in human populations (Doherty and Roth, 2018). While post-mortem studies can help us to reach a more mechanistic view of the effects of maltreatment on epigenetic regulation in the brain, these too suffer from a number of limitations, including the use of small samples with mixed clinical presentation, the reliance on cross-sectional data, difficulties assessing maltreatment exposure and incomplete phenotyping (see Bakulski et al., 2016 for a review). Overall, research to date comparing multiple peripheral and brain tissues has found that although DNAm patterns are largely tissue and cell-type specific, a considerable proportion of DNAm sites – particularly those that are highly-variable – show cross-tissue concordance (e.g. Hannon et al., 2015; Islam et al., 2019; Smith et al., 2015). A handful of studies included in this review also found concordant associations between maltreatment and DNAm across multiple tissues (Hecker et al., 2017; Houtepen et al., 2016; Kaminsky et al., 2015) – although all focused on single genes so correspondence in genome-wide DNAm patterns in relation to maltreatment is presently unknown. Finally, it is unclear to what extent statistical associations between maltreatment and DNAm imply a functional effect. In other words, observed associations can be highly significant, but involve very small

changes in DNAm levels, with unknown biological consequence. To move towards a more biologically informed approach, we provide the following recommendations:

4.2.3.1. Make use of cross-tissue designs and tools. Although the focus on single tissue sources can provide valuable insights (e.g. examining blood given its potential role in neuroimmunology or buccal cells given that they originate from the same germ layer as the brain), ideally, studies should aim to collect DNA from multiple tissue sources, in order to establish the extent to which maltreatment-related DNAm patterns may be tissue-specific vs systemic. Furthermore, studies should seek to bridge findings from peripheral tissues in living individuals with those based on post-mortem brain samples. When such strategies may not be feasible, for example due to cost and logistic constraints, researchers may draw on publicly available resources to help make cross-tissue inferences. For example, several freely accessible resources exist that enable researchers to compare how DNAm levels in CpG sites of interest correlate between peripheral and brain tissue (e.g., <https://han-lab.org/methylation/default/imageCpG#>, Braun et al., 2019; <https://redgar598.shinyapps.io/BECon/>, Edgar et al., 2017; <http://epigenetics.iop.kcl.ac.uk/bloodbrain/>, Hannon et al., 2015), some of which also provide useful metrics in addition to cross-tissue correlations, such as the degree of variability and influence of cell-type composition on DNAm in blood vs brain (Edgar et al., 2017). It is important to note here, that peripheral DNAm patterns may still be useful within research and practice even in the absence of cross-tissue concordance with the brain. For example, peripheral tissues may be the most suitable target for studying other biological systems that have been found to be dysregulated in response to maltreatment, such as immune, cardiovascular and metabolic function (Berens et al., 2017). Furthermore, to be useful markers, DNAm patterns need not to show cross-tissue correspondence, so long as they are reliably and robustly associated with the exposure or outcome of interest (Felix and Cecil, 2019).

4.2.3.2. Test functional effects at different biological levels. It will be important in future to integrate DNAm data with information at other biological levels in order to test the functional effect of maltreatment-associated epigenetic changes. Again, this may be done directly, by profiling gene expression and additional omics data from the same samples as the DNAm. Indeed, procedures for co-ordinated RNA and DNA extraction are now widely available, which enable to assess concurrently epigenetic and transcriptional activity from the same biological samples (although at a higher cost than kits for DNA extraction only). The study by Lutz and colleagues (2017), provides a compelling example of how integration of biological data can provide key insights into the functional role of maltreatment-related epigenetic patterns. In cases where such data is not available, researchers may use online resources to check whether the identified DNAm loci overlap with established key regulatory elements, such as transcription factor binding sites, hypersensitive genomic regions and histone marks (e.g. using data from ENCODE: <http://genome.ucsc.edu/ENCODE/> and ROADMAP: <http://www.roadmapepigenomics.org/tools>). More work is also needed to clarify the biological pathways linking maltreatment, epigenetics and mental health. One approach is to examine how maltreatment-related DNAm changes associate with intermediate phenotypes of psychiatric risk. To this end, an increasing number of studies are utilizing neuroimaging data to examine how DNAm patterns of interest may relate to *in vivo* brain structure and function in circuits implicated in psychopathology (Walton et al., 2017; Vukojevic et al., 2014). In future, it will be interesting to test whether peripheral DNAm sites associated with brain structure and function also show higher cross-tissue concordance, making them potentially useful markers of brain processes *in vivo*.

4.2.3.3. Collaborate with fundamental scientists. Finally, researchers in

the field of maltreatment are encouraged to connect with fundamental scientists to help gain biological insights and enhance interpretation of their findings. For example, researchers may seek to collaborate with others carrying out animal models and in vitro experiments (e.g. cell cultures), which permit experimental manipulation of DNAm levels to characterize downstream functional effects. Such multi-disciplinary partnerships can be equally fruitful for fundamental scientists, who are often faced with the challenge of establishing how findings from their highly controlled, experimental conditions extend to humans in more naturalistic and ecologically-valid settings. For advice on ways to facilitate collaboration between animal and human studies of early life stress, we recommend the review by [Watamura and Roth \(2019\)](#).

4.3. Implications and translational potential

In conclusion, DNA methylation is fast emerging as a promising mechanism for biological embedding of maltreatment exposure. Yet, as we gain an appreciation of the challenges facing epigenetic research in this field, we must be mindful to manage expectations. In future, the use of strategies such as (i) careful selection of research design (e.g. appropriate tissue, sample size, time points); (ii) use of longitudinal and integrative data, (iii) consideration of unmeasured environmental and genetic influences, (iv) transparency in reporting and replication, (v) functional characterization of epigenetic findings, and (vi) the application of causal inference methods, will mark an important step for overcoming current hurdles and moving the field forward. Bearing this in mind, the knowledge generated from epigenetic studies may hold important implications for the way that we understand – and in future perhaps even intervene on – childhood maltreatment and its sequelae.

In the first instance, findings may be used to refine existing models of maltreatment. For example, DNAm may help us to better understand how environmental exposures, such as maltreatment, interact with genetic factors to shape long-term trajectories of child development, behavior and disease risk. DNAm may also help us to understand how the body adapts to adverse, threatening and unpredictable early environments – and how such adaptations may be potentially protective in the short-term, but detrimental in the long-term. Finally, DNAm can provide a mechanism for latent vulnerability; in other words, how maltreatment engenders vulnerability for psychiatric and physical health problems even decades after the exposure itself has ceased.

In the medium-term, as replication efforts increase and meta-analyses become more feasible, it may be possible to identify robust and reproducible associations between maltreatment exposure and DNAm. This could pave the way for a new wave of studies testing the potential of DNAm as a biomarker of maltreatment exposure across clinical and research settings. Such a biomarker could be used, for example, to overcome issues common to other reporting methods of maltreatment (e.g. recall bias) and to inform risk assessment and treatment formulation. Furthermore, the comparison of DNAm pre- vs post-intervention (e.g. via environmental enrichment, psychological therapy, etc), could lend insights into the potential reversibility of maltreatment-related patterns and how best to promote resilience among exposed individuals. Indeed, a small number of prospective studies to date have utilized repeated sampling of DNAm to predict clinical outcomes. These have shown, for example, that changes in DNAm patterns associate with symptom severity following a traumatic exposure (e.g. military deployment, [Rutten et al., 2018](#)), but also with symptom change following psychological intervention (e.g. [Roberts et al., 2018](#); [Yehuda et al., 2013](#); [Ziegler et al., 2016](#)). Longitudinal research that investigates the timing of maltreatment effects on epigenetic regulation could also be used to identify specific windows of biological vulnerability or opportunity that could benefit most from preventive action. Any potential benefit of DNAm as a biomarker, however, would likely also be balanced by potential risks, raising important ethical implications that would need careful consideration and safeguarding. Ultimately, establishing causal pathways between childhood maltreatment and DNAm

could lead to the development of novel strategies for alleviating the societal burden of maltreatment and treating its psychological and physical sequelae.

Declaration of Competing Interest

All authors report no competing interests.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.neubiorev.2020.02.019>.

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