Child Abuse & Neglect 122 (2021) 105304

Contents lists available at ScienceDirect

Child Abuse & Neglect

journal homepage: www.elsevier.com/locate/chiabuneg

Invited review

Child maltreatment and *NR3C1* exon 1_F methylation, link with deregulated hypothalamus-pituitary-adrenal axis and psychopathology: A systematic review

D.L. Wadji [a,*], T. Tandon [a], G.J.M. Ketcha Wanda [b], C. Wicky [c], A. Dentz [a], G. Hasler [d], N. Morina [e], C. Martin-Soelch [a]

[a] *I-Reach Lab, Unit of Clinical and Health Psychology, University of Fribourg, Switzerland*
[b] *Clinical psychology Lab, Department of Psychology, University of Yaoundé I, Cameroon*
[c] *Department of Biology, University of Fribourg, Switzerland*
[d] *Department of Psychiatric, University of Fribourg, Fribourg, Switzerland*
[e] *Department of Consultant-Liaison Psychiatry and Psychosomatic Medicine, University Hospital Zurich, University of Zurich, Switzerland*

ARTICLE INFO

Keywords:
Child maltreatment
NR3C1 exon 1_F
DNA methylation
HPA axis
Psychopathology

ABSTRACT

Background: Epigenetics offers one promising method for assessing the psychobiological response to stressful experiences during childhood. In particular, deoxyribonucleic acid (DNA) methylation has been associated with an altered hypothalamus–pituitary–adrenal (HPA) axis and the onset of mental disorders. Equally, there are promising leads regarding the association between the methylation of the glucocorticoid receptor gene (*NR3C1*-1_F) and child maltreatment and its link with HPA axis and psychopathology.

Objective: The current study aimed to assess the evidence of a link among child maltreatment, *NR3C1*-1_F methylation, HPA axis deregulation, and symptoms of psychopathology.

Methods: We followed the Prisma guidelines and identified 11 articles that met our inclusion criteria.

Results: We found that eight studies (72.72%) reported increased *NR3C1*-1_F methylation associated with child maltreatment, specifically physical abuse, emotional abuse, sexual abuse, neglect, and exposure to intimate partner violence, while three studies (27.27%) found no significant association. Furthermore, a minority of studies (36.36%) provided additional measures of symptoms of psychopathology or cortisol in order to examine the link among *NR3C1*-1_F methylation, HPA axis deregulation, and psychopathology in a situation of child maltreatment. These results suggest that *NR3C1*-1_F hypermethylation is positively associated with higher HPA axis activity, i.e. increased production of cortisol, as well as symptoms of psychopathology, including emotional lability-negativity, externalizing behavior symptoms, and depressive symptoms.

Conclusion: NR3C1-1_F methylation could be one mechanism that links altered HPA axis activity with the development of psychopathology.

* Corresponding author at: Department of Psychology, University of Fribourg, Rue P.-A.-de Faucigny 2, CH-1700 Fribourg, Switzerland.
E-mail address: dany.nkonlack@unifr.ch (D.L. Wadji).

https://doi.org/10.1016/j.chiabu.2021.105304
Received 8 October 2020; Received in revised form 10 June 2021; Accepted 26 August 2021
Available online 3 September 2021

1. Introduction

Child maltreatment is a social problem affecting the lives of millions of children (Tarantola, 2018). Approximately 40 million children worldwide below the age of 15 are subjected to child maltreatment each year (Butchart & Harvey, 2006). Child maltreatment includes all forms of physical abuse, sexual abuse, emotional abuse (or psychological abuse), neglect, and exploitation (Bellis & Thomas, 2003). New research avenues have begun to consider exposure to intimate partner violence as a form of child maltreatment (Wathen & Macmillan, 2013). Child maltreatment has been regarded as a major factor in the development of several psychopathological disorders, such as depression (Targum & Nemeroff, 2019), post-traumatic stress disorder (Moore et al., 2013) and anxiety (Rehan et al., 2017).

Epigenetics offers one promising method for assessing the biological response to stressful experiences during childhood. One epigenetic mechanism that has been widely studied is the deoxyribonucleic acid (DNA) methylation of genes regarded as the addition of a methyl group to specific DNA sites (Wiley et al., 2016). Previous research has reported an association of hypermethylation of genes with child maltreatment (Beach et al., 2010; Hecker et al., 2016; Suderman et al., 2014).

The nuclear receptor subfamily 3, group C, member 1 (*NR3C1*) gene has been extensively studied in the field of epigenetics because the protein encoded by this gene binds the stress hormone cortisol to create a negative feedback loop within the hypothalamic–pituitary–adrenal (HPA) axis, which regulates the body's neuroendocrine response to stress (Cecil et al., 2020; Laryea et al., 2013; Liu et al., 2020). Although the *NR3C1* gene contains nine exons, the focus of research has been placed on the methylation patterns of the exon 1_F (Schür et al., 2018; Weaver et al., 2004). The discovery by Weaver et al. (2004) that increased pup licking and grooming and arched-back nursing by rat mothers altered the offspring epigenome at a glucocorticoid receptor gene promoter, particularly at the exon 17, prompted a new research field that has since been extended to human populations. Special attention has subsequently been paid to human exon 1_F due to its homology with rat exon 17. This review focuses specifically on the exon 1_F because substantial research has shown it to be susceptible to environmentally induced DNA methylation, which leads to reduced gene expression (Daskalakis & Yehuda, 2014; Witzmann et al., 2012). Furthermore, hypermethylation of the *NR3C1*, in particular exon 1_F, has been associated with altered regulation of the HPA axis and negative long-term mental-health outcomes (Holmes et al., 2019; Liu & Nusslock, 2018).

Recently, Cecil et al. (2020) conducted the first systematic review of human studies linking childhood maltreatment with DNA methylation. Their findings supported an association between childhood maltreatment and altered patterns of DNA methylation in several genes or across the whole genome. However, exposure to intimate partner violence was not considered a form of child maltreatment, and DNA methylation included several candidate genes (e.g., NR3C1, *SLC6A4*, *FKBP5*, *BDNF*, and *OXTR*). To our knowledge, no prior systematic review has addressed *NR3C1*-1_F methylation in cases of child maltreatment, i.e. child neglect, child abuse, or exposure to intimate partner violence, with particular attention paid to its link with HPA axis deregulation and psychopathology (see Table 1).

The current study aimed to assess evidence of a link between child maltreatment and *NR3C1*-1_F methylation, in particular the relationship among *NR3C1*-1_F methylation associated with child maltreatment, its link with HPA axis, and psychopathology.

2. Method

We used the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (Prisma) statement (Moher et al., 2009).

2.1. Study variables

The study variables were child maltreatment, i.e. child exposure to intimate partner violence, child neglect, or child abuse, and *NR3C1*-1_F methylation.

2.2. Identification of all primary research

To identify all primary research findings, we consulted two databases before 18 June 2020: PubMed and Web of Science.

In PubMed, we searched for ((child exposure to domestic violence) OR (child maltreatment) OR (child neglect) OR (child abuse)) AND (DNA methylation) (135 results) and ((child abuse) OR (child neglect) OR (child maltreatment) OR (child exposure to domestic violence)) AND (methylation of NR3C1 gene) (34 results). In Web of Science, we searched for ((child exposure to domestic violence) OR (child maltreatment) OR (child neglect) OR (child abuse)) AND (DNA methylation) (139 results) and ((child abuse) OR (child neglect) OR (child maltreatment) OR (child exposure to domestic violence)) AND (methylation of NR3C1 gene) (26 results).

Additional articles relating to our keywords were also obtained through the analysis of references cited in systematic reviews, meta-analyses, and research reports or articles consulted (10 results).

2.3. Inclusion criteria

To ensure a comparison of the results, the inclusion criteria were as follows: (1) studies must report empirical evidence published in peer-reviewed journals; (2) they must be written in English; (3) they must focus on human epigenetics; (4) they must measure DNA methylation levels quantitatively; (5) they must focus solely on the *NR3C1* exon 1_F because substantial research has shown it to be modulated by stressful life experiences; and, finally, (6) they must examine childhood maltreatment, i.e. child abuse, child neglect, or

Table 1
Descriptive summary of studies investigating NR3C1 exon 1_F methylation associated with child maltreatment.

Study	Nature of adversity	Objective	Sample	Instrument	Design	Tissue	Genes studied	Cortisol or psychopathology outcome measures	Epigenetic analysis	Findings
(Perroud et al., 2011)	Childhood sexual abuse	Investigated the effect of childhood maltreatment and its severity on methylation of the exon 1_F *NR3C1* promoter	101 BPD and 114 MDD	CTQ and interviews	Cross-sectional and retrospective design with 3 groups: BPD, MDD, and MDD with PTSD	Blood samples	*NR3C1* exon 1_F	No assessment	DNA bisulfite treatment; PCR amplification followed by pyrosequencing	Increased methylation
(McGowan et al., 2009)	Childhood maltreatment	Examined epigenetic differences in the *NR3C1* promoter of suicide victims with a history of childhood maltreatment and those without any history of childhood maltreatment	12 suicide victims with HCA, 12 suicide victims with a negative HCA and 12 controls	Structured interviews using the Childhood Experience of Care and Abuse questionnaire adapted for psychological autopsies	Cross-sectional study with exposed and control groups	Hippocampal samples in the brain	*NR3C1* exon 1_F	No assessment	DNA bisulfite treatment, PCR amplification, cloning, and sequencing	Increased methylation, decreased *NGFI-A* binding on methylated *NR3C1* promoter in vitro
(Martín-Blanco et al., 2014)	Childhood maltreatment	Evaluated the association between *NR3C1* methylation status, childhood maltreatment, and current clinical severity	281 participants	CTQ: 28 items	Cross-sectional study with no control group	Blood samples	*NR3C1* exon 1_F	No assessment	Bisulfite treatment; PCR amplification followed by pyrosequencing	Increased methylation
(Romens et al., 2015)	Physical maltreatment	Examined methylation of exon 1_F of *NR3C1* from leukocytes in children who experienced physical childhood maltreatment	56 children	CPS	Cross-sectional study with 2 groups: maltreated children and non-maltreated children	Blood samples	*NR3C1* exon 1_F	No assessment	Bisulfite treatment; PCR amplification followed by pyrosequencing	Increased methylation
(Steiger et al., 2013)	Childhood abuse	Explored in women with bulimia nervosa the extent to which methylation of the GR gene promoter corresponds to childhood abuse	64 women with BN (32 with history of severe childhood abuse and 32 selected as having no such history) and 32 women in the comparison group with no ED or history of childhood abuse	Childhood Trauma Interview consisting of 30-min structured interviews	Cross-sectional study with a control group	Blood samples	*NR3C1* exon 1_F	No assessment	Bisulfite treatment followed by EpiTyper processing	No significant difference
(Bustamante et al., 2016)	Childhood maltreatment	Investigated the association of childhood maltreatment and major depressive disorder with DNA methylation and gene expression of the GR	147 adult participants	CTS and CTQ	Cross-sectional study with a control group	Blood samples	*NR3C1* exon 1_F	Assessment of psychopathology outcome measures	Bisulfite treatment; PCR amplification followed by pyrosequencing	Increased methylation at the *NGFI-A* binding site
(Radtke et al., 2011)	Exposure to intimate partner violence during pregnancy	Analyzed methylation of the GR gene in mothers and their children	25 dyads mother-child	Structured interview	Cross-sectional study with no control group	Blood samples	*NR3C1* exon 1_F	No assessment	DNA bisulfite treatment; PCR amplification, cloning and sequencing	Increased methylation

(*continued on next page*)

Table 1 (*continued*)

Study	Nature of adversity	Objective	Sample	Instrument	Design	Tissue	Genes studied	Cortisol or psychopathology outcome measures	Epigenetic analysis	Findings
(Vangeel et al., 2015)	Childhood trauma	Examined DNA methylation of the GR gene (*NR3C1*) in CFS and associations with childhood sexual and physical trauma	76 female patients (46 with no/mild and 30 with moderate/severe childhood trauma) and 19 healthy controls	Interviews using the Dutch version of the Structured Trauma Interview and questionnaires inquiring about 29 types of potential trauma subdivided into 5 subscales	Cross-sectional study with a control group	Blood samples	*NR3C1* exon 1_F	Assessment of cortisol measures	Bisulfite treatment followed by EpiTyper processing	No significant difference
(Vangeel et al., 2018)	Childhood trauma	Investigated the association between *NR3C1*-1_F methylation and chronic fatigue syndrome symptom severity in carefully screened chronic fatigue syndrome patients compared to a control group; used a well-validated tool to retrospectively assess childhood trauma	80 female CFS patients and 91 female controls	CTQ-SF: 25 items	Cross-sectional study with a control group	Blood samples	*NR3C1* exon 1_F	No assessment	Bisulfite treatment followed by EpiTyper processing	No significant difference
(Farrell et al., 2018)	Emotional abuse	Investigated whether cortisol concentrations, evaluated in 25 depressed patients and 20 controls, and measures of early life adversity were associated with the degree of methylation of these candidate gene regions	67 individuals (33 depressed patients and 34 controls)	CTQ	Cross-sectional study with a control group	Blood samples	*NR3C1* exon 1_F and the FKBP5 gene intron 7	Assessment of cortisol measures	Bisulfite treatment; PCR amplification followed by pyrosequencing	Increased methylation
(Cicchetti & Handley, 2017)	Childhood maltreatment	Examined the effect of various dimensions of child maltreatment on methylation of the glucocorticoid receptor gene	534 children divided in maltreated (53.4%) and non-maltreated (46.6%) children	Maltreatment Classification System	Cross-sectional study with a control group	Salivary samples	*NR3C* exon 1_F	Assessment of psychopathology outcomes measures	Bisulfite treatment and whole genome methylation analysis using the HumanMethylation450 BeadChip from Illumina	Increased methylation

Abbreviations. BD = Bipolar Disorder; BN = Bulimia Nervosa; BPD = Borderline Personality Disorder; CFS = Chronic Fatigue Symptoms; CpG = CG sites or CpG islands (or CG islands); CTQ = Child Trauma Questionnaire; CTQ-SF = Child Trauma Questionnaire short form; CTS = Conflict Tactics Scale; DNA = Deoxyribonucleic Acid; ED = Eating Disorder; EpiTYPER = Mass Spectrometry-Based Bisulfite Sequencing Method; GR or *NR3C1* = Glucocorticoid receptor gene; HCA = History of Childhood Abuse; IPV = intimate partner violence; IPV = interpersonal violence; PTSD = Post-Traumatic Stress Disorder.

exposure to intimate partner violence.

Studies were excluded if (1) they had the structure of review books, presentations, theses, literature reviews, meta-analyses, or magazines; (2) they were published in a language other than English; (3) they were conducted in animals, excluding humans; (4) they examined other types of epigenetic mechanisms, like histone modifications and non-coding RNAs; (5) they investigated methylation across the whole genome or methylation with other candidate genes, such as *SLC6A4*, *FKBP5*, *BDNF*, *OXTR*, *MAOA*, or *5-HT3A*; (6) they examined child maltreatment simultaneously with other forms of early life adversity or calculated an adverse composite score; and, lastly, (7) they examined child maltreatment simultaneously with genetic polymorphism.

2.4. Screening and eligibility

After removal of the duplicates, the first (D.W.) and second (T.T.) authors screened the abstracts of all included studies and selected potentially eligible studies. All identified publications were written in English. Therefore it was not necessary to apply the language exclusion criterion. Next, both authors (D.W. and T.T.) read full-text studies to assess them for eligibility. Disagreements between the two authors were resolved by discussion and consensus based on inclusion and exclusion criteria. No actions were taken to seek additional information from the original researchers.

2.5. Data processing, extraction, and synthesis

We collected data from all eligible studies based on our variables: namely, child maltreatment (i.e. exposure to domestic violence, child neglect, or child abuse) and *NR3C1* gene exon 1_F methylation. We were interested in the percentage of methylation at CpGs in promoter regions (1_F). The percentage of methylation at CpGs in other promoter regions, like 1_B, 1_C, and 1_H, was not included in this review. Data extraction was performed based on ten variables: 1) nature of adversity, 2) objective, 3) sample, 4) instrument, 5) design, 6) tissue investigated, 7) gene studied, 8) cortisol or psychopathology outcome measures, 9) epigenetic analysis, and 10) findings. One author (D.W.) extracted the main information from the included studies, and this information was independently reviewed and cross-checked for accuracy and completion by the second author (T.T.). Finally, we were interested in the statistical summary in terms of the percentage of strength, as well as the results of the statistical tests performed.

3. Results

Our search generated 344 records: 334 studies from a database search (169 from PubMed and 165 from Web of Science) and 10 additional studies from lists of references. After filtering out the duplicates, we were left with 256 studies. Based on the available title abstract, a first screening gave us 90 studies. Full-text articles permitted us to exclude 79 of the 90 studies because they concerned other genes (42), intergenerational transmission (4), child maltreatment and gene polymorphism or other early life adversity (22), gene-environment (7) or were carried out on animals (4). A total of 11 original studies on child maltreatment and *NR3C1*-1_F methylation were included in the systematic review. Fig. 1 illustrates the selection process of this systematic review.

3.1. Descriptive summary

We identified 11 studies reporting on the association between child maltreatment and *NR3C1* exon 1_F. Child maltreatment included sexual abuse, physical abuse, neglect, exposure to intimate partner violence during pregnancy, and emotional abuse. Notably, most studies were performed on a clinical population ($n = 7$), including patients with borderline personality disorder (Martín-Blanco et al., 2014; Perroud et al., 2011), major depressive disorder (Bustamante et al., 2016; Farrell et al., 2018; Perroud et al., 2011), bulimia nervosa (Steiger et al., 2013), or chronic fatigue syndrome (Vangeel et al., 2015; Vangeel et al., 2018), while other researchers ($n = 4$) investigated a non-clinical population (Cicchetti & Handley, 2017; McGowan et al., 2009; Radtke et al., 2011; Romens et al., 2015). Most studies used a cross-sectional research design based on retrospective measures with an exposed and a control group (Bustamante et al., 2016; Cicchetti & Handley, 2017; Farrell et al., 2018; McGowan et al., 2009; Perroud et al., 2011; Romens et al., 2015; Steiger et al., 2013; Vangeel et al., 2015; Vangeel et al., 2018). Only two studies did not include a control group (Martín-Blanco et al., 2014; Radtke et al., 2011). Child maltreatment was assessed primarily using a self-reported instrument: the Child Trauma Questionnaire (CTQ; Bustamante et al., 2016; Farrell et al., 2018; Martín-Blanco et al., 2014; Perroud et al., 2011; Vangeel et al., 2018), structured interviews (McGowan et al., 2009; Radtke et al., 2011; Steiger et al., 2013; Vangeel et al., 2015), Child Protective Services (Romens et al., 2015) or the Maltreatment Classification System (Cicchetti & Handley, 2017). The type of tissue used to examine DNA methylation was usually a blood sample (Bustamante et al., 2016; Farrell et al., 2018; Martín-Blanco et al., 2014; Perroud et al., 2011; Radtke et al., 2011; Romens et al., 2015; Steiger et al., 2013; Vangeel et al., 2015; Vangeel et al., 2018). Other types of tissues were hippocampal samples in the brain (McGowan et al., 2009) and saliva samples (Cicchetti & Handley, 2017). Concerning the epigenetic analysis technique used, most of the included studies used the pyrosequencing technique (Bustamante et al., 2016; Farrell et al., 2018; Martín-Blanco et al., 2014; Perroud et al., 2011; Romens et al., 2015), chromatin immunoprecipitation assays (McGowan et al., 2009), EpiTYPER assays (Steiger et al., 2013; Vangeel et al., 2015), bisulfite sequencing (Cicchetti & Handley, 2017; Radtke et al., 2011), or a combination of pyrosequencing and EpiTYPER (Vangeel et al., 2018).

3.2. Association between child maltreatment and NR3C1-1_F methylation

3.2.1. Increased NR3C1-1_F methylation associated with child maltreatment

Of the 11 human studies examining *NR3C1*-1_F methylation, eight (72.72%) reported increased promoter methylation related with child maltreatment, namely physical abuse, emotional abuse, sexual abuse, neglect and exposure to domestic violence (Table 2). Specifically, Perroud et al. (2011) found a significant association between childhood sexual abuse and methylation status. McGowan et al. (2009) reported an increased site-specific methylation of the exon 1_F *NR3C1* promoter in suicide victims with a history of childhood abuse. Martín-Blanco et al. (2014) indicated a significant positive correlation between *NR3C1* methylation status and physical abuse. Radtke et al. (2011) provided evidence that methylation in the offspring was directly affected by maternal exposure to

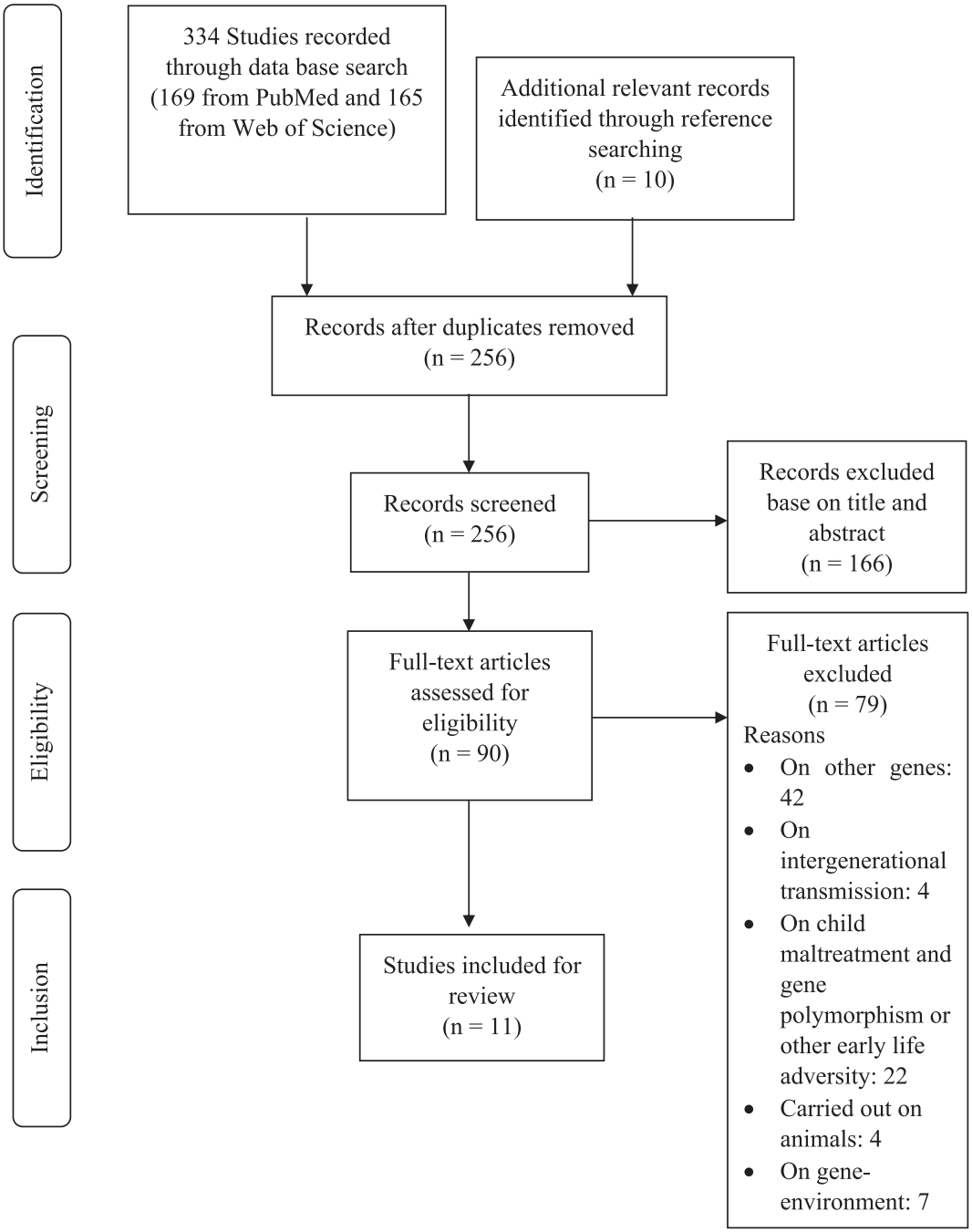

Fig. 1. Flow chart of the selection process according to Prisma diagram (Moher et al., 2009).

Table 2
Studies reporting an increased NR3C1-1_F methylation associated with child maltreatment.

Study	Nature of adversity	Sample size (N)	Genes studied	Findings	Results
(Perroud et al., 2011)	Childhood sexual abuse	$N = 215$	*NR3C1*-1_F	Increased methylation	($p = 6.16 \times 10^{-8}$)
(McGowan et al., 2009)	Childhood maltreatment	$N = 36$	*NR3C1*-1_F	Increased methylation	($F = 3.47$, $p < 0.05$)
(Martín-Blanco et al., 2014)	Childhood maltreatment	$N = 281$	*NR3C1*-1_F	Increased methylation	($\beta = 0.06$, $p = 0.009$)
(Romens et al., 2015)	Physical maltreatment	$N = 56$	*NR3C1*-1_F	Increased methylation	($F = 2.46$, $p = 0.02$)
(Bustamante et al., 2016)	Childhood maltreatment	$N = 147$	*NR3C1*-1_F	Increased methylation	At CpG sites 1–4 ($\beta = 0.038$, SE = 0.015, p corrected = 0.001)
(Radtke et al., 2011)	Exposure to IPV during pregnancy	$N = 25$ dyads	*NR3C1*-1_F	Increased methylation	($p < 0.05$)
(Farrell et al., 2018)	Emotional abuse	$N = 67$	*NR3C1*-1_F	Increased methylation	Exon 1_F CG37 ($r = 0.53$, $p = 0.01$) and CG38 ($r = 0.43$, $p = 0.04$)
(Cicchetti & Handley, 2017)	Child maltreatment	$N = 534$	*NR3C1*-1_F	Increased methylation	($F(1) = 5.58$, $p = 0.019$)

Abbreviations. β = standardized beta coefficient; CpG = CG sites or CpG islands (or CG islands); F = F-test coefficient; n = frequency; *NR3C1*-1_F = nuclear receptor subfamily 3, group C, member 1 gene exon 1_F; p = p-value; r = correlation coefficient; SE = standard error.

intimate partner violence during gestation. Romens et al. (2015) reported that children exposed to physical maltreatment had greater methylation within exon 1_F in the *NR3C1* promoter region of the gene compared to non-maltreated children. Bustamante et al. (2016) showed that exposure to childhood maltreatment was associated with an increase in DNA methylation in the upstream region of *NR3C1* (CpG sites 1–4). Farrell et al. (2018) indicated that DNA methylation levels at specific CG sites within the *NR3C1* exon 1_F were related to childhood emotional abuse severity. Lastly, Cicchetti and Handley (2017) reported that mean *NR3C1*-1_F methylation in maltreated children evidenced hypermethylation compared to non-maltreated children.

3.2.2. No significant association between NR3C1-1_F methylation and child maltreatment

Three studies (27.27%) reported no significant association of methylation *NR3C1*-1_F with child maltreatment (Table 3). Steiger et al. (2013) used a cross-sectional study with an exposed and control group and showed no significant group × promoter-region site interaction in the 1_F region. Vangeel et al. (2015) compared all *NR3C1*-1_F CpG units in the no-trauma/mild trauma group with those in the moderate to severe trauma group and found no methylation differences between the groups. With the aim of replicating and extending their previous findings, Vangeel et al. (2018) found no significant correlation between the scores obtained on the Child Trauma Questionnaire (CTQ) and *NR3C1*-1_F methylation.

3.3. Links among NR3C1-1_F methylation, HPA axis deregulation, and psychopathology in a situation of child maltreatment

Of the 11 studies reporting on child maltreatment and its association with *NR3C1*-1_F methylation, four provided additional measures of symptoms of psychopathology or cortisol in order to examine the links among *NR3C1*-1_F methylation, HPA axis deregulation, and psychopathology (Bustamante et al., 2016; Cicchetti & Handley, 2017; Farrell et al., 2018; Vangeel et al., 2015). Hypermethylation here is thought to lead to less GC receptor expression and thus to a higher cortisol response and a higher risk of developing symptoms of psychopathology.

3.3.1. Child maltreatment, NR3C1-1_F methylation and cortisol

Farrell et al. (2018) investigated whether cortisol concentration and measures of child maltreatment were associated with the degree of methylation at *NR3C1*-1_F. Saliva samples were collected in Salivette tubes (Sarstedt, Nümbrecht, Germany) at five time points throughout the day: 0, 30, and 60 min after waking (to assess cortisol awakening response) and 12 and 12.5 h after waking (to assess diurnal variation). Their results showed that *NR3C1*-1_F methylation was positively associated with higher basal HPA axis activity: i.e., morning cortisol concentrations.

Vangeel et al. (2015) reported on *NR3C1*-1_F methylation and its associations with the HPA axis function and childhood trauma. The HPA axis function was assessed via salivary cortisol concentrations collected seven times using a cotton wool Salivette (Sarstedt, Nümbrecht, Germany). Overall methylation and selective CpG units were positively correlated with salivary cortisol. Their results showed that *NR3C1* promoter hypomethylation was consistent with lower production of cortisol, indicating the hypofunction of the HPA axis.

Taken together, these studies reflect a persistent dysregulation of the neurobiological stress system via the hyper- or hypofunction of the HPA axis associated with the *NR3C1*-1_F methylation.

3.3.2. Child maltreatment, NR3C1-1_F methylation and symptoms of psychopathology

Bustamante et al. (2016) investigated the link between *NR3C1*-1_F methylation and symptoms of depression assessed using the Patient Health Questionnaire (PHQ-9) (Kroenke et al., 2001). They reported increased *NR3C1*-1_F methylation associated with major

Table 3
Studies reporting no significant association between NR3C1-1_F methylation and child maltreatment.

Study	Nature of adversity	Sample size (N)	Genes studied	Findings	Results
(Steiger et al., 2013)	Childhood abuse	$N = 96$	*NR3C1*-1_F	No significant difference	($p > 0.05$)
(Vangeel et al., 2015)	Childhood trauma	$N = 95$	*NR3C1*-1_F	No significant difference	(All $p > 0.05$ in all *NR3C1*-1_F CpG units)
(Vangeel et al., 2018)	Childhood trauma	$N = 171$	*NR3C1*-1_F	No significant difference	(All $p > 0.060$ in all *NR3C1*-1_F CpG units)

Abbreviations. *NR3C1*-1_F = nuclear receptor subfamily 3, group C, member 1 gene exon $1_{F;}$ $p = p$ value.

depressive disorder.

Cicchetti and Handley (2017) investigated the relationship between *NR3C1*-1_F methylation associated with child maltreatment and child outcomes, in particular emotional lability–negativity as measured by the Emotion Regulation Checklist (Shields & Cicchetti, 1997, 1998), externalizing and internalizing behavior assessed at the end of the week through completion of the Teacher Report Form (Achenbach, 1991), depressive symptoms in the past two weeks using the Children's Depression Inventory (Kovacs, 1982), and anxiety symptoms assessed using the Revised Child Manifest Anxiety Scale (Reynolds & Richmond, 1985). Their results indicated that mean *NR3C1*-1_F hypermethylation was related to higher levels of emotional lability–negativity, greater externalizing behavior symptoms and higher levels of child-reported depressive symptoms. *NR3C1*-1_F methylation was not, however, associated with child-reported anxiety symptoms or internalizing behavior symptoms.

In sum, these studies reported poor mental health, which correlates positively with *NR3C1*-1_F hypermethylation.

4. Discussion

The aim of this systematic review was to assess evidence of the relationship between *NR3C1*-1_F methylation associated with child maltreatment (i.e., child neglect, child abuse, or exposure to domestic violence) and its link with HPA axis and psychopathology. We found 11 studies that meet our inclusion criteria. Eight studies (72.72%) reported increased *NR3C1*-1_F methylation associated with child maltreatment, in particular physical abuse, emotional abuse, sexual abuse, neglect, and exposure to domestic violence, while three studies (27.27%) found no significant association. Furthermore, a minority of studies (36.36%) provided additional measures of symptoms of psychopathology or cortisol to examine the links among *NR3C1*-1_F methylation, HPA axis deregulation, and psychopathology in a situation of child maltreatment. Their results suggest that *NR3C1*-1_F methylation is positively associated with higher HPA axis activity, i.e. increased production of cortisol and symptoms of psychopathology, including emotional lability–negativity, externalizing behavior symptoms, and depressive symptoms. These studies aid in understanding that child maltreatment as a form of stress could induce continuous cortisol production, which will eventually lead to a decreased response of the HPA axis through methylation of the *NR3C1* receptor and symptoms of psychopathology.

We found that most of the included studies (72.72%) indicated that child maltreatment is associated with increase epigenetic methylation *NR3C1*-1_F. These results are similar to those of Turecki and Meaney (2016), who indicated that negative early-life environments was associated with hypermethylation of the *NR3C1*-1_F promoter in 89% of human early-life adversity studies and 70% of animal early-life adversity studies. Three studies, however, found no significant association between *NR3C1*-1_F methylation and child maltreatment (27.27%). One possible factor accounting for the failure to detect epigenetic alterations associated with childhood abuse could be the sample characteristic and sample size. Whereas most studies performed to date have been conducted on borderline personality disorder (BPD) patients or major depressive disorder (MDD) patients, Steiger et al. (2013) investigated a clinical population of bulimia nervosa patients ($n = 96$), while Vangeel et al. (2015) and Vangeel et al. (2018) examined chronic fatigue syndrome (CFS) patients (respectively ($n = 95$) and ($n = 171$)). The main reason most studies preferred BPD or MDD patients is that MDD is very common, so people take it as an outcome (Burcusa & Iacono, 2007), and patients with BPD, which is rare, frequently have a trauma history (Perroud et al., 2013; Prados et al., 2015). Another important aspect to be taken into consideration is the type of epigenetic analysis technique used. Whereas the majority of the included studies assessed DNA methylation via pyrosequencing, Steiger et al. (2013) and Vangeel et al. (2015) examined the methylation of *NR3C1* exon 1_F using EpiTYPER assays, while Vangeel et al. (2018) combined EpiTYPER essay and pyrosequencing. Though the use of EpiTYPER assays has several advantages, it is particularly suitable for projects that require the measurement of larger numbers of samples or regions, since a single EpiTYPER run yields 126 triplicate measurements (Suchiman et al., 2015). Equally, compared to pyrosequencing, which is the gold standard for detecting DNA methylation at a single CpG or single gene-promoter level, the disadvantage of using the EpiTYPER technique is that sometimes it cannot discriminate between individual nearby CpG sites, grouping them together into units (Vangeel et al., 2018). Still, by combining EpiTYPER essay and pyrosequencing, Vangeel et al. (2018) could not find a significant relationship between child maltreatment and methylation *NR3C1*-1_F.

Most of the included studies investigated child maltreatment, such as sexual abuse, physical abuse, emotional abuse, or neglect. Of note, only one study (Radtke et al., 2011) stood out by examining the effect of exposure to intimate partner violence as a form of child maltreatment on the methylation of *NR3C1*-1_F. Generally, exposure to intimate partner violence is not considered a form of child maltreatment. This is perhaps why systematic-review papers (see, e.g., the review by Cecil et al., 2020), which have assessed methylation *NR3C1*-1_F in relation to child maltreatment, did not report this study, which nonetheless provided evidence that methylation in offspring (now aged 10–19 years) was directly affected by the adverse experiences of the mother with intimate partner violence during gestation. The results obtained by Radtke et al. (2011) suggested that intimate partner violence could produce fetal programming of the HPA-axis through methylation of the GR gene and that gestational exposure to psychological stressors can have a

lasting effect on methylation status in human offspring. Regrettably, this study was characterized by the absence of a control group. More research is needed on the effect of exposure to intimate partner violence on *NR3C1*-1_F methylation.

Very few studies ($n = 4$) provided additional measures of symptoms of psychopathology or cortisol to examine the links among *NR3C1*-1_F methylation, HPA axis deregulation, and psychopathology in a situation of child maltreatment. While Farrell et al. (2018) and Vangeel et al. (2015) showed that *NR3C1*-1_F hypermethylation (hypomethylation) was positively associated with higher (lower) HPA axis activity, i.e. increased (decreased) production of cortisol, Bustamante et al. (2006) and Cicchetti and Handley (2017) provided evidence that mean *NR3C1*-1_F hypermethylation was related to symptoms of psychopathology, including emotional lability–negativity, externalizing behavior symptoms, and depressive symptoms. Taken together, the results from these studies provide crucial information about the early gene-environment mechanisms that may be involved in life-span health development, as they suggest that *NR3C1*-1_F hypermethylation in cases of child maltreatment could be responsible for the deregulated HPA axis function associated with elevated cortisol levels and the development of mental disorders. As mentioned in previous reviews (Cecil et al., 2020; Queirós & Caseiro, 2018), DNA methylation of GC receptor gene promoters could therefore be one epigenetic mechanism responsible for altered hypothalamus–pituitary–adrenal (HPA) axis and subsequently associated with the risk of the development of psychopathology. Child maltreatment as a form of stress could induce continuous cortisol production, which will eventually lead to a decreased response of the HPA axis through methylation of the *NR3C1* receptor, which would subsequently lead to psychopathologies.

The results of this systematic review might also have clinical implications. Although there is to our knowledge no data on child maltreatment investigating the impact of any intervention(s) on *NR3C1* methylation, previous studies on post-traumatic stress disorder (PTSD) indicated that *NR3C1* methylation levels may serve as a potential marker to predict PTSD treatment outcome. For example, Pape et al. (2018) showed that baseline methylation of *NR3C1* was associated with better PTSD treatment response particularly in patients who had experienced child abuse. Yehuda et al. (2013) indicated that methylation of the *NR3C1* exon 1F promoter assessed at pre-treatment predicted treatment outcome to prolonged exposure therapy for PTSD in combat veterans. Further research is needed to understand how *NR3C1* epigenetic alteration can impact treatment response. Understanding this may be essential for the development of innovative and individually tailored interventions to improve the health of maltreated children.

When interpreting the results of this systematic review, it is important to take into account the limitations of the included studies. One first notable finding is that most researchers investigated a clinical population, as child maltreatment is a risk factor for psychopathology, specifically the development of mental disorders, such as borderline personality disorder (Herman et al., 1989; Zanarini et al., 1997) or major depressive disorder (Widom et al., 2007). Secondly, we could not find any data that demonstrate the impact of any intervention(s) on adversity-induced/associated methylation. Most studies adopted a cross-sectional research design based on retrospectives measures (like the Child Trauma Questionnaires or structured interviews), which are subject to bias. It is possible that the length of time since the last exposure to abuse may affect the recall of the experience. Thirdly, the relatively small sample size ($n = 25$ to $n = 534$) limits the statistical power of the analyses by decreasing the confidence interval of the study, as well as the findings' representativeness. Fourthly, it is important to note that most studies collected tissue samples during adulthood. It is therefore difficult to tell when exactly the observed epigenetics changes occurred. Longitudinal follow-up studies will be crucial in order to assess and monitor the long-term effects of the DNA methylation patterns. Fifthly, as mentioned, another bias may be related to the type of epigenetic analysis technique, as each technique has its own limitations. Sixthly, most studies were conducted in a Western context (e.g., Switzerland, Canada, Spain, and Belgium). Therefore, the results cannot be generalized to non-Western contexts, where a child's education or parenting style may be understood differently (Peterson & Bush, 2013). Lastly, most researchers failed to control for the effect of confounding factors like coping or resilience strategies, which are central to understanding an individual's response to adverse life events (Jackson et al., 2018).

5. Limitations

There are some limitations to be noted regarding this review. Despite the relevant number of included studies ($n = 11$), we did not carry out a meta-analysis to assess the strength of the relationship quantitatively because very few studies ($n = 4$) have examined *NR3C1*-1_F methylation and its links with symptoms of psychopathology or cortisol. Furthermore, these four studies were not sufficiently similar to justify generating a combined statistical analysis of the data.

6. Conclusion

The current study examines the relationship between *NR3C1*-1_F methylation associated with child maltreatment as well as its link with the HPA axis and symptoms of psychopathology. The results mainly show a significant correlation between child maltreatment, including sexual abuse, physical abuse, emotional abuse, neglect, and exposure to intimate partner violence and methylation at the CpG site located at exon 1_F of the human glucocorticoid receptor gene *NR3C1*. Interestingly, the results suggest a link between *NR3C1*-1_F methylation, higher HPA axis activity with increased production of cortisol and symptoms of psychopathology. Further research is needed to understand the mechanisms underlying this relationship. In addition, studies of intervention(s) on *NR3C1* methylation induced/associated with adversity are warranted. Understanding these mechanisms may be essential for the development of new therapeutic and preventive interventions in the field of child maltreatment. Future longitudinal studies will likely provide a better understanding of the crucial role of DNA methylation in relation to maltreatment. It is also necessary that future methodological design in the field of epigenetics include psychobiological correlates such as markers of HPA axis activity like cortisol awakening response or area under the curve and symptoms of psychopathology. The use of a design with an exposed and a control group as well as the control measures for differences between both groups is one important aspect to be taken in consideration by future studies. Controlling for

this aspect can help control for potential bias and allow for comparisons between groups. Researchers are encouraged to measure all types of maltreatment like psychological, sexual and physical abuse; emotional and physical neglect and exposure to intimate partner violence, ideally using several scales, so as to control for the consistency of results across the scales.

Funding

This work was funded by a grant from the Swiss Confederation (2018.0801) awarded to the first author and by the research funds of the University of Fribourg (TE-40505 Project 734 Epigenetic and psychological factors). It was also supported by the I-Reach Lab, Unit of Clinical and Health Psychology, University of Fribourg.

Declaration of competing interest

None.

Acknowledgments

We thank Madeleine Viviani and Chia Oliver Ankiambom for their relevant suggestions and comments which improved the writing quality of this text.

References

Achenbach, T. M. (1991). *Manual for the teacher's report form and 1991 profile*. Burlington, VT: University of VT, Department of Psychiatry. Retrieved from https://scholar.google.com/citations?user=gfIVlCIAAAAJ&hl=en.

Beach, S. R. H., Brody, G. H., Todorov, A. A., Gunter, T. D., & Philibert, R. A. (2010). Methylation at SLC6A4 is linked to family history of child abuse: An examination of the Iowa adoptee sample. *American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics: The Official Publication of the International Society of Psychiatric Genetics, 153B*(2), 710–713. https://doi.org/10.1002/ajmg.b.31028

Bellis, M. D.d., & Thomas, L. A. (2003). Biologic findings of post-traumatic stress disorder and child maltreatment. *Current Psychiatry Reports, 5*(2), 108–117.

Burcusa, S. L., & Iacono, W. G. (2007). Risk for recurrence in depression. *Clinical Psychology Review, 27*(8), 959–985. https://doi.org/10.1016/j.cpr.2007.02.005

Bustamante, A. C., Aiello, A. E., Galea, S., Ratanatharathorn, A., Noronha, C., Wildman, D. E., & Uddin, M. (2016). Glucocorticoid receptor DNA methylation, childhood maltreatment and major depression. *Journal of Affective Disorders, 206*, 181–188. https://doi.org/10.1016/j.jad.2016.07.038

Butchart, A., & Harvey, A. P. (2006). *Preventing child maltreatment: A guide to taking action and generating evidence*. Copenhagen: World Health Organization.

Cecil, C. A. M., Zhang, Y., & Nolte, T. (2020). Childhood maltreatment and DNA methylation: A systematic review. *Neuroscience and Biobehavioral Reviews, 112*, 392–409. https://doi.org/10.1016/j.neubiorev.2020.02.019

Cicchetti, D.[. D.]., & Handley, E. D. (2017). Methylation of the glucocorticoid receptor gene, nuclear receptor subfamily 3, group C, member 1 (NR3C1), in maltreated and nonmaltreated children: Associations with behavioral undercontrol, emotional lability/negativity, and externalizing and internalizing symptoms. *Development and Psychopathology, 29*(5), 1795–1806. https://doi.org/10.1017/S0954579417001407

Daskalakis, N. P., & Yehuda, R. (2014). Site-specific methylation changes in the glucocorticoid receptor exon 1F promoter in relation to life adversity: Systematic review of contributing factors. *Frontiers in Neuroscience, 8*, 369. https://doi.org/10.3389/fnins.2014.00369

Farrell, C., Doolin, K., O'Leary, N., Jairaj, C., Roddy, D., Tozzi, L., … O'Keane, V. (2018). Dna methylation differences at the glucocorticoid receptor gene in depression are related to functional alterations in hypothalamic-pituitary-adrenal axis activity and to early life emotional abuse. *Psychiatry Research, 265*, 341–348. https://doi.org/10.1016/j.psychres.2018.04.064

Hecker, T., Radtke, K. M., Karl, M., Hermenau, K., Papassotiropoulos, A., & Elbert, T.[. T.]. (2016). Associations among child abuse, mental health, and epigenetic modifications in the proopiomelanocortin gene (POMC): A study with children in Tanzania. *Development and Psychopathology, 28(4pt2*, 1401–1412. https://doi.org/10.1017/S0954579415001248

Herman, J. L., Perry, J. C., & van der Kolk, B. A. (1989). Childhood trauma in borderline personality disorder. *The American Journal of Psychiatry, 146*(4), 490–495. https://doi.org/10.1176/ajp.146.4.490

Holmes, L., Shutman, E., Chinaka, C., Deepika, K., Pelaez, L., & Dabney, K. W. (2019). Aberrant Epigenomic modulation of glucocorticoid receptor gene (NR3C1) in early life stress and major depressive disorder correlation: Systematic review and quantitative evidence synthesis. *International Journal of Environmental Research and Public Health, 16*(21). https://doi.org/10.3390/ijerph16214280

Jackson, L., Jackson, Z., & Jackson, F. (2018). Intergenerational resilience in response to the stress and trauma of enslavement and chronic exposure to institutionalized racism. *Journal of Clinical Epigenetics, 04*(03). https://doi.org/10.21767/2472-1158.1000100

Kovacs, M. (1982). *The children's depression inventory: A self-rated depression scale for school-aged youngsters*. Pittsburgh: University of Pittsburgh.

Kroenke, K., Spitzer, R. L., & Williams, J. B. (2001). The PHQ-9: Validity of a brief depression severity measure. *Journal of General Internal Medicine, 16*(9), 606–613. https://doi.org/10.1046/j.1525-1497.2001.016009606.x

Laryea, G., Schütz, G., & Muglia, L. J. (2013). Disrupting hypothalamic glucocorticoid receptors causes HPA axis hyperactivity and excess adiposity. *Molecular Endocrinology (Baltimore, Md.), 27*(10), 1655–1665. https://doi.org/10.1210/me.2013-1187

Liu, L., Wu, J., Qing, L., Li, J., Yang, H., Ji, A., … Nie, S. (2020). Dna methylation analysis of the NR3C1 gene in patients with schizophrenia. *Journal of Molecular Neuroscience: MN*. https://doi.org/10.1007/s12031-020-01525-8. Advance online publication.

Liu, P. Z., & Nusslock, R. (2018). How stress gets under the skin: Early life adversity and glucocorticoid receptor epigenetic regulation. *Current Genomics, 19*(8), 653–664. https://doi.org/10.2174/1389202919666171228164350

Martín-Blanco, A., Ferrer, M., Soler, J., Salazar, J., Vega, D., Andión, O., … Pascual, J. C. (2014). Association between methylation of the glucocorticoid receptor gene, childhood maltreatment, and clinical severity in borderline personality disorder. *Journal of Psychiatric Research, 57*, 34–40. https://doi.org/10.1016/j.jpsychires.2014.06.011

McGowan, P. O., Sasaki, A., D'Alessio, A. C., Dymov, S., Labonté, B., Szyf, M., … Meaney, M. J. (2009). Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. *Nature Neuroscience, 12*(3), 342–348. https://doi.org/10.1038/nn.2270

Moher, D., Liberati, A., Tetzlaff, J., & Altman, D. G. (2009). Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. *PLoS Medicine, 6*(7), Article e1000097. https://doi.org/10.1371/journal.pmed.1000097

Moore, E., Gaskin, C., & Indig, D. (2013). Childhood maltreatment and post-traumatic stress disorder among incarcerated young offenders. *Child Abuse & Neglect, 37*(10), 861–870. https://doi.org/10.1016/j.chiabu.2013.07.012

Pape, J. C., Carrillo-Roa, T., Rothbaum, B. O., Nemeroff, C. B., Czamara, D., Zannas, A. S., … Binder, E. B. (2018). DNA methylation levels are associated with CRF1 receptor antagonist treatment outcome in women with post-traumatic stress disorder. *Clinical Epigenetics, 10*(1), 136. https://doi.org/10.1186/s13148-018-0569-x

Perroud, N.[. N.]., Paoloni-Giacobino, A., Prada, P., Olié, E., Salzmann, A., Nicastro, R., … Malafosse, A. (2011). Increased methylation of glucocorticoid receptor gene (NR3C1) in adults with a history of childhood maltreatment: A link with the severity and type of trauma. *Translational Psychiatry, 1*, Article e59. https://doi.org/10.1038/tp.2011.60

Perroud, N.[. N.]., Salzmann, A., Prada, P., Nicastro, R., Hoeppli, M. E., Furrer, S., … Malafosse, A. (2013). Response to psychotherapy in borderline personality disorder and methylation status of the BDNF gene. *Translational Psychiatry, 3*, Article e207. https://doi.org/10.1038/tp.2012.140

Peterson, G. W., & Bush, K. R. (Eds.). (2013). *Handbook of marriage and the family* (3. ed.). New York: Springer.

Prados, J., Stenz, L., Courtet, P., Prada, P., Nicastro, R., Adouan, W., … Perroud, N.[. N.]. (2015). Borderline personality disorder and childhood maltreatment: A genome-wide methylation analysis. *Genes, Brain, and Behavior, 14*(2), 177–188. https://doi.org/10.1111/gbb.12197

Queirós, M., & Caseiro, J. (2018). *Child maltreatment and mental disorders – The role of epigenetics*. Advance online publication. https://doi.org/10.25753/BirthGrowthMJ.v27.i3.13431.

Radtke, K. M.[. K. M.]., Ruf, M., Gunter, H. M., Dohrmann, K., Schauer, M., Meyer, A., & Elbert, T.[. T.]. (2011). Transgenerational impact of intimate partner violence on methylation in the promoter of the glucocorticoid receptor. *Translational Psychiatry, 1*, Article e21. https://doi.org/10.1038/tp.2011.21

Rehan, W., Antfolk, J., Johansson, A., Jern, P., & Santtila, P. (2017). Experiences of severe childhood maltreatment, depression, anxiety and alcohol abuse among adults in Finland. *PLoS One, 12*(5), Article e0177252. https://doi.org/10.1371/journal.pone.0177252

Reynolds, C. R., & Richmond, B. O. (1985). *Revised children's manifest anxiety scale (RCMAS) manual*. Los Angeles, CA: Western Psychological Services.

Romens, S. E., McDonald, J., Svaren, J., & Pollak, S. D. (2015). Associations between early life stress and gene methylation in children. *Child Development, 86*(1), 303–309. https://doi.org/10.1111/cdev.12270

Schür, R. R., van Leeuwen, J. M. C., Houtepen, L. C., Joëls, M., Kahn, R. S., Boks, M. P., & Vinkers, C. H. (2018). Glucocorticoid receptor exon 1F methylation and the cortisol stress response in health and disease. *Psychoneuroendocrinology, 97*, 182–189. https://doi.org/10.1016/j.psyneuen.2018.07.018

Shields, A., & Cicchetti, D.[. D.]. (1997). Emotion regulation among school-age children: The development and validation of a new criterion Q-sort scale. *Developmental Psychology, 33*(6), 906–916. https://doi.org/10.1037//0012-1649.33.6.906

Shields, A., & Cicchetti, D.[. D.]. (1998). Reactive aggression among maltreated children: The contributions of attention and emotion dysregulation. *Journal of Clinical Child Psychology, 27*(4), 381–395. https://doi.org/10.1207/s15374424jccp2704_2

Steiger, H., Labonté, B., Groleau, P., Turecki, G., & Israel, M. (2013). Methylation of the glucocorticoid receptor gene promoter in bulimic women: Associations with borderline personality disorder, suicidality, and exposure to childhood abuse. *The International Journal of Eating Disorders, 46*(3), 246–255. https://doi.org/10.1002/eat.22113

Suchiman, H. E. D., Slieker, R. C., Kremer, D., Slagboom, P. E., Heijmans, B. T., & Tobi, E. W. (2015). Design, measurement and processing of region-specific DNA methylation assays: The mass spectrometry-based method EpiTYPER. *Frontiers in Genetics, 6*, 287. https://doi.org/10.3389/fgene.2015.00287

Suderman, M., Borghol, N., Pappas, J. J., Pinto Pereira, S. M., Pembrey, M., Hertzman, C., … Szyf, M. (2014). Childhood abuse is associated with methylation of multiple loci in adult DNA. *BMC Medical Genomics, 7*, 13. https://doi.org/10.1186/1755-8794-7-13

Tarantola, D. (2018). Child maltreatment: Daunting and universally prevalent. *American Journal of Public Health, 108*(9), 1119–1120. https://doi.org/10.2105/AJPH.2018.304637

Targum, S. D., & Nemeroff, C. B. (2019). The effect of early life stress on adult psychiatric disorders. *Innovations in Clinical Neuroscience, 16*(1–2), 35–37.

Turecki, G., & Meaney, M. J. (2016). Effects of the social environment and stress on glucocorticoid receptor gene methylation: A systematic review. *Biological Psychiatry, 79*(2), 87–96. https://doi.org/10.1016/j.biopsych.2014.11.022

Vangeel, E. B., Kempke, S., Bakusic, J., Godderis, L., Luyten, P., van Heddegem, L., … Claes, S. (2018). Glucocorticoid receptor DNA methylation and childhood trauma in chronic fatigue syndrome patients. *Journal of Psychosomatic Research, 104*, 55–60. https://doi.org/10.1016/j.jpsychores.2017.11.011

Vangeel, E. B., van den Eede, F., Hompes, T., Izzi, B., Del Favero, J., Moorkens, G., … Claes, S. (2015). Chronic fatigue syndrome and DNA Hypomethylation of the glucocorticoid receptor gene promoter 1F region: Associations with HPA Axis Hypofunction and childhood trauma. *Psychosomatic Medicine, 77*(8), 853–862. https://doi.org/10.1097/PSY.0000000000000224

Wathen, C. N., & Macmillan, H. L. (2013). Children's exposure to intimate partner violence: Impacts and interventions. *Paediatrics & Child Health, 18*(8), 419–422.

Weaver, I. C. G., Cervoni, N., Champagne, F. A., D'Alessio, A. C., Sharma, S., Seckl, J. R., … Meaney, M. J. (2004). Epigenetic programming by maternal behavior. *Nature Neuroscience, 7*(8), 847–854. https://doi.org/10.1038/nn1276

Widom, C. S., DuMont, K., & Czaja, S. J. (2007). A prospective investigation of major depressive disorder and comorbidity in abused and neglected children grown up. *Archives of General Psychiatry, 64*(1), 49–56. https://doi.org/10.1001/archpsyc.64.1.49

Wiley, J. W., Higgins, G. A., & Athey, B. D. (2016). Stress and glucocorticoid receptor transcriptional programming in time and space: Implications for the brain-gut axis. *Neurogastroenterology and Motility: The Official Journal of the European Gastrointestinal Motility Society, 28*(1), 12–25. https://doi.org/10.1111/nmo.12706

Witzmann, S. R., Turner, J. D., Mériaux, S. B., Meijer, O. C., & Muller, C. P. (2012). Epigenetic regulation of the glucocorticoid receptor promoter 1(7) in adult rats. *Epigenetics, 7*(11), 1290–1301. https://doi.org/10.4161/epi.22363

Yehuda, R., Daskalakis, N. P., Desarnaud, F., Makotkine, I., Lehrner, A. L., Koch, E., … Bierer, L. M. (2013). Epigenetic biomarkers as predictors and correlates of symptom improvement following psychotherapy in combat veterans with PTSD. *Frontiers in Psychiatry, 4*, 118. https://doi.org/10.3389/fpsyt.2013.00118

Zanarini, M. C., Williams, A. A., Lewis, R. E., Reich, R. B., Vera, S. C., Marino, M. F., … Frankenburg, F. R. (1997). Reported pathological childhood experiences associated with the development of borderline personality disorder. *The American Journal of Psychiatry, 154*(8), 1101–1106. https://doi.org/10.1176/ajp.154.8.1101