# EARLY ADVERSITIES AND EPIGENETIC MODIFICATIONS OF HPA-AXIS GENES

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# Abstract

The early life marks a particularly important period for the development of an individual during which adverse experiences can have lifelong implications for health. The experience of early adversities – even before birth – increases the risk to develop behavioral problems and mental illness. The hypothalamic pituitary adrenal (HPA) axis, a neuroendocrine axis enabling the adaptation to environmental stressors, seems to be at the core of this association. The activity of the HPA axis highly depends on experiences early in life and its dysregulation associates with a range of psychopathological conditions. Epigenetic modifications, i.e. environmentally inducible mechanisms that control gene expression, may translate past experiences into physiological changes and consequently altered behavior and impaired mental health. This thesis investigates epigenetic modifications of HPA axis genes, DNA methylation, established in the aftermath of early life adversities and how these relate to psychological wellbeing.

Following a general introduction about the involvement of epigenetic modifications in the etiology of stress induced mental illness in chapter I, chapter II provides a review on the current literature addressing association between epigenetic modifications and prenatal stress. While the second and the third chapter focus on stress experienced before birth, the fourth and the fifth chapter address maltreatment experienced in childhood.

Chapter III demonstrates a long-lasting effect of gestational psychological stress on DNA methylation in human offspring. By then, such results were only reported in the context of nutrition or in non-human organisms. In this study, the methylation of the *glucocorticoid receptor* (*GR*) gene, a key regulator of the HPA-axis, was increased in the blood of 10 to 19 year old children whose mothers experienced intimate partner violence (IPV) during pregnancy.

Chapter IV evidences an effect of the interaction between the epigenome and the environment on psychological health. In this study, the simultaneous occurrence of methylation at the *GR* gene and the experience of childhood abuse increased the vulnerability to develop psychopathology in general and to borderline personality disorder (BPD) in particular. In order to more accurately depict the subjects' psychological condition, this study conceptualized psychopathology as general concept including symptoms of several psychopathological conditions and behavioral and emotional problems.

Chapter V further highlights the detrimental consequences of childhood abuse regardless of the societal and cultural embedding. In societies, in which specific forms of child abuse are frequent and socially accepted, its consequences have been argued to be less harmful. The study in chapter V took place in such a cultural setting – in Tanzania – and demonstrated an association of childhood abuse with both a worsened mental health and differential DNA methylation of HPA axis genes, especially the *pro-opiomelanocortin* (*POMC*) gene. Differential DNA methylation by means of childhood abuse could be seen in both blood and saliva, thus suggesting a system-wide epigenetic response to early adversities.

In summary, all studies conducted in this thesis report persistent epigenetic modifications in HPA axis genes in response to early adversities. These effects were evident in peripheral tissues, blood and saliva. These tissues most likely reflect the epigenetic constitution of the brain, where epigenetic modifications are thought to affect psychological function. Thus this thesis improves the understanding of how early adversities may program later psychosocial function.

# Zusammenfassung

Das frühe Leben kennzeichnet eine für die Entwicklung des Individuums bedeutsame Phase, in der sich bestimmte Ereignisse ein Leben lang auf die Gesundheit auswirken können. So erhöhen aversive Erlebnisse in der Kindheit und sogar schon vor der Geburt das Risiko Verhaltensprobleme oder psychische Krankheiten zu entwickeln. Der Hypothalamus-Hypophysen-Nebenrinden (engl. HPA) Achse, die es dem Individuum ermöglicht auf Umweltstressoren zu reagieren, kommt dabei eine zentrale Funktion zu. Stress während früher Lebensphasen moduliert die Aktivität der HPA Achse und kann zu deren Fehlregulation führen, ein Kennzeichen vieler psychopathologischer Zustände. Epigenetische Modifikationen – durch die Umwelt induzierbare Mechanismen, welche die Genaktivität bestimmen – könnten frühe Erfahrungen in eine veränderte Physiologie und folglich verändertes Verhalten und psychische Konstitution überführen. Die vorliegende Arbeit untersucht epigenetische Modifikationen, DNA Methylierung, in HPA Achsen Genen infolge aversiver Erfahrungen in frühen Lebensphasen und deren Einfluss auf das psychische Wohlbefinden.

Kapitel I und II bieten eine allgemeine Einleitung über die Involvierung epigenetischer Modifikationen in die Ätiologie stressinduzierter psychischer Erkrankungen sowie einen Überblick über die gegenwärtige Literatur bzgl. der Assoziation zwischen epigenetischen Modifikationen und vorgeburtlichem Stress. Während sich das zweite und das dritte Kapitel mit vorgeburtlichem Stress beschäftigt, werden im vierten und fünften Kapitel Misshandlungen in der Kindheit behandelt.

Kapitel III zeigt anhand von Blutproben 10-19-jähriger Personen, dass psychologischer Stress während der Schwangerschaft einen nachhaltigen Einflusses auf die DNA Methylierung des Nachwuchses ausübt. Ein Befund, der zu diesem Zeitpunkt ausschließlich im Kontext der Ernährung bzw. in nicht-humanen Organismen gezeigt werden konnte. In dieser Studie konnte bei Kindern deren Mütter partnerschaftliche Gewalt während der Schwangerschaft erfahren haben eine erhöhte Methylierung des *Glukokortikoid-Rezeptor* (*GR*) Gens, ein wichtiges Gen in der Regulation der HPA Achse, nachgewiesen werden.

Kapitel IV belegt einen Einfluss der Interaktion zwischen dem Epigenom und der Umwelt auf die psychische Gesundheit. In dieser Studie führte das gemeinsame Auftreten von Methylierung im *GR* Gen und die Erfahrung von Kindesmissbrauch zu einer allgemeinen Beeinträchtigung der psychischen Gesundheit

sowie einer erhöhten Anfälligkeit für eine Borderline-Persönlichkeitsstörung. Um die psychische Gesundheit der Studienteilnehmer möglichst akkurat darzustellen, wurde Psychopathologie in dieser Studie als ein mehrdimensionales Konstrukt aufgefasst, dass sowohl Symptome verschiedener psychischer Krankheitsbilder als auch Verhaltensauffälligkeiten beinhaltet.

Kapitel V zeigt, dass Kindesmissbrauch auch in Ländern, in denen bestimmte Formen von Gewalt gegenüber Kindern gesellschaftlich akzeptiert und üblich sind, verheerende Folgen hat. Einige Autoren argumentieren, dass der Einfluss von Kindesmissbrauch in solchen Gesellschaften weniger schädlich sei. Die in Kapitel V beschriebene Studie fand in solch einem kulturellen Umfeld statt und belegt einen Zusammenhang von Kindesmissbrauch mit sowohl verschlechterter Gesundheit als auch veränderter Methylierung in Genen der HPA Achse, insbesondere des *Proopiomelanocortin* (*POMC*) Gens. Kindesmissbrauch beeinflusste die DNA Methylierung sowohl im Blut als auch im Speichel und scheint somit eine systemübergreifende epigenetische Reaktion hervorzurufen.

Zusammenfassend zeigen alle im Rahmen dieser Arbeit ausgeführten Studien nachhaltige epigenetische Modifikationen in HPA Axis Genen infolge aversiver Erfahrungen in frühen Entwicklungsphasen. Diese Effekte traten in peripheren Geweben, Blut und Speichel, zutage, welche vermutlich die epigenetische Konstitution des Gehirns wiederspiegeln. Somit trägt diese Arbeit zu einem besserem Verständnis des Einflusses von Stress während früher Entwicklungsphasen auf die spätere psychische Konstitution bei.

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# Abbreviations

| | |
|---|---|
| ACTH | adrenocorticotropic hormone |
| ADHD | attention deficit hyperactivity disorder |
| AVP | arginine vasopressin |
| BDNF | brain-derived neurotrophic factor |
| BPD | borderline personality disorder |
| BPD | borderline personality disorder |
| CD | conduct disorder |
| CRH | corticotropin-releasing hormone |
| CSA | childhood sexual abuse |
| ELS | early life stress |
| FKBP5 | FK506 binding protein 5 |
| GBD | global burden of disease |
| GC | glucocorticoid |
| GR | glucocorticoid receptor |
| HDAC | histone deacetylase |
| HDCAi | histone deacetylase inhibior |
| hGR | Human glucocorticoid receptor |
| HPA-axis | hypothalamic pituitary adrenal axis |
| LTP | long-term potentiation |
| MDB | methyl-CpG-binding domain |
| miRNA | micro RNA |
| POMC | pro-opiomelanocortin |
| ncRNA | noncoding RNA |
| NMDA | N-methyl-D-aspartate |
| ODD | oppositional defiant disorder |
| PTSD | post traumatic stress disorder |
| PVN | paraventricular nucleus |
| siRNA | small interfering RNA |
| sncRNA | small noncoding RNA |
| TF | transcription factor |
| TSA | trichostatin A |
| WHO | World Health Organization |

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# I Introduction

Mental health problems are associated with impaired wellbeing and role functioning, and thus pose major challenges to both the affected individual and the society at large (WHO, 2003). In accordance with generally high prevalence rates (Demyttenaere *et al.*, 2004), mental illness accounted for 22.9% of the global non-fatal burden of disease[1] and thus was classified as its leading cause in 2010 (Whiteford *et al.*, 2013). Depending on the calculation, mental illness was ranked the 3rd (Ferrari *et al.*, 2014) or 5th (Whiteford *et al.*, 2013) most prevalent category amongst the global disease burden, which includes both non-fatal and fatal components of disease-burden[2]. Projections of the World Health Organization (WHO) predict that in 2030, depressive disorders alone will be the most prominent cause of overall disease burden (Whiteford *et al.*, 2013). These alarming dimensions of mental illness emphasize the urgent need for understanding its etiology.

There is a substantial body of empirical evidence showing that exposure to adversities during early child development, even prenatally, is associated with vulnerability to mental illness (Heim *et al.*, 2001; Talge *et al.*, 2007). In addition to severe forms of early adversities such as childhood maltreatment including abuse and neglect, seemingly milder forms of child adversities such as bullying, parental loss or low socioeconomic status constitute risk factors for the development of mental illness (Dube *et al.*, 2003; Kessler *et al.*, 2010). When experienced prenatally, maternal mood disturbances or stressful events are associated with increased vulnerabilities for psychopathological states later in life (Stein *et al.*, 2014). From birth onwards, the early life is characterized by dynamic changes of the body and the brain. The brain is highly plastic during this period and thus particularly receptive to environmental stimuli. Adversities during this time might elicit neurodevelopmental paths eventually resulting in disease. Indeed, all of the aforementioned early life adversities have been reported to change the neuroendocrine system governing the release of stress hormones. Epigenetic modifications of genes related to the hypothalamic-pituitary-adrenal (HPA) may be a driving force of how early life stress (ELS) manifests in such "stress-

[1] As measured by years lived with disability (YLD)
[2] As measured by disability adjusted life years (DALYs). DALYs combine YLD and premature mortality as years of life lost (YLLs).

induced" disorders. The following chapters will explore theses findings and potential mechanisms in greater detail.

## I.1. Early life stress and mental illness

To date, most research on postnatal stress has mainly focused on stressors such as maternal postpartum depression or anxiety, childhood maltreatment, poverty, loss of a parent and exposure to family conflict and violence (Provençal *et al.*, 2015). Investigated prenatal stressors are somewhat more diverse in nature but always relate to *in utero* exposure to maternal experiences, mental states or habits. These include social exclusion, mood disturbances such as those related to anxiety or depression, bereavement (Khashan *et al.*, 2008) or the experience of natural- (Laplante *et al.*, 2008) or man-made disasters (Kleinhaus *et al.*, 2013; Yehuda *et al.*, 2005), daily hassles (Grizenko *et al.*, 2012; Rice *et al.*, 2010), smoking (Cornelius *et al.*, 2012; Wakschlag *et al.*, 2010) or malnutrition (Painter *et al.*, 2006). While a wide range of potential health outcomes mirrors the diversity of prenatal stress-categories, no specific cause-disease patterns has appeared (see chapter II). However, the detrimental impact of prenatal stress on psychological function is well established. As outlined in chapter II prenatal stress associates with later behaviroal or emotional problems, impaired, cognitive function, severe psychopathological states such as schizophrenia or autism, as well as sleep problems, obesity, dermatoglyphic asymmetry, mixed handedness and immune function (for reviews see Glover, 2014; Talge *et al.*, 2007).

While most studies report neonatal outcomes, prenatal stress can also affect the individual through to adulthood (Betts *et al.*, 2015; Brown *et al.*, 1995; Buchmann *et al.*, 2014; Entringer *et al.*, 2009b; Entringer *et al.*, 2008; Neugebauer *et al.*, 1999; Painter *et al.*, 2006). Unsurprisingly, the undesirable effects of postnatal stressors may last into adulthood, too. Extensive literature substantiates a tendency towards adult psychopathology following childhood maltreatment. A common definition of childhood maltreatment refers to "*any act of commission or omission by a parent or other caregiver that result in harm, potential harm, or threat of harm to a child*" (Leeb *et al.*, 2008). Besides the potential for immediate physical or emotional harm, child maltreatment causes widespread and lifelong consequence for both physical and mental health. As repeatedly confirmed by longitudinal studies (Kaplan *et al.*, 2014; Widom *et al.*, 2007), child maltreatment is associated with emotional and behavioral problems that begin in childhood and can persist throughout adolescence and adulthood (for a review see

Carr *et al.*, 2013) including depression, anxiety disorders, posttraumatic stress disorder (PTSD), reduced self-esteem, suicidal behavior, conduct disorder, and aggressive or delinquent behavior (Catani *et al.*, 2008; Dube *et al.*, 2003; Hermenau *et al.*, 2014; Sugaya *et al.*, 2012). These conditions in turn might explain the increased rates of health-risk behavior amongst these individuals, such as smoking alcohol and/or drug abuse, overeating or sexual behaviors and thus may indirectly expose them to a higher vulnerability to peripheral medical conditions (Felitti *et al.*, 1998). Accordingly, child abuse is associated with the later development of chronic diseases such as lung cancer (Brown *et al.*, 2010), liver disease (Dong *et al.*, 2003) and ischemic heart disease (Dong *et al.*, 2004).

As described in the previous paragraph, early life stress adversely affects various functions and behaviors. The variety of brain structures and functions affected by early life stress might explain for such non-specific burden. Functional and volumetric changes in the brain areas related to emotionality and behavioral control are consistently reported in association with childhood maltreatment, thus providing a link to a range of psychopathological conditions (for a review see McCrory *et al.*, 2010). For example adults, but not children, with a history of childhood maltreatment consistently present with reduced hippocampal volume (Carrion *et al.*, 2007; De Bellis *et al.*, 1999; Tupler *et al.*, 2006). The hippocampus plays a central role in learning and memory (Gould *et al.*, 1999) and is likely to be the most stress-sensitive structure in the brain. Moreover, the amygdala and the prefrontal cortex, which are involved in processes such as emotional- and cognitive processing (Ochsner *et al.*, 2005; Phelps *et al.*, 2005) also appear to be affected, however the results of previous studies are mixed. While volumetric reductions and increases have been reported for both structures after childhood maltreatment, some studies have failed to replicate these findings (for reviews see McCrory *et al.*, 2010; Teicher *et al.*, 2014b). Although humans are born with relatively mature brains due to rapid brain growth in the 3$^{rd}$ trimester of intrauterine life, brain development is far from complete at birth (Dobbing *et al.*, 1979). The hippocampal formation, the amygdala and the prefrontal cortex undergo major growth periods that occur until 2 years of age, during until the late 20s and in adolescence between 8 and 14 years of age, respectively (for a review see Lupien *et al.*, 2009). These developmental windows have been suggested to represent sensitive periods during which the respective brain regions are most susceptible to environmental influences (Andersen *et al.*, 2008a; Lupien *et al.*, 2009). Recent research supports this hypothesis by roughly linking reduced brain volume to the incidence of childhood maltreatment

in these sensitive periods; depending on whether sexual abuse was experienced between the ages of 3 to 5 years or between the ages between 14 to 16 years the hippocampal- or the prefrontal cortex volume was reduced, respectively (Andersen *et al.*, 2008b). The neurotoxicity hypothesis postulates that these neurodegenerative processes result from excessive exposure to stress-released glucocorticoids (GC) (McEwen *et al.*, 2011; Sapolsky *et al.*, 1986). Intriguingly, the previously mentioned brains structures, especially the hippocampus, are critically involved in regulating the hypothalamic pituitary adrenal (HPA) axis and consequently the release of glucocorticoids, its downstream effectors. Dysregulation of HPA-axis presents a likely candidate in mediating early life stress (ELS) associated mental illness, as it is a robust finding in both individuals suffering from symptoms of mental illness and/or a history of ELS. Thus stress exposure might initiate a downward spiral of degeneration and disease.

## I.2. HPA-axis and ELS

The HPA-axis is a major neuroendocrine axis that allows the organism to deal with stressors, defined as any condition that threatens physical or psychological integrity. The HPA-axis includes the hypothalamus, the pituitary and the adrenal glands (Chrousos *et al.*, 1992; de Kloet *et al.*, 2005). Cortisol, which is the main effector of the HPA-axis in humans, mobilizes energy resources from stored glucose and lipids and contributes to multiple physiological changes such as inhibition of the immune system and the reproductive system (for a review see Tsigos *et al.*, 2002). Thus it prepares the individual to meet the energy demands created by a stressful situation such as increased vigilance and preparedness to employ defensive strategies. Upon stress perception, a hormonal cascade is initiated: Corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) are released from the paraventricular nucleus (PVN) of the hypothalamus. These hormones in turn trigger the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary into the bloodstream. Finally, ACTH acts on the adrenal gland to synthesize and release cortisol. As indicated above, chronically elevated cortisol levels are associated with detrimental health outcomes. Thus, HPA-axis activity is tightly regulated via negative feedback loops at the level of the hypothalamus and the pituitary. Cortisol binds to glucocorticoid receptors (GR), which then regulate gene expression and finally dampen HPA-axis activity. In addition to direct feedback regulation, other brain

regions such as the prefrontal cortex, the amygdala and especially the hippocampus modulate HPA-axis activity in response to cortisol.

Early life stress (ELS), including prenatal stress, has repeatedly been shown to enduringly alter the basal and stress-related activity of the HPA-axis, although the directions of these changes vary considerably. For example, a flattened diurnal cortisol profile was demonstrated in infants of mothers who experienced the 9/11 terror attacks and subsequently developed PTSD (Yehuda *et al*, 2005), and in adolescents exposed to prenatal maternal anxiety (Van den Bergh *et al*, 2008; O'Donnell *et al*, 2013). On the other hand, exposure to prenatal maternal anxiety or depression is associated with increased awakening cortisol levels in children (O'Connor *et al*, 2005) and elevated cortisol responses to acute stress in newborns (Oberlander *et al.*, 2008), respectively. Childhood stress exerts analagous effects on HPA-axis function. Both increased (Halligan *et al*, 2004), and decreased (King *et al*, 2001) basal HPA-axis activities folowing childhood adversities have been reported. Furthermore, adults with a history of childhood maltreatment were shown to exhibit elevated (Heim, 2000) as well as decreased HPA-axis responses to stress (Carpenter *et al.*, 2007). A recent study demonstrated a decreased stress-related but increased basal HPA-axis activity in individuals that were sexually abused during childhood (Schalinski *et al*, 2015). Although these results seemingly contradict each other, they all point towards a programming of HPA-axis function by early experiences bearing a devastating potential for current and future health. Yet, as outlined in further detail in chapter II, from an evolutionary perspective this process might reflect adaptations to a hostile environment.

Some studies report decreased hippocampal *GR* expression that is related to the advent of childhood abuse in human post-mortem brain tissue (McGowan *et al*, 2009)(Labonté *et al.*, 2012b; Suderman *et al.*, 2012). However, only animal models enabled considerable insights into the molecular mechanisms that link pre- and postnatal stress to mental ill health later in. As in humans, ELS evokes endocrine, neuronal and behavioral alterations in rodents. Rats exposed to either prenatal or postnatal stress displayed more anxiety- and depressive like behavior and HPA-axis hyperactivity (Caldji *et al.*, 1998; Francis *et al.*, 1999; Liu *et al.*, 1997; for a review see Maccari *et al.*, 2014; Morley-Fletcher *et al.*, 2003a; Morley-Fletcher *et al.*, 2003b), in addition to differential gene expression in the brain. Various studies have shown that prenatally or postnatally stressed rodents display decreased hippocampal *GR* expression, increased *CRH* and *AVP* expression in the paraventricular nucleus (PVN)

of the hypothalamus, increased basal or stress-induced corticosterone levels and increased anxiety- and depressive-like behaviors (Brunton *et al.*, 2010; Maccari *et al.*, 2014; Murgatroyd *et al.*, 2009). Also structural and functional alterations in the hippocampus in the aftermath of exposure to ELS have been reported. Consistent with impaired spatial learning, prenatally-stressed rodents present with reduced neurogenesis in the dentate gyrus (Lemaire *et al*, 2000) and reduction of N-methyl-D-aspartate receptor (NMDA) receptor induced long-term potentiation (LTP) in CA1 neurons *in vitro* (Son *et al*, 2006). Correspondingly, decreased hippocampal volume and inhibition of neurogenesis in the dentate gyrus is associated with increased emotionality and HPA-axis responses in prenatally stress rhesus monkeys (Coe *et al*, 2003). Postnatal stress, e.g. by means of low maternal care in rats, results in similar effects on the hippocampus as evidenced by decreased survival in the hippocampal dentate gyrus (Bredy *et al*, 2003), shorter dendritic branch length and lower spine density in CA1 neurons (Champagne *et al*, 2008). Again these structural alterations are mirrored in hippocampal functioning, as low maternal care associated with a reduced capacity to induce LTP in the CA1 area and dentate region *in vitro* (Bagot *et al*, 2009; Champagne *et al*, 2008). Interestingly these effects only hold under basal conditions. In these experiments, corticosterone application enhanced hippocampal LTP in rats, which received low levels of maternal care (Bagot *et al*, 2009; Champagne *et al*, 2008). This suggests a programming effect of maternal care, which ensures cognitive functioning in a threatening environment facilitating memory formation in a highly emotional context (see also chapter II).

In addition to genes related to the HPA-axis, differential expression in genes involved in brain plasticity has been reported. For example, expression of brain-derived neurotrophic factor (BDNF), which is implicated in neuronal differentiation, proliferation and plasticity (Park *et al.*, 2013), was reported to be decreased in the hippocampus of rats that experienced early life stress (Boersma *et al*, 2014; Liu *et al*, 2000). ELS also leads to downregulation of NDMA- and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, both of which are important modulators of LTP (Malenka and Nicoll, 1999) in the hippocampus, as evidenced by reduced expression of their subunits (Liu *et al*, 2000; Yaka *et al*, 2007).

The afore-mentioned consequences of ELS were evident in adult animals, thus highlighting their long-lasting legacy. In the past decade epigenetic modifications have emerged as a promising mechanism by which experiences can be embedded in changes in gene activity and thus long-term changes in physiology and behavior.

## I.3. Epigenetics

The term epigenetics was first coined by the embryologist Conrad H. Waddington as the "*the branch of biology, which studies the causal interactions between genes and their products, which bring the phenotype into being*" (Waddington, 1942, 1968). He hypothetically described how the genotype of the zygote gives rise to multiple cell types such as skin or nerve cells through interacting with differentiated cytoplasm and the external environment (Waddington, 1939). Although, those cells share the same genome, they vary drastically in terms of their structure and function. Epigenetics can be regarded as an extra layer of information superimposed onto the genetic information, which defines patterns of gene activity and thus phenotypic properties, such as cell identity. During cycles of cell division, the cellular identity is reliably copied to the daughter cells. A more recent and widely used definition of epigenetics incorporates this dimension of inheritance: "*The study of mitotically and/or meiotically heritable changes in gene function that cannot be explained by changes in DNA sequence.*" (Russo *et al.*, 1996)

The epigenetic mechanisms provoking these varying patterns of genome activity can roughly be divided into chromatin modifications and noncoding RNA- (ncRNA) mediated processes. Chromatin modifications include chemical modifications of the DNA, DNA methylation, and associated proteins, histone modifications (Fig. II-1). The various epigenetic mechanisms do not operate in isolation but rather interact in determining the chromatin structure and patterns of gene activity. DNA methylation is probably the most widely studied epigenetic mechanism. This thesis exclusively investigates DNA methylation, however all of the epigenetic mechanism will be briefly introduced in order to provide a comprehensive picture.

**Histone modifications**

Although the DNA contained in the genome would measure 2 meters if linearly stretched out, it fits into a cell nucleus of microscopic dimensions. The structural organization into chromatin, the complex of DNA and proteins, enables such a high degree of compaction. DNA is wound around eight histone proteins (each containing two copies of four histone types: H2A, H2B, H3 and H4) to build a structure called the nucleosome. Like a string of beads, successive repeats of

nucleosomes create the chromatin fiber. Chromatin modifications, covalent modifications of the histones at amino acid residues on their N-terminal tails, control the degree to which the nucleosomes are condensed and thus the accessibility of the DNA strand to the transcriptional machinery. Histone covalent modifications include acetylation, ubiquitination, or SUMOylation at lysine residues, methylation at lysine or arginine residues, and phosphorylation at serine or threonine residues, among others (Kouzarides, 2007). In general, histoneacetylation for example leads to a more open chromatin structure, euchromatin, and marks transcriptionally active DNA, whereas covalent addition of methyl groups usually results in the opposite effect, heterochromatin (Jenuwein *et al.*, 2001).

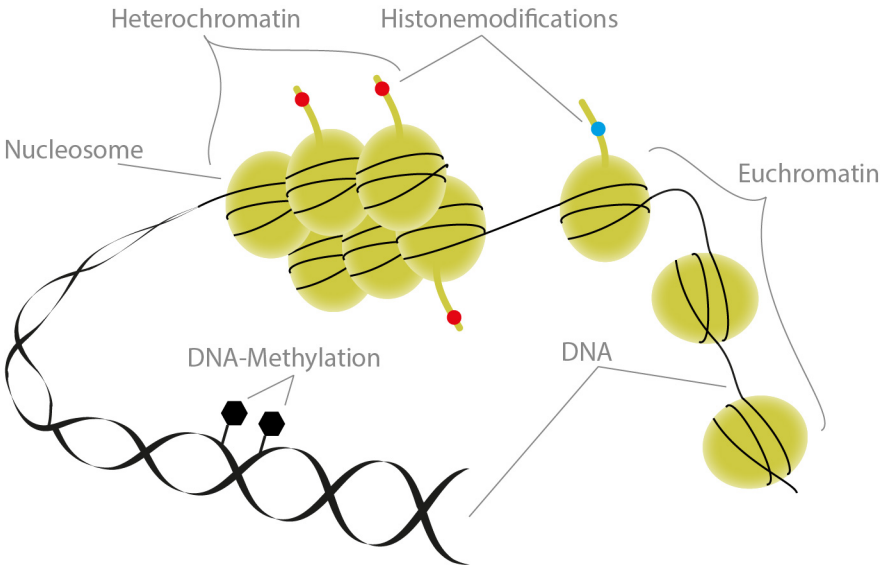


**Fig. I-1: Chromatin modifications**

### DNA methylation

DNA methylation, the covalent addition of a methyl residue to a cytosine, marks a further type of chromatin modifications. It predominantly occurs in the context of CpG-sites, a cytosine nucleotide followed by a guanine nucleotide, but has also been identified in a non-CpG-context (Lister *et al.*, 2009). The majority of human gene-promoters are characterized by regions of high CpG density, CpG islands, which are usually unmethylated (for a review see Jones, 2012). Methylation of a CpG-site does not alter the genetic code itself, but it does alter the activity of the respective gene. While DNA methylation in gene bodies is associated with transcribed genes, DNA methylation in the regulatory region of a gene generally dampens its activity via direct and indirect mechanisms (Jones, 1999). DNA methylation inhibits gene

transcription either by preventing transcription factors (TF) from binding their target sequence (Weaver *et al.*, 2004) or by recruiting methyl-CpG-binding domain (MDB) proteins. MDB proteins in turn recruit histone modifying enzymes resulting in transcriptional silencing (Lopez-Serra *et al.*, 2008). Thus DNA methylation and histone modifications are highly intertwined mechanisms.

**Non coding RNA**

Traditionally, RNA including mRNA, tRNA and rRNA has been considered to be the intermediate between DNA and protein, providing the template and infrastructural platform for protein synthesis. Recently, various classes of small noncoding (sncRNA) and long noncoding RNAs (lncRNA), i.e. RNA molecules not being translated to proteins, have been post-transcriptionally implicated in regulating gene expression (Morris *et al.*, 2014). In a process called RNA interference, sncRNAs of the classes small interfering RNA (siRNA) and mircoRNA (miRNA) bind to complementary mRNA transcripts, preventing them from being translated into proteins and/ or causing their degradation (Novina *et al.*, 2004). Their sequence complementarity to both mRNA and DNA renders sncRNA as good candidates to guide chromatin modifiaction enzymes to specific loci. Indeed, the association of an sncRNA with an mRNA transcript can cause DNA methylation and histone modifications in the corresponding gene (Morris *et al.*, 2004; Morris *et al.*, 2014; Volpe *et al.*, 2011).

## I.4. Epigenetics and stress related disease

Epigenetic processes aren't limited to cellular differentiation, but also respond to signals triggered by the external physical and social environment particularly during early life. Thus, epigenetics can be regarded as an adaptation of the genome or its activity to environmental conditions (Szyf, 2012). However, under certain circumstances this mechanism might turn maladaptive and result in a sequela of physical and mental illness. An elegant series of studies in rats illustrates these relationships. Rats that experienced low levels of maternal care in their first days of postnatal life show reduced hippocampal *GR* expression, increased HPA-axis responses to stress and increased anxiety related behavior in adulthood (Francis *et al*, 1999; Liu *et al*, 1997). These changes have been causally related to increased methylation and decreased histone acetylation at the *GR* promoter (Weaver *et al*, 2004). These results have been replicated in a human context; postmortem hippocampi of

suicide completers with a history of childhood abuse present with increased *GR* methylation and correspondingly decreased *GR* expression (McGowan *et al*, 2009). Following these initial reports, an increasing amount of evidence substantiates the interaction between epigenetics and the early environment. Accordingly, several of the afore-mentioned examples of differential gene expression were accompanied by epigenetic modifications. The *BDNF* gene displayed increased methylation in the prefrontal cortex of adult rats exposed to abusive caretakers (Roth *et al*, 2009) and in both the hippocampus and amygdala of prenatally stressed adult rats (Boersma *et al*, 2014). In addition to the findings in the *GR* (Mueller *et al.*, 2008; Weaver *et al.*, 2004), which have been extensively replicated (Palma-Gudiel *et al.*, 2015; Turecki *et al.*, 2016), sustained epigenetic modifications due to ELS in further genes related to the HPA axis have also been well established. For example, decreased methylation of the *CRH* gene was found in the hippocampus and the hypothalamus in rats exposed to maternal separation (Chen *et al*, 2012; Wang *et al*, 2014) and in the amygdala of prenatally stressed rats (Mueller *et al.*, 2008). Furthermore, maternal separation is associated with decreased *POMC* and *AVP* methylation in the pituitary and the hypothalamus in rats, respectively (Murgatroyd *et al*, 2009; Wu *et al*, 2014).

These studies demonstrate how early adversities become biologically embedded and thus might interfere with brain development and consequently set the affected individual at a heightened risk for the development of psychopathology. Correspondingly, Teicher and colleagues suggested that epigenetic modifications established in the aftermath of early adversities might reduce synaptic overproducing resulting in a progressive loss of hippocampal synapses first apparent after puberty (Andersen *et al.*, 2004; Andersen *et al.*, 2008b; Teicher *et al.*, 2014a). This might explain the reduced hippocampal volume after childhood sexual abuse (CSA), which only becomes appearant in adolescence and for the delayed onset of depressive symptoms that appear at adolescence, even though CSA might have already abated (Teicher *et al.*, 2009).

**Epigenetics and Therapy**

Such a delay between the experience of adverstities and resulting disease is also seen in other psychopathological condtiotions such as PTSD and borderline personality disorder (BPD) and might represent sensitive periods in which interventive measures might alleviate the consequences of early adversities. Indeed peripubertal enrichment was shown to restore initially reduced levels of NMDA-receptor

expression, synaptic density in the hippocampus (Bredy *et al*, 2004) and cognitive abilities in rats that received low levels of maternal care (Bredy *et al*, 2003a). Moreover, cross-fostering of rats to high caring dams reversed the effects intially created by low-levels of maternal care, such as increased hippocampal *GR* methylation, decreased hippocampal *GR* expression and increased HPA-responses and anxious-like behaviour (Weaver *et al*, 2004). Interestingly the effects induced by low levels of maternal care could also be reversed by central application of the histone deacetylase (HDAC) inhibitor trichostatin A (TSA) (Weaver *et al*, 2004).

There are some promising preliminary results that suggest the monitoring and manipulation of epigenetic modifications in humans could serve as effective therapeutic tools. Although in need of replication and validation, a recent pilot study suggests that prolonged exposure psychotherapy is associated with decreased methylation of the *FK506 binding protein 5* (*FKBP5*) gene and PTSD remission (Yehuda *et al*, 2013). Similarly, decreased *BDNF* methylation has been observed in the blood of BPD subjects responding to intensive behavior therapy (Perroud *et al*, 2013). These results highlight the potential of epigenetic modifications to serve as biomarkers for therapeutic success. Furthermore, the administration of HDAC inhibitors (HDCAi) has been discussed in order to assist psychotherapeutic approaches in anxiety and fear-related disorders such as phobias or PTSD (Whittle and Singewald, 2014). Enhanced histone acetylation is involved in long-term memory of fear extinction (Bredy *et al*, 2007), which is important in exposure therapies targeting anxiety and fear-related disorders. Impaired extinction of learned fear is suggested to be impaired in PTSD-patients (Blechert *et al.*, 2007; Milad *et al.*, 2009). Rather than erasing learned stimulus-fear associations, extinction of fear responses probably occurs through inhibition via brain regions such as the hippocampus or the prefrontal cortex. Thus, it represents a form of learning and relies on changes in gene expression in the respective brain regions. Although human data showing HDCA inhibitors to enhance fear extinction induced by exposure based therapy are limited to healthy individuals (Kuriyama *et al*, 2011, 2013), similar results were obtained in an animal model of PTSD. HDAC inhibitor application enhanced fear extinction. This was accompanied by enhanced levels of hippocampal histone acetylation and expression in memory related genes such as the *NMDA* receptor and *calcium/calmodulin kinase II* (*CaMKII*) (Matsumoto *et al*, 2013).

**Relevance of peripheral Epigenetics**

Although some studies investigated DNA methylation in post-mortem brain tissue of suicide completers, only very few included early life adversities (Klengel *et al.*, 2014). Differential methylation of the *GR* gene and the *rRna* genes was found in the hippocampus of persons with a history of childhood abuse (Labonté *et al.*, 2012b; McGowan *et al.*, 2009; McGowan *et al.*, 2008; Suderman *et al.*, 2012). Furthermore, genome-wide analyses revealed differential methylation as a result of childhood abuse in several hundred promoters in the hippocampus (Labonté *et al.*, 2013; Labonté *et al.*, 2012a). However, due to inherent limitations in obtaining brain tissue, human epigenetic research has mainly utilized peripheral tissue such as blood and saliva, and has repeatedly identified associations between early life adversities and epigenetic modifications (Klengel *et al.*, 2015). Whether findings in peripheral tissues represent purely biomarkers or correlate with the epigenetic constitution of the brain needs to be further investigated. However, as *GR* genes are expressed in almost every cell, glucocorticoids released due to ELS could simultaneously act on the same genes in different tissues (Klengel *et al*, 2014; Szyf, 2012, 2013). In this fashion ELS could evoke system-wide epigenetic patterns that are correlated across tissues. In support of this theory, treatment of hippocampal progenitor cells with glucocorticoids led to demethylation in the same region of the *FKBP5* gene that was observed to be less methylated in the blood adults with a history of childhood trauma (Klengel *et al*, 2013). Correspondingly, differential DNA methylation by means of differential maternal care could not only be detected in both blood and the prefrontal cortex but also overlapped in some CpG sites in rhesus macaques (Provençal *et al*, 2012). Davies *et al* reported inter-individual differences in DNA methylation that were reflected in both blood and brain tissue, however these were exceeded by intra-individual differences between these tissues (Davies *et al*, 2012). Additionally, associations between peripheral DNA methylation and memory functions (Vukojevic *et al.*, 2014), as well as *in vivo* measures of brain serotonin synthesis (Wang *et al*, 2012) suggest that peripheral epigenetic measures provide useful information about brain function and are thus relevant in epidemiological research. A causal involvement of peripheral epigenetic modifications in psychopathological conditions might be inferred from to the crosstalk between the immune system and the HPA-axis (Miller *et al*, 2005). An increase in proinflammatory cytokines for example is discussed in the etiology of depression or PTSD (Gola *et al*, 2013; Köhler *et al*, 2014).

## I.5. Scope of this thesis

This thesis aims to investigate epigenetic modifications of HPA-axis genes established in the aftermath of early adversities, including both prenatal stress and stress during childhood, and how these relate to psychological wellbeing. A long-lasting effect on DNA methylation in peripheral tissues has been reported for various forms of early adversities such as low socio-economic-status (Borghol *et al.*, 2012), parental stressors (Essex *et al.*, 2013), bullying victimization (Ouellet-Morin *et al.*, 2013) or childhood maltreatment (McGowan *et al.*, 2009; McGowan *et al.*, 2008; Tyrka *et al.*, 2012). Although epigenetic research concerning early adversities has to date, focused mainly on postnatal stressors, epigenetic modifications following prenatal stress are becoming increasingly investigated, too. Chapter II reviews the current literature on this topic. The great majority of these studies utilized cord blood samples and can thus demonstrate epigenetic modifications at birth. Additionally, studies on prenatal food shortage performed in adults up to 6 decades after the event of undernutrition evidence long-lasting epigenetic modifications in response to prenatal stress. Only within the last year, persistent epigenetic modifications due to prenatal exposure to psychological stress have been reported (Cao-Lei *et al.*, 2015). Chapter III investigates long-lasting epigenetic modifications after intrauterine exposure to maternal psychological stress.

In addition to early adversities, differential DNA methylation reflects several psychiatric diagnoses or emotional/behavioral disturbances including for example suicide attempt (Kang *et al.*, 2013; Murphy *et al.*, 2013), depression (Fuchikami *et al.*, 2011; Kim *et al.*, 2013; Uddin *et al.*, 2011) and depressive symptoms (Zhao *et al.*, 2013) and PTSD (Klengel *et al.*, 2014; Rusiecki *et al.*, 2013; Rusiecki *et al.*, 2012). As outlined in more detail in the following chapters, differential methylation of the *GR* gene is for example associated with both early adversities and impaired psychological wellbeing (for reviews see Palma-Gudiel *et al.*, 2015; Turecki *et al.*, 2016). These parallel findings suggest a key role of epigenetic modifications in mediating the negative consequences of early life stress on psychological wellbeing. However, apart from rare exceptions (see for example Klengel *et al.*, 2013), these mutual relationships have not properly been tested, as previous research usually focused exclusively on associations between epigenetic modifications and either early adversities or impaired psychological wellbeing (for a review see Klengel *et al.*, 2014). Chapter IV investigates how the interplay between early adversities and DNA methylation affects psychological

wellbeing. So far, research investigating associations between epigenetic modifications and mental health has been limited to a single measurement of mental health. However, the specific psychopathology an individual might develop in the aftermath of early adversities is highly variable and likely depends on various factors such as timing, type and severity of exposure, genetic background and the presence of moderating factors, such as the degree of parental support or involvement (Teicher *et al.*, 2014a). Therefore, in chapter IV impaired psychological wellbeing refers to an increase of symptoms related to several psychopathological conditions (e.g. symptoms associated with depression or conduct disorder) and behavioral/emotional problems.

Despite the well documented adverse consequences of childhood abuse (Heim *et al.*, 2001), it has been argued that specific forms of childhood abuse are less harmful if taking place in societies or cultural groups in which such practices are common, socially accepted and legal (Lansford *et al.*, 2005). Chapter V investigates the consequences of childhood abuse on both psychological wellbeing and DNA methylation in a country — Tanzania — in which abusive acts towards children are commonly employed and considered as efficient disciplinary strategy (Tanzania Daily News, 2013).

Specifically, this thesis addresses the following hypotheses:

1) Both prenatal and postnatal stressors lead to long-lasting epigenetic modifications in HPA-axis genes. (Chapter III – V)
2) Interactions between the epigenome and the environment determine an individual's risk to develop mental illness. More specifically, the simultaneous occurrence of methylation at the *GR* gene and childhood maltreatment constitutes a risk factor for developing psychopathology. (Chapter IV)
3) These stress-induced epigenetic modifications are also apparent in societies or social groups, in which specific forms of child abuse are common practice and socially accepted. (Chapter V)

# II Epigenetic biomarkers of maternal stress

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## II.1. Abstract

Diverse maternal experiences or mood disturbances before birth pose a substantial risk for poor lifetime mental health outcomes. DNA methylation variation in response to prenatal stress has been shown in animal model studies. Although prenatal time represents a sensitive period of development, little is known about the impact of maternal stress during pregnancy on DNA methylation during the life span in humans. In this review we provide a brief summary of key human studies that bring evidence of DNA methylation in association with prenatal stress. We discuss common findings in the studies such as the type of maternal stress associated to offspring's DNA methylation and plasticity/stability of epigenetic variations. We also suggest the contribution of additional candidate gene approaches and genome-wide DNA methylation profile, in order to further explore and define the relationship between early social environment, epigenetics and long term outcomes. The implications of

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maternal care on DNA methylation as well as the importance of maternal well being during pregnancy to prevent future health problems are considered.

## II.2. Prenatal stress and its consequences for health

Adversities affecting children during early development, even prenatally, pose major risk factors for psychopathological development, ranging from depression, anxiety and substance use disorders to axes II diagnoses (personality disorders). Compared to non-maltreated individuals, psychiatric patients with a history of childhood maltreatment are characterized by earlier disease onset, greater symptom severity, more comorbidities and poorer responses to treatments. Adversities during prenatal life have reliably proven to elicit long-term effects. Epigenetic modifications in stress-response systems, such as the hypothalamic-pituitary-adrenal (HPA) axis, may be a driving force producing these maltreatment-induced disorders. There appears to be limited specificity in terms of disease etiology, as various types of prenatal adversities have been associated with various types of outcomes. Factors include social exclusion, maternal mood disturbances such as those related to anxiety or depression, bereavement (Khashan *et al.*, 2008) or the experience of natural- (Laplante *et al.*, 2008) or men-made disasters (Kleinhaus *et al.*, 2013; Yehuda *et al.*, 2005), daily hassles (Grizenko *et al.*, 2012; Rice *et al.*, 2010), smoking (Cornelius *et al.*, 2012; Wakschlag *et al.*, 2010) or malnutrition (Painter *et al.*, 2006).

While most studies assess infant or child outcomes, manifestations of prenatal stress in adulthood also occur in adulthood, highlighting the long-term impact of prenatal stress (Betts *et al.*, 2015; Brown *et al.*, 1995; Buchmann *et al.*, 2014; Entringer *et al.*, 2009b; Entringer *et al.*, 2008; Neugebauer *et al.*, 1999; Painter *et al.*, 2006). The diverse nature of stressor types is mirrored by a wide range of different health outcomes. Several studies report an association between prenatal stress and later behavioral or emotional problems, such as asocial behavior (Zohsel *et al.*, 2014), hyperactivity disorder (Grizenko *et al.*, 2012) aggression (Buchmann *et al.*, 2014) or internalizing and externalizing behavior (Betts *et al.*, 2015). Further consequences, the findings of which have been well replicated, include impaired cognitive function (Buitelaar *et al.*, 2003; Entringer *et al.*, 2009a; Field *et al.*, 2002; Huizink *et al.*, 2003; King *et al.*, 2005; Laplante *et al.*, 2008; Mennes *et al.*, 2006; Niederhofer *et al.*, 2004; Van den Bergh *et al.*, 2005) and severe psychopathological states such as schizophrenia (Khashan *et al.*, 2008; Susser *et al.*, 1992; Susser *et al.*, 1996; van Os *et al.*, 1998) or

autism (Beversdorf *et al.*, 2005; Kinney *et al.*, 2008). Finally, prenatal stress is associated with diverse findings such as sleep problems (Field *et al.*, 2002; O'Connor *et al.*, 2007), obesity (Ravelli *et al.*, 1998; Ravelli *et al.*, 1999), dermatoglyphic asymmetry (King *et al.*, 2009) mixed handedness (Glover *et al.*, 2004) or immune function (Veru *et al.*, 2014; Wright *et al.*, 2010).

Stress acts on several pathways such as the neuroendocrine and immune systems, which are in turn highly interwoven (Chrousos *et al.*, 1992). Stressful early life events such as childhood physical and sexual abuse have been associated to long lasting alteration of hypothalamic–pituitary–adrenal (HPA)-axis activity (e.g. Carpenter *et al.*, 2007; Heim *et al.*, 2000; Schalinski *et al.*, 2015). Furthermore trauma related disorders such as PTSD present with both a marked reduction of regulatory T cells (Sommershof *et al.*, 2009) as well as altered brain function (Elbert *et al.*, 2011). Moreover dysregulation of these pathways is associated with various diseases and behavioral problems (de Kloet *et al.*, 2005). Accordingly, rather than focusing on a specific disease, many studies focused on investigating the physiological or neurological consequences of prenatal stress that place an individual at higher risk for disease development. Many studies report altered HPA-axis function in response to prenatal stress, which can be tilted the one way (elevated Diego *et al.*, 2004; Huizink *et al.*, 2008; Van den Bergh *et al.*, 2008) or the other (flattening of the rhythym, Buchmann *et al.*, 2014; O'Donnell *et al.*, 2013; Yehuda *et al.*, 2005). Also brain development seems to be affected, as indicated by increased right frontal asymmetry (Diego *et al.*, 2004), reduced gray matter density (Buss *et al.*, 2010) or differential white matter microstructural organization (Sarkar *et al.*, 2014). Finally, physiological conditions increasing the vulnerability for metabolic or cardiovascular diseases, such as insulin resistance, decreased glucose tolerance (Ravelli *et al.*, 1998) or increased blood pressure (Painter *et al.*, 2006) have been reported after prenatal stress exposure.

This wide range of health outcomes highlights the necessity of having a multidisciplinary, etiology-based approach in prenatal stress research, incorporating findings from fields such as mental health, immunology and physiology in order to more accurately gauge the averse effects of prenatal stress (O'Connor *et al.*, 2014). Understanding how maternal experience in the social environment can be embedded in offspring's biology is of fundamental importance for creating effective interventions.

## II.3. Adaptive value of prenatal stress?

Via the placenta, which directly connects the *in utero* environment to the maternal physiology, the fetus receives signals about the maternal environment. Fetal programming denotes the concept that such signals can permanently alter the development of the fetus and the child. Although research has mainly focused on the adverse aspects in terms of increased disease susceptibility, this process is thought to be adaptive as it enables the fetus to acclimate to both its current environment and the one into which it will be born. Maternal constraint, i.e. mechanisms avoiding the outgrowth of the fetus from the pelvic channel, exemplifies a classic example of the adaptive values gained by maternal signals (Gluckman *et al.*, 2004b). In the context of nutrition, the developmental (or fetal) origins of adult disease have been proposed. This theory states that poor nutrition during fetal development places the individual at higher risk for the development of cardiovascular diseases and metabolic disorders in adulthood (Barker, 2004). Rather than exclusively attributing pathological consequences to physiological alterations induced by prenatal stress, the predictive adaptive response model assumes that these may be beneficial if the organism continues to be exposed to stressful conditions in later life (Gluckman *et al.*, 2004a; Gluckman *et al.*, 2005). However, if the *in utero* environment fails at predicting the future environment, the organism is at heightened risk for developing unfavorable health conditions. Compelling evidence for this model was gathered when comparing the offspring of women pregnant during either one of two civilian famines of world war two, the Dutch Hunger Winter (1944-45) and the Siege of Leningrad (1941-44). In the former case, food was soon available again while the population remained starving in the latter case. Accordingly, in the Dutch cohort, those children were more susceptible to a range of metabolic diseases including insulin resistance or obesity while the latter cohort seemed to be unaffected (Stanner *et al.*, 1997). These contradictory effects were hypothesized to result from fetal adaptations to a poorly nourished environment such as insulin resistance ensuring energy allocation towards the brain. However, in a situation of plenty such conditions rather increase the vulnerability to metabolic diseases such as diabetes or obesity in individuals in a situation of plenty (Hales *et al.*, 2001). While the aforementioned concepts and studies focus on maternal prenatal food availability and cardiovascular and metabolic consequences, the effects of prenatal stress on neurodevelopment are also likely to reflect a predictive role in assisting the individual to survive in a dangerous

environment. From an evolutionary perspective, it has been debated, for example, that increased levels of aggression, a feature found to be associated with prenatal stress (Buchmann *et al.*, 2014), may be adaptive in dangerous environments. However, in our modern society, we are usually not confronted with the types of stressors that demand an increased willingness for the display of aggressive behavior (for a review see Glover, 2011). Accordingly, adverse effects of incongruence between the pre- and postnatal environment have been found in a model of maternal depression. Infants of mothers experiencing inconsistent levels of depressive symptoms show signs of impaired motor and mental development, even though the incongruence was due to improving maternal mental health following delivery (Sandman *et al.*, 2012).

## II.4. Transmission of prenatal stress to the fetus

A crucial question in understanding the contribution of the maternal wellbeing during gestation in determining the children's health concerns the transmission of maternal stress to the fetus, i.e. the biological correlate of maternal stress. The maternal HPA-axis constitutes a likely candidate. Glucocorticoids are critical in normal development of the fetus as they are involved in the growth and maturation of many organ systems. However exposure of the fetus to increased levels of cortisol, resulting for example from maternal stress, can lead to long-term "programming" of HPA function and behavior (Moisiadis *et al.*, 2014). Indeed elevated cortisol concentration in amniotic fluid were associated with blunted HPA-axis responses to acute stress and higher basal cortisol (O'Connor *et al.*, 2013). Furthermore, it predicted decreased cognitive abilities (Bergman *et al.*, 2010). However, due to the dynamics of placental CRH expression, the maternal HPA-axis functions differently during gestation rendering maternal cortisol exclusively mediating the effects of prenatal stress unlikely. In contrast to the cortisol-mediated feedback inhibition of the HPA-axis, cortisol triggers placental CRH expression culminating in exponentially increasing maternal CRH and concomitantly cortisol-levels over the course of pregnancy. Especially during later stages of pregnancy, the maternal HPA-axis becomes less responsive and the association between gestational stress and maternal cortisol levels is highly debated (for a review see O'Donnell *et al.*, 2009). Moreover, the placental enzyme 11β-dehydrogenase 2 (11β-HSD2) prevents the fetus from excess exposure to maternal cortisol by converting it into its inactive form corticosterone. Consequently, *in utero* cortisol levels can only partially reflect the

mother's cortisol levels and are substantially lower (Gitau *et al.*, 1998; Seckl, 2004). Lower placental 11β-HSD2-expression causing increased *in utero* cortisol concentrations has also been suggested to be involved into the transmission of prenatal stress. Indeed, placental 11β-HSD2 expression has been shown to be down regulated by means of prenatal maternal stress (O'Donnell *et al.*, 2012), which seems to be epigenetically regulated (Conradt *et al.*, 2013; Marsit *et al.*, 2012). A simplified model of the HPA and Placental Stress System during pregnancy is illustrated in Fig. II-1.

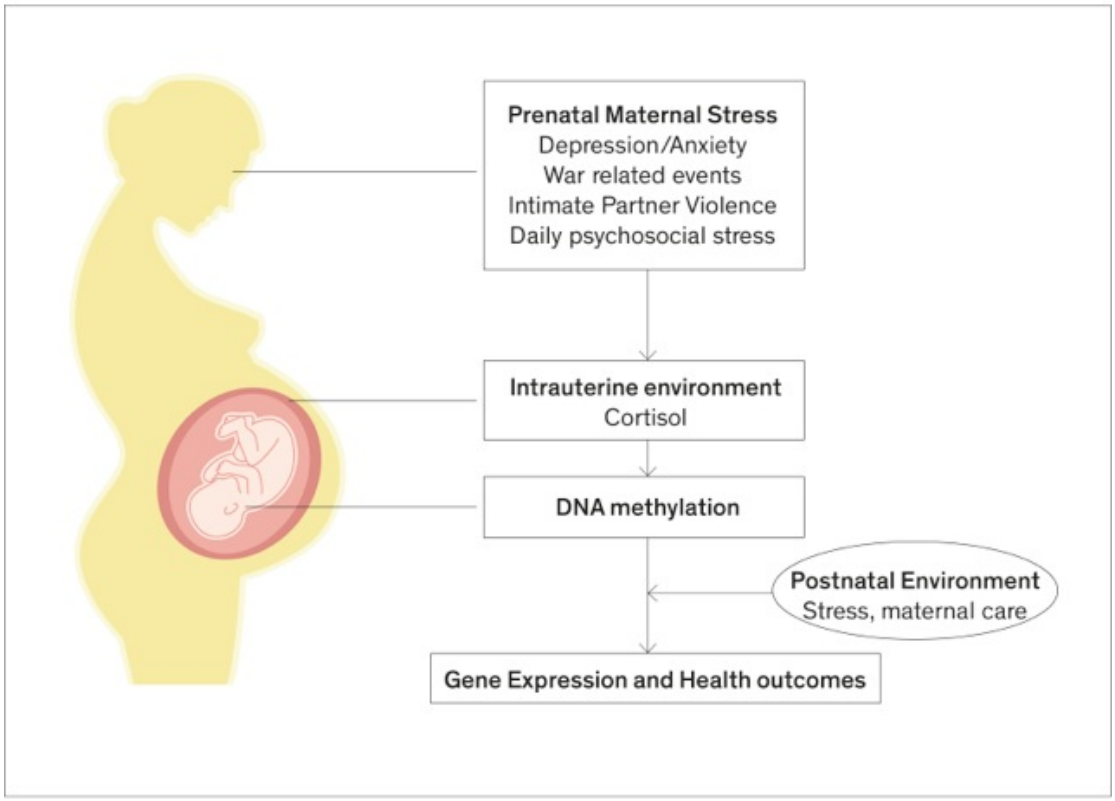


**Fig. II-1: Prenatal Stress**

Mood disorders (Oberlander *et al.*, 2008), intimate partner violence (Radtke *et al.*, 2011), war related events and daily psychosocial stress (Mulligan *et al.*, 2012), have been reported to affect DNA methylation and thus gene expression and lifetime health. Furthermore aspects of the postnatal environment such as stress and maternal care are known to induce changes in DNA methylation (Champagne *et al.*, 2008; Provençal *et al.*, 2012; Tung *et al.*, 2012; Weaver *et al.*, 2004).

The impact of maternal stress during pregnancy on the fetus involves complex pathways. In addition to the maternal HPA-axis, other mechanisms are also considered to meditate the effects of gestational maternal stress. However, whether these can account for later health outcomes remains to be determined. For instance, reduced uterine blood flow potentially restricting fetal oxygen supply has been considered a possible correlate of prenatal stress (Teixeira *et al.*, 1999). Via blood vessel

constriction, maternal catecholamines released following distress may account for this phenomenon (Resnik *et al.*, 1979). However, recent studies report only low or missing associations between prenatal maternal stress and uterine blood flow (Mendelson *et al.*, 2011; Monk *et al.*, 2012), thus questioning the latter as a correlate for maternal stress. Levels of inflammatory cytokines, which have been shown to be elevated in gestational stressed women (Blackmore *et al.*, 2011; Coussons-Read *et al.*, 2007), constitute another alternative. Indirect evidence for the implications of those in the offspring's development might arise from findings showing that maternal influenza in pregnancy is associated with schizophrenia (Brown *et al.*, 2000).

## II.5. Epigenetic modifications – molecular mirror of the prenatal environment

Epigenetic mechanisms have been suggested to underlie the interplay between the environment and gene expression. Epigenetics involves a broad range of phenomena (dosage compensation, genomic imprinting) and mechanisms (chromatin organization and histone modifications) whereby DNA methylation is to date the most extensively investigated mechanism and the focus of this review. Although DNA methylation was thought to be restricted to early embryonic development, it has been shown to be plastic throughout life. Exposure to nutritional changes (Vucetic *et al.*, 2010), chemicals (Onishchenko *et al.*, 2008) and a broad range of environmental stressors (Roth *et al.*, 2011b; Weaver *et al.*, 2004) occurring during pre- and postnatal development have suggested that epigenetic regulation of gene expression is a critical target of experience-dependent changes. Epigenetic reprogramming, when occurring in primordial cells and during pre-implantation development, leads to almost complete genome-wide DNA demethylation (Hackett *et al.*, 2012). Nevertheless, evidence suggests that some epigenetic marks seem to persist across generations (Gapp *et al.*, 2014). Furthermore, in line with the fetal programming concept, it has been debated that epigenetic modifications during the intra uterine period responding to adversities could serve as a possible genome adaptation mechanism, adapting the genome function to changes in the early environment, occurring in multiple tissues and across many regions in the genome. A model illustration of prenatal maternal stress, DNA methylation and gene expression association is summarized in Fig II-2.

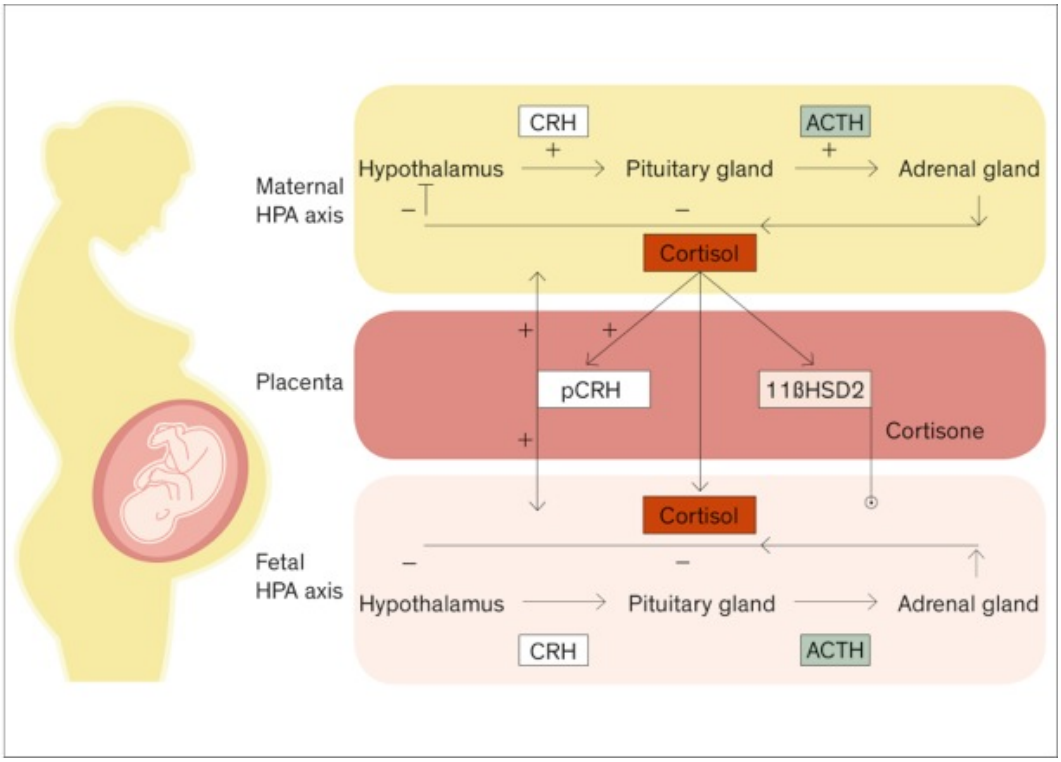


**Fig. II-2: Transmission of prenatal Stress**

During pregnancy, CRH is released from the placenta into both the maternal and fetal compartments. In contrast to the negative feedback regulation of hypothalamic CRH, cortisol increases the production of CRH from the placenta. Placental CRH (pCRH) concentrations rise exponentially over the course of gestation. In addition to its effects on pCRH, maternal cortisol passes through the placenta. However, the effects of maternal cortisol on the fetus are modulated by the presence of p11ß-HSD2 which oxidizes it into an inactive form, cortisone (modified version from Sandman *et al.*, 2011).

## II.6. Candidate gene approach

In candidate studies, the investigated genes are selected based on prior knowledge concerning their biological function or involvement in certain traits. The candidate approach allows for gaining further insights on the molecular and biological mechanisms that underlie the association between DNA methylation and the environmental stressors. For instance, it can provide information on whether a specific set of candidate genes is differentially methylated by means of early life stress or on the effects of epigenetic modification on downstream processes such as gene expression.

A particular endocrine pathway affected by early adverse experiences is the "élite defence force" against stressors, i.e., the HPA-axis. This pathway involves hormones secreted by the hypothalamus, pituitary and adrenal glands as well as their receptors and plays a major role in stress responses. When functioning properly, the HPA-axis helps in responding to a crisis. When unremitting stress forces the HPA-axis to tilt one way, the result can be anything from a long-lasting head cold to depression. When

tilted the other way towards a flattening of the rhythm of stress hormones, the undesirable consequences may be abdominal fat, loss of muscle mass and mental ailing (McEwen *et al.*, 2002).

The first line of evidence in an association between early-life experience and changes in DNA methylation came from a candidate gene study focusing on the glucocorticoid receptor (GR) in rats. The GR is critically involved in the negative feedback regulation of the HPA axis. Weaver *et al.* (2004) showed that variation in maternal care during the first week of the rats' life regulates the level of methylation of the GR gene. In mice, the GR gene was also shown to be differentially methylated in response to prenatal stress (Champagne *et al.*, 2011).

The impact of prenatal stress on the methylation of the glucocorticoid receptor gene has also been investigated in humans. DNA methylation has been associated with different types of prenatal adversities, such as maternal mood disorders and traumatic events (Turecki *et al.*, 2016) but also the absence of an effect has been reported (van der Knaap *et al.*, 2014). In a prospective study, maternal suffering from depression symptoms was reported to be associated with both DNA methylation of the *GR* gene in neonatal cord blood and infant cortisol stress responses at three months (Oberlander *et al.*, 2008). Conradt *et al.* (2013) extended this work using placenta tissue and found maternal depression during pregnancy associated with increased methylation of both the *GR* and the *11β-HSD2* gene suggestive of elevated intrauterine cortisol levels and thus a potential mechanism by which maternal stress is transmitted to the fetus. Accordingly, Hompes *et al.* (2013) reported an additive effect of gestational maternal cortisol levels and emotional state on *GR* methylation in cord blood. Recently Murgatroyd *et al.* (2015) reported an interactive effect of prenatal and postnatal maternal depression. Methylation of the *GR* gene in infants was elevated in the presence of increased maternal postnatal depression following low prenatal depression. More interesting those effects were reported to be reversed by maternal stroking of the infants over the first weeks of life (Murgatroyd *et al.*, 2015). Furthermore, an association between war related events during pregnancy and *GR* methylation in newborns has been shown (Mulligan *et al.*, 2012). While the studies cited above investigated differential methylation at birth, these changes seem to persist into adulthood. In a retrospective study we found that maternal exposure to Intimate Partner Violence during pregnancy is associated with increased methylation of the *GR* gene in children aged 10-19 year old in peripheral blood samples (Radtke *et al.*, 2011).

The methylation status of several other candidate genes have been investigated in association to adversities experienced during the prenatal period. For example in infants' cord blood, maternal depression during pregnancy was correlated with DNA methylation at the serotonin transporter gene *SLC6A4* but not *BDNF* (Devlin *et al.*, 2010) as well as at the differentially methylated region (DMR) controlling the imprinted *IGF2*- and *H19*-genes *(*Chen *et al., 2014)*. Another study investigating imprinted genes reported increased methylation at the *MEG3* DMR by means of gestational maternal depression or anxiety but not at the *IGF2* DMR (Liu *et al.*, 2012). However, methylation of the *IGF2* DMR in this study was associated with low birth weight indicative of prenatal stress. Prenatal exposure to the Dutch Hunger Winter was associated with adult *IGF2* DMR methylation (Heijmans *et al.*, 2008) and in the following genes *GNASAS*, *MEG3*, *INSIGF*, *IL10, ABCA1* and *LEP* (Tobi *et al.*, 2009). Perceived stress during pregnancy (Vidal *et al.*, 2014) was associated with higher infant DNA methylation at *MEST*, a gene relevant to abnormal maternal behavior and obesity in studies with mice.

While the majority of human epigenetic research investigating the effects of prenatal stress on DNA methylation focused on molecular findings, a few also incorporated phenotypic measures further implicating DNA methylation in mediating the association with prenatal stress. Differential DNA methylation evoked by prenatal stress was associated with low birth weight (Liu *et al.*, 2012; Mulligan *et al.*, 2012; Murphy *et al.*, 2012), altered HPA-axis function (Oberlander *et al.*, 2008; Yehuda *et al.*, 2014), decreased GR expression (Yehuda *et al.*, 2014) and impaired neonatal behavior and lethargy (Conradt *et al.*, 2013). It has been suggested that the impact of environmental factors on the type and extent of epigenetic variations is dependent on the developmental stage of occurrence (Provencal *et al.*, 2015), and particularly early pregnancy (Heijmans *et al.*, 2008; Hompes *et al.*, 2013; Tobi *et al.*, 2014; Tobi *et al.*, 2009) seems to play a critical role, although the third trimester has also been implicated (Oberlander *et al.*, 2008). Interestingly, different types of adversities have been reported in association with offspring's DNA methylation: not only maternal exposure to severe traumatic events (e.g. war related events) or mood disorders (e.g. depression/anxiety symptoms) but also more common prenatal stressful events, such as daily psychosocial stress (e.g. Mulligan *et al.*, 2012; Vidal *et al.*, 2014).

## II.7. Epigenome-wide association studies

With recent technological advancements it is now possible to identify epigenetic variants across the genome and test their association with complex traits (Tsai *et al.*, 2012). Inspired by the forward genetic approach of genome-wide association studies (GWAS), epigenome-wide association studies (EWAS) query DNA methylation at thousands of genomic locations in order to identify associations with a trait of interest. Unlike candidate gene approaches a genome-scale analysis of differential DNA methylation enables the unbiased detection of new regulatory mechanisms that are susceptible to environmental changes. Many of these associated regions are unsuspected *a priori*. For example, DNA methylation in novel genes involved in diverse developmental processes that had not been previously implicated in responses to tobacco smoke was reported in association with tobacco smoke during pregnancy (Joubert *et al.*, 2012). Non et al. (2014) performed an EWAS in umbilical cord blood of neonates exposed to maternal depression and anxiety during pregnancy and described a gene (*COL7A1)* linked with a skin condition (dystropich epidermolysis bullosa) that had not yet been associated with depression or other psychological experiences. Interestingly biomarkers for prenatal adversity in genome-wide DNA methylation profiles have been reported also in adolescence and adulthood (Cao-Lei *et al.*, 2014; Tobi *et al.*, 2014). For instance, Cao-Lei *et al.* (2014) reported the patterns of DNA methylation in children whose mothers were pregnant when exposed to the Quebec ice storm in 1998 and found an association to pathways involved in the immune system thirteen years later. DNA methylation changes in *SCG5* and *LTA*, both highly correlated with maternal objective stress, were comparable in T cells, peripheral blood mononuclear cells (PBMCs) and saliva cells. Tobi *et al.* (2014) performed a genome-scale analysis in whole blood of individuals who were exposed to famine during early gestation at the Dutch Hunger Winter and identified key pathways related to growth and metabolism associated with malnutrition more than six decades later. Interestingly, as reported by the candidate gene studies, differentially DNA methylation was observed in different tissues (e.g. saliva, blood, T cells). The results support the "system wide" response hypotheses, based on evidence that phenotypic response to early-life adversity involves multiple phenotypes and thereby involves multiple systems (Szyf 2012). Concordantly, a recent study compared differential methylation by means of maternal lifetime depression in two cohorts using cord blood T lymphocytes and adult hippocampi. This study reported an overlap of 33 genes with

changes in DNA methylation in the two tissues supporting the idea of lifelong epigenetic effects in various tissues (Nemoda *et al.*, 2015). Table II-1 summarizes the list of candidate gene and EWAS studies with humans reported in this review.

**Table I-1: Prenatal stress and DNA methylation in Humans**

| Gene | Study design | maternal stressor | Age at tissue collection | Tissue | Authors |
|---|---|---|---|---|---|
| *GR* | Candidate gene | Depression | Newborn | Cord blood | Oberlander *et al.* (2008) |
| *IGF2* | Candidate gene | War-time famine | 6 decades after famine prenatally exposure | Whole blood | Heijmans *et al.* (2008) |
| *GNASAS*, *MEG3*, *INSIGF*, *IL10* and *LEP* | Candidate gene | War-time famine | 51 – 62 years old | Whole blood | Tobi *et al.* (2009) |
| *SLC6A4* | Candidate gene | Depression | Newborn | Cord blood (leukocytes) | Devlin *et al.* (2010) |
| *GR* | Candidate gene | Intimate Partner Violence | 10 - 19 years old | Whole blood | Radtke *et al.* (2011) |
| *GR* | Candidate gene | War related events | Newborn | Cord blood | Mulligan *et al.* (2012) |
| *MEG3* | Candidate gene | Depression | Newborn | Cord blood | Liu *et al.* (2012) |
| *GR* and *11ß-HSD2* | Candidate gene | Depression, anxiety | Newborn | Placenta | Conradt *et al.* (2013) |
| *GR* | Candidate gene | Emotional well-being, cortisol | Newborn | Cord blood | Hompes *et al.* (2013) |
| *IGF2*, *H19* | Candidate gene | Depression, anxiety, perceived stress | Newborn | Placenta, umbilical cord blood | Chen *et al.* (2014) |
| *MEST* | Candidate gene | Perceived stress | Newborn | Cord blood | Vidal *et al.* (2014) |
| Genes associated to regulation of transcription, translation and cell division | EWAS | Depression, anxiety | Newborn | Cord blood | Non *et al.* (2014) |
| Biological pathways prominently featured in immune function | EWAS | Ice storm | 13 years old | T cells | Cao-Lei *et al.* (2014) |
| Biological pathways related to growth and metabolism associated | EWAS | War-time famine | 51 – 62 years old | Whole blood | Tobi *et al.* (2014) |
| Genes involved in immune system functions | EWAS | Depression | Newborn | Cord blood (T lymphocytes) | Nemoda *et al.* (2015) |
| *GR* | Candidate gene | Depression and postnatal maternal depression and stroking | 5 weeks – 14 months | Saliva | Murgatroyd *et al.* (2015) |

## II.8. Maternal rearing as predictor of variation in DNA methylation

Timescale for maternal effects on epigenetic variations is suggested to not be restricted to the *intrauterine* environment. Although the prenatal period represents a sensitive period of development (Pirini *et al.*, 2015), association of parenting and variations in DNA methylation is suggested to be extended to the entire lifespan, including before conception, such as nutritional and smoking influence on the gametes (for a review see Lane *et al.*, 2014) and postnatal period (Champagne, 2008). Indeed parental care is a strong predictor of postnatal variation in DNA methylation (for a review see Champagne 2008). It is known that maternal behavior can influence critical aspects of development such as offspring's neurobiology and behavior (Harlow *et al.*, 1974; Sato *et al.*, 1998; Taborelli *et al.*, 2013). Evidence from experiments with animal models manipulating maternal care received by offspring have been reinforcing the plasticity idea of epigenetic states in response to variation in the social environment (Provençal *et al.*, 2012; Tung *et al.*, 2012; Weaver *et al.*, 2004). For instance, as mentioned before in the experiment of *Weaver et al.* (2004), maternal care caused differential DNA methylation of the *GR* promoter region and correspondingly altered hippocampal GR expression and behavior. A cross-fostering design excluding genetic artifacts confirms that these effects were mediated by the quality of the postnatal environmental, i.e. maternal care. This provides a dynamic mechanism for maintaining long-term changes in the gene expression and behavior of offspring (Champagne 2008). Consistent with Weaver *et al.* (2004), rearing was associated with differential DNA methylation in an experimental investigation of early maternal deprivation on adult rhesus monkeys (Provençal *et al.* 2012). Interestingly the differences in DNA methylation were observed in adulthood, supporting the hypothesis that the response to early-life adversity can persist into adulthood. Epigenetic changes during adulthood were also reported in female monkeys when exposed to experimentally controlled changes in social status (i.e. dominance rank) during adulthood (Tung *et al.* 2012). Moreover, when the monkeys moved to a new rank, their gene expression profiles changed as well, suggesting epigenetic flexibility in responses to the changes in the social environment (Tung et al. 2012).

In humans, the disruption or lack of adequate nurturing and inadequate parental care was associated with increased *GR* promoter methylation (Tyrka *et al.*, 2012). In addition, epigenetic changes in the proopiomelanocortin (POMC) gene may

promote HPA axis dysfunction (Ehrlich *et al.*, 2010). In a very recent study we found that inadequate parental care and maltreatment was associated with children's differential methylation in the *POMC* gene measured in saliva and blood (Hecker *et al.*, 2016). In a sample of former indentured child laborers who suffered severe childhood adversities functional annotation clustering suggested several gene clusters were differentially methylated (Marinova et al. 2015). The genes with hypermethylated CpGs were related to cellular morphogenesis, neuronal and cell development and differentiation. Genes with hypomethylated CpGs were enriched in clusters related to impaired connectivity of neurons. The study indicated that distinct differences in DNA methylation associated with childhood trauma could be detected in late adulthood. These findings emphasize the detrimental consequences of inadequate parental care and child maltreatment.

## II.9. Implications of Epigenetic plasticity

The notion of epigenetic plasticity and thus the reversibility of epigenetically determined health conditions might open new avenues for therapy by either pharmacological agents or social interventions and the evaluation thereof (Szyf, 2011). Specific drugs such as histone deacetylase inhibitor trichostatin A and methionine have been shown to reverse early experience associated changes in DNA methylation and behavioral changes in adulthood (Weaver *et al.*, 2006). Furthermore, a recent study suggested that the epigenetic state might be changed after psychotherapy (Yehuda *et al.*, 2013). Therefore, at least certain trauma-associated epigenetic changes seem to be reversible and positive environment might induce epigenetic variation that would impact the response to stress (Provencal *et al.*, 2015).

These observations point to a seemingly contradictory scenario. On the one hand, prenatal stress can have long lasting impact on DNA methylation. On the other hand, epigenetic modifications are also sensitive to environmental changes throughout the lifespan. Whether the nature of epigenetic changes is dependent on windows of sensitivity or is specific to a genomic region (Daskalakis *et al.*, 2014) and the complex interactions involved in methylation stability, remains to be investigated. Although evidence of prenatal studies has shown an association between fetal exposure and maternal stress, there is also a possibility that this influence is indirect. Maternal stress experienced during the prenatal period compromises maternal care during the postnatal period and thus influences offspring development (Champagne 2008).

As reviewed above, a variety of adverse conditions increase the risk for an impaired development of the children, as reflected at the molecular level. This implies a great burden not only on the affected individual but also on the greater society e.g. due to the costs arising from the complications stemming from mental ill health and the associated mental health care treatments (Soni, 2009). Therefore, preventative interventions improving the well being of pregnant mothers as means to circumvent future health problems in their children is crucial and most likely more economically beneficial than interventions targeting these children after unfavorable health conditions or molecular patterns have already manifested. While physical attributes such as diet or avoidance of toxin exposure have been incorporated and proven to be beneficial in the care of pregnant women (Kirkham *et al.*, 2005), psychosocial risk factors have been largely neglected. Instruments to identify women confronted with psychosocial risk factors are fundamental in filling this void (e.g. Austin *et al.*, 2013; Harrison *et al.*, 2008; Reid *et al.*, 1998; Spyridou *et al.*, 2014, 2015).

Taken together, both candidate gene approach and EWAS can bring valuable contributions to the field. Focus on target genes contributes to the understanding of biological events that mediate connections between early stress and biological function. Furthermore, understanding methylation patterns at the level of specific candidate genes may be beneficial when assessing the effectiveness of clinical treatments. Describing the DNA methylation signatures from a genome-scale analysis associated with a trait is an important step in establishing DNA signatures of prenatal stress, uncovering new regulatory mechanisms and providing a better understanding of complex pathways involved in different biological functions that are susceptible to environmental changes. Hence knowledge of the factors that are involved in the stability and reversibility of DNA methylation status associated to prenatal stress is fundamental to contribute to the development of novel specific therapeutic and preventive interventions.

## II.10. Acknowledgments

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# III Transgenerational impact of intimate partner violence on methylation in the promoter of the glucocorticoid receptor

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## III.1. Abstract

Prenatal exposure to maternal stress can have lifelong implications for psychological function, such as behavioural problems and even the development of mental illness. Previous research suggests that this is due to transgenerational epigenetic programming of genes operating in the hypothalamic-pituitary-adrenal (HPA) axis, such as the glucocorticoid receptor (GR). However, it is not known whether intrauterine exposure to maternal stress affects the epigenetic state of these genes beyond infancy. Here we analyse the methylation status of the GR gene in mothers and their children, at 10 to 19 years after birth. We combine these data with a retrospective evaluation of maternal exposure to intimate partner violence (IPV). Methylation of the mothers' GR gene was not affected by IPV. For the first time, we show that methylation status of the GR gene of adolescent children is influenced by

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their mother's experience of IPV during pregnancy. As these sustained epigenetic modifications are established in utero we consider this to be a plausible mechanism by which prenatal stress may program adult psychosocial function.

## III.2. Introduction

Prenatal stress can have a lasting detrimental impact on psychological health; however, the molecular mechanisms that transmit this experience to adult behaviour are not fully characterized (St Clair *et al.*, 2005; Susser *et al.*, 1992; Susser *et al.*, 1996; Talge *et al.*, 2007; Watson *et al.*, 1999). The hypothalamic-pituitary-adrenal (HPA) axis is critical for homeostasis – it controls growth, reproduction, metabolism and behaviour; it is also the primary line of the "defence cascade" which helps humans to deal with crises. Hyperactivity of the HPA-axis can cause anything from a long-lasting head cold to depression. Hypoactivity of the HPA-axis can cause undesirable consequences such as abdominal fat, loss of muscle mass and mental ill-health (Elbert *et al.*, 2003; McEwen *et al.*, 2002). Tuning of the HPA-axis and its physiological pathways is highly susceptible to the influence of early life events. Prenatal stress such as antenatal exposure to maternal anxiety has sustained effects on HPA-axis function (O'Connor *et al.*, 2005) and is associated with behavioural and emotional problems arising during development (O'Connor *et al.*, 2002; O'Connor *et al.*, 2003).

DNA methylation could be a mechanism by which prenatal stress is translated into changes in gene expression and physiology (Murgatroyd *et al.*, 2009), and ultimately psychologically vulnerable phenotypes (Gregory *et al.*, 2009; Uddin *et al.*, 2010). The glucocorticoid receptor (GR), a major regulator of the HPA-axis (de Kloet *et al.*, 2005), could be involved in such a transmission, controlling many aspects of development, metabolism and immune function. Landmark experiments in rodents show that both the hippocampal expression of this gene and the behavioural responses to stress are modulated by the amount of care mothers invest into their offspring in the first days of postnatal life (Francis *et al.*, 1999; Liu *et al.*, 1997). This is likely to be the result of epigenetic modifications, specifically, through methylation of exon 1F of the GR promoter (Weaver *et al.*, 2004). This exon contains a response element for the nerve growth factor-inducible protein A (NGFI-A) (McCormick *et al.*, 2000) and binding of NGFI-A to its response element increases GR expression.

Methylation of the NGFI-A response element inhibits the association with its 'ligand' thereby decreasing GR expression(Szyf *et al.*, 2005; Weaver *et al.*, 2007). Also the human GR gene is affected by aversive social environments as childhood abuse leads to increased methylation in exon 1F of the GR promoter (McGowan *et al.*, 2009).

The vast majority of studies analysing the influence of stress on the epigenetic regulation of the HPA-axis have focussed on postnatal stressors. However, recent research suggests that the intrauterine environment can also impact the epigenetic state of HPA-axis genes – prenatal exposure to maternal depressive mood was shown to correlate with GR promoter methylation in newborns (Oberlander *et al.*, 2008). Furthermore, increased GR promoter methylation was associated with higher cortisol responses to stress in this study and could therefore represent fetal programming of the HPA-axis. However, this study focused on methylation status in umbilical cord blood. Therefore it is not known, whether epigenetic marks that are established in utero due to the psychosocial situation of the mother are maintained beyond infancy.

Here we investigate whether gestational maternal aversive experiences can have a prolonged effect, 10 to 19 years after birth, on DNA methylation of the offspring based on bisulfite sequencing of DNA from whole blood. Both intrauterine exposure to marital discord and GR promoter methylation can predict psychopathology (McGowan *et al.*, 2009; Stott, 1973; Ward, 1991). We aimed to determine whether prenatal exposure to intimate partner violence (IPV) leads to increased GR promoter methylation later in life, which might mediate increased susceptibility to psychopathology.

## III.3. Materials and Methods

**Participants**

The study cohort represents a convenience sample, as there were no specific criteria that the participants had to fulfil. Mother-child pairs were either recruited via advertisements or taken from another study (Roth *et al.*, 2011a).

**Psychological parameters**

We studied the impact of exposure to intimate partner violence (IPV) in women from a variety of ethnic backgrounds (e.g. Kosovo, Russia, Turkey, etc.), who reside in Germany (Table III-1). IPV was evaluated using the Composite Abuse Scale (CAS) (Hegarty *et al.*, 1999) applied as structured interview by experienced clinical

psychologists. The CAS is a validated (Hegarty *et al.*, 2005) tool, which measures the degree of domestic violence experienced by an individual in the four dimensions of severe combined abuse, physical abuse, emotional abuse and harassment (Hegarty *et al.*, 1999). It consists of 30 items, which are scaled from 0 (never) to 5 (daily). Missing items, due to problems concerning the translation, were replaced with the mean of the existing items (1 of 25 interviews). According to previously described methods (MacMillan *et al.*, 2009; Taft *et al.*, 2009), a resulting 'sumscore' of 7 or higher was used as the criterion for exposure to IPV. In order to match the design of our study, the CAS was conducted three times separately focusing on the period before, during and after the pregnancy with the particular child whose blood was analyzed in this study. For the periods before and after pregnancy, participants were asked to report acts of domestic violence whenever these happened before or after pregnancy with the relevant child. These periods were not limited to an absolute time span.

**Table III-1: Maternal IPV and sociodemographics**

| label | maternal exposure to IPV in relation to pregnancy | | | | childs' gender | childs' age [years] | mode of birth | mothers' age at birth [years] | mothers' country of origin |
|---|---|---|---|---|---|---|---|---|---|
| | before | during | after | total | | | | | |
| 1 | no | no | no | no | male | 19 | spont. | 19 | Rus |
| 2 | no | no | no | no | male | 17 | spont | 31 | Pl |
| 3 | no | no | no | no | female | 13 | emerg. C-sect. | 25 | Tr |
| 4 | no | no | no | no | male | 10 | spont. | 29 | Ger |
| 5 | no | no | yes | yes | female | 16 | spont. | 19 | Irq |
| 6 | no | no | no | no | female | 14 | elect. C-sect. | 28 | Ger |
| 7 | no | no | no | no | male | 13 | spont. | 28 | Rus |
| 8 | no | no | no | no | female | 14 | elect. C-sect. | 27 | Rus |
| 10 | no | yes | yes | yes | female | 15 | other | 29 | Ger |
| 11 | no | no | yes | yes | female | 12 | spont. | 31 | Rc |
| 12 | no | no | yes | yes | male | 17 | emerg. C-sect. | 21 | Ir |
| 13 | no | no | no | no | female | 17 | spont. | 26 | Cs |
| 14 | no | yes | no | yes | female | 13 | spont. | 38 | Rus |
| 17 | n.a | n.a. | yes | yes | female | 12 | emerg. C-sect. | 18 | Irq |
| 18 | no | no | no | no | female | 13 | spont. | 29 | Srb |
| 20 | no | no | no | no | female | 12 | spont. | 26 | Srb |
| 21 | yes | yes | yes | yes | male | 11 | elect. C-sect. | 23 | Srb |
| 22 | no | no | no | no | male | 12 | spont. | 20 | Scg |
| 23 | no | yes | no | yes | female | 10 | spont. | 19 | Irq |
| 24 | no | no | no | no | male | 13 | spont. | 29 | Srb |
| 25 | yes | yes | yes | yes | female | 14 | spont. | 24 | Srb |
| 26 | no | no | yes | yes | female | 17 | spont. | 16 | Srb |
| 27 | n.a. | yes | n.a | yes | female | 14 | spont. | 22 | Tr |

| label | maternal exposure to IPV in relation to pregnancy | | | | childs' gender | childs' age [years] | mode of birth | mothers' age at birth [years] | mothers' country of origin |
|---|---|---|---|---|---|---|---|---|---|
| | before | during | after | total | | | | | |
| 28 | yes | yes | yes | yes | female | 18 | spont. | 22 | Tr |
| 29 | n.a. | yes | n.a. | yes | female | 17 | spont. | 30 | Tr |
| n | 3 (22) | 8 (24) | 9 (23) | 13 (25) | 25 | 25 | 25 | 25 | 25 |
| range | | | | | | 10-19 | | 16-38 | |
| mean ± s.e.m. | | | | | | 14.1±.5 | | 25.2±1. | |

Abbreviations: spont., spontaneous; emerg C.-sect, emergency caesarean section; elect. C-sect, elective caesarean section; Cs., Czechoslovakia; Ger., Germany; IPV, intimate partner violence; Ir., Iran; Irq., Iraq; Pl., Poland; Rus., Russia; Scg., Serbia and Montenegro; Sos., Kosovo; Tw., Taiwan; Tr., Turkey.
'Total' indicates exposure to IPV regardless of timing of exposure. Whenever IPV was experienced before, during or after pregnancy 'total' was computed as 'yes'. In three cases we could not obtain CAS-scores across all periods as indicated by 'n.a.'.

**Sodium bisulfite sequencing**

Sodium bisulfite ($NaHSO_3$)-conversion was performed according to previously described methods(Clark *et al.*, 1994; Frommer *et al.*, 1992) on DNA extracted from whole blood (DNeasy Blood and Tissue Kit, Qiagen). Briefly, 5 µg DNA was denatured by incubating in 0.4 M NaOH (Riedel-deHaën) for 30 min at 42°C. After the addition of hydroquinone (Sigma-Aldich) and $NaHSO_3$ (Sigma-Aldrich) to a final concentration of 0.5 mM and 2.6 mM respectively, the reaction was incubated at 55°C for 18 h. To remove free $NaHSO_3$, the reaction was cleaned up using a silica-based method (Boyle *et al.*, 1995), and was eluted in 100 µl $ddH_2O$. To remove the bisulfite adduct, 11 µl of 4.0 M NaOH was added and the reaction was incubated at 37°C for 15 min. The reaction was then neutralized by adding 111 µl of 7.5 M ammonium acetate, pH 7.0, (Riedel-deHaën). DNA was precipitated with EtOH (Sambrook *et al.*, 2001) and resuspended in 30 µl $ddH_2O$.

The converted DNA was amplified with PCR, using FastStart Taq DNA Polymerase (Roche), with the previously published PCR-primers (Moser *et al.*, 2007): fwd, -142763905-5'-GTTGTTATTYGTAGGGGTATTGG-3'-142763883 and rev, 142763770-5'-AAACCACCRAATTTCTCCAA-3'-142763789 (sequence numbering is according to the published nucleotide position on chromosome 5; GenBank accession number: AJ877168). PCR conditions: 94°C for 5 min, then 34 cycles of denaturation (30 s, 95°C), annealing (2 min, 56°C) and elongation (30 s, 72°C), plus a final elongation step (72°C, 7 min). The PCR was designed to amplify a 93 bp fragment in the GR promoter spanning 10 CpG sites. Products were purified (QIAquick, Qiagen) and then cloned into pCR4-TOPO-vector using the TOPO TA Cloning kit (Invitrogen). Positive clones were identified with colony PCR using the primers

designed to the plasmid: M13-Fwd and M13-Rev primers (Invitrogen). Between 14 and 25 clones were sequenced using the same forward and reverse primers used for colony PCR (BigDye Terminator 3.1 Cycle Sequencing kit, Applied Biosystems). The sequences for each individual clone were aligned and analyzed in Sequencher (version 4.2.2., Gene Codes Cooperation).

**Statistical Analysis**

Statistical analysis was conducted using PASW statistics (version 18.0, SPSS incorporation). To assign a potential association between maternal exposure to intimate partner violence (IPV) and the presence of methylation in either the mothers or the children we conducted Fisher's exact tests thereby treating methylation as a bimodal variable. The GR promoter was referred to as being methylated, if a methylated CpG site was detected in at least one of up to 25 clones that were sequenced. For the mothers, we did not distinguish between different periods of exposure to IPV. Therefore we computed an overall variable for exposure to IPV assigning exposure to IPV whenever it happened before, during or after pregnancy. For the children, as we were interested in the relationship between methylation and maternal exposure to IPV before, during and after pregnancy, we performed tests independently for each interval.

To test for an association between the methylation status of the GR promoter in the mothers and in the children we used Fisher's exact test.

To further analyze the direction of the relationship between methylation and maternal exposure to IPV during pregnancy, we conducted a Man-Whitney-U test with the exposure to IPV during pregnancy as the between group factor and the percentage of methylated clones in the children as the dependent variable. The percentage of methylated clones was calculated according to previously described methods (McGowan *et al.*, 2009) as the number of clones containing at least one methylated CpG site divided by the total number of clones. Additionally, we analyzed the relationship between maternal exposure to IPV before and after gestation and the percentage of methylated clones in the children in the same way.

## III.4. Results

**Maternal stress profiling**

Twenty-five women of various ethnic backgrounds (Table III-1) participated in the study. They were between 29 and 51 years old. All of them had children aged between 10 and 19 years (Table III-1). We used the Composite Abuse Scale (CAS) to screen the mothers, but not the children, for exposure to IPV before, during and after their pregnancies. We identified three women, who were exposed to IPV in the period before pregnancy, eight women, who were exposed during and nine after pregnancy. It should be noted, that these groups were overlapping, i.e. for some women exposure to IPV was apparent in more than one period. In three cases we could not obtain CAS-values for each period, therefore sample sizes varied slightly in the statistical analyses (Table III-1).

**GR promoter methylation**

We examined the methylation status of 10 CpG sites in the GR promoter, to determine whether there is a link between exposure to IPV and methylation in a gene that is involved in the HPA-axis. This region was selected because it was previously shown to contain transcription factor binding sites, whose methylation statuses are influenced by early life stress (McGowan *et al.*, 2009). The GR promoter was referred to as being methylated if a methylated CpG site was detected in at least one of up to 25 clones that were sequenced. We characterized GR promoter methylation of 25 women and of 24 of their children (for one mother, we could not obtain a blood sample of any of her children). We detected methylation in 7 of 10 CpG sites (sites 01, 02, 03, 07, 08, 09 and 10) in the GR promoter in our samples. The degree of methylation in these CpG sites ranged from 0 to 20% of all analyzed clones (Fig. III-1).

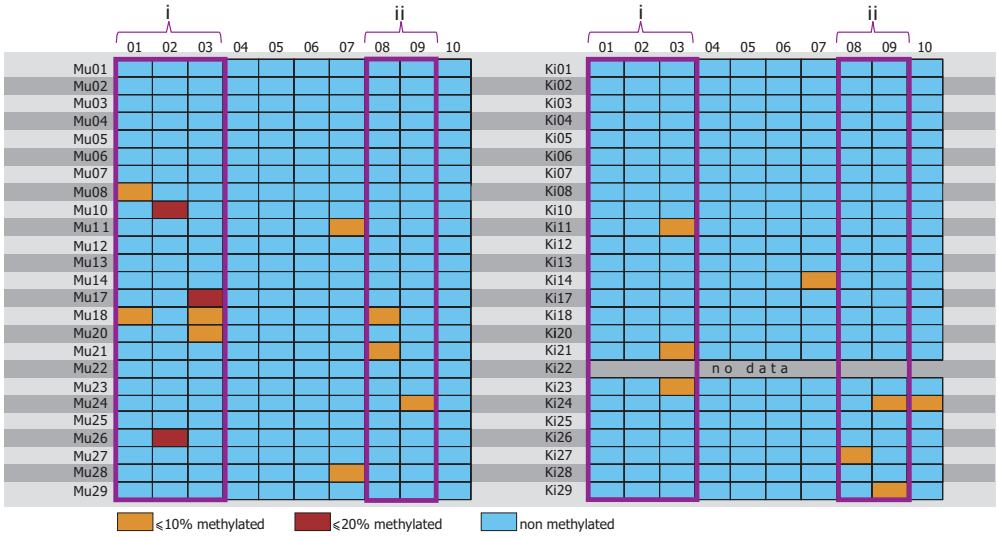


**Fig. III-1: Degree of methylation per individual and CpG site**

Each line represents the analyzed fraction of the glucocorticoid receptor gene of a woman and her child. The rows correspond to individual CpG sites. The degree of methylation for each individual CpG site, i.e. the number of clones containing methylation in the particular site divided by the total number of sequenced clones, is colour-coded. In the case of one child we could not obtain a blood sample as indicated by "no data".
i: putative NGFI-A-binding site; ii: known NGFI-A binding site

We detected methylation in the GR promoter of 10 mothers and of 7 children in at least 1 clone. There was no association between maternal methylation status and the methylation status of the children (n=24; p=1.0).

### Impact of IPV on maternal methylation pattern

Using fisher's exact test, we did not find a significant relationship between exposure to IPV and maternal methylation status (n=25, p=.7), irrespective of when IPV was experienced.

### Impact of IPV on methylation patterns in offspring

The presence of methylated residues was significantly associated with maternal exposure to IPV during pregnancy (n=23, p<.05). Remarkably, there was no association between maternal exposure to IPV and the presence of methylation in the offspring, when IPV was experienced either before or after pregnancy (before: n= 21, p=1.0; after: n=22, p=1.0). Furthermore, we tested the influence of a variety of third variables (country of origin; maternal age; age of children; marital status; graduation from high school, completion of vocational training or academic degree; pregnancy problems; smoking, alcohol or drug consumption during pregnancy; use of painkillers

during birth; anxiety, panic or helplessness during birth; skin contact after birth; severe diseases of children; birth weight; gestational age at birth; mode of birth) on the methylation status, but did not find a significant relationship (Table S-III-1 and S-III-2). According to the guidelines of the world health organization (WHO) none of the analysed children were of extreme immaturity (less than 28 completed weeks of gestation, WHO) or extreme low birth weight (birth weight of 999 g or less, WHO). However, one children was born premature (28 completed weeks or more but less than 37 completed weeks of gestation, WHO) and four were born with low birth weights (1000-2499 g, WHO). These children did not show methylation at the GR promoter. Furthermore, the vast majority of the children (n=17) were delivered spontaneously, indicating that most mothers did not take any measures to delay or induce labor. However, six were delivered via caesarean section and one via other (Table S-III-2). Neither the birth weight, the gestational age or the mode of birth affected the methylation status of the GR gene (Table S-III-1). This provides evidence that methylation in the offspring is directly affected by adverse experiences of the mother during gestation.

We then sought to determine whether gestational exposure to IPV influenced the degree of methylation in the GR promoter in the offspring. To achieve this we evaluated the percentage of methylated clones (i.e. the number of clones containing at least one methylated CpG site divided by the total number of clones). This value ranged from 0 to 20 percent across all of our experimental subjects. In line with our previous analysis, we found a significant relationship between the percentage of methylated clones and IPV during pregnancy (n=23, U=30.5, p=.015; Fig III-2; S-III-3). Additionally, we

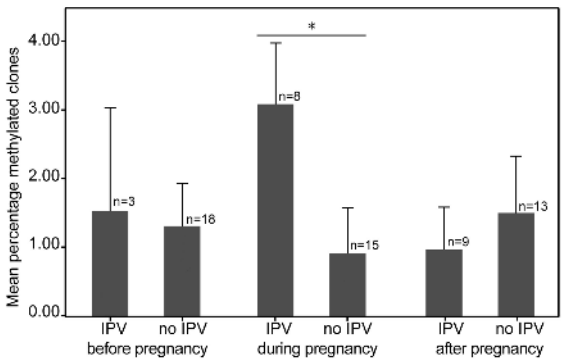


**Fig. III-2: Gestational effects of IPV on methylation of the GR promoter in the children.**

Mean ± s.e.m. of percentage of methylated clones for the children of women exposed to IPV. IPV only associates with increased methylation if maternal exposure occurred during pregnancy. The percentage of methylated clones was calculated as the number of clones containing at least one methylated CpG site divided by the total number of clones. *: $p<0.05$

did not find an association between the percentage of methylated residues and maternal exposure to IPV before or after pregnancy (before: n=21, U=25.0, p=.9; after: n=22, U=55.0, p=.7; Fig. III-2; Fig S-III-2; Fig. S-III-3).

## III.5. Discussion

We examined the methylation pattern of the Glucocorticoid Receptor (GR) promoter in 25 women and their children, and analyzed its association with maternal stress during gestation. We find a positive relationship between methylation of the children's GR promoter and maternal exposure to intimate partner violence (IPV) during pregnancy, defined as "any behaviour within an intimate relationship that causes physical, psychological or sexual harm to those in the relationship (Krug *et al.*, 2002)". We present evidence that GR methylation is related to maternal stress during pregnancy, as when IPV is experienced before or after pregnancy, it has no impact on the children's methylation. To the best of our knowledge, this is the first study to demonstrate that prenatal psychological stress can result in sustained, rather than just short-term alteration of methylation in a regulator gene of the HPA-axis.

We found that maternal gestational IPV is associated with methylation of exon 1F in the GR promoter of the offspring, which is hence a transgenerational effect that may exert a lifelong influence on HPA-axis regulation in these individuals. This is consistent with previous observations that prenatal anxiety is associated with a sustained elevation of basal HPA activity (O'Connor *et al.*, 2005) as well as with behavioural/emotional problems that may continue throughout the lifetime (O'Connor *et al.*, 2002; Seckl *et al.*, 2006). It seems likely that the intrauterine environment that the fetus experiences can differentially affect the methylation pattern. Maternal stress may lead to changes in HPA regulatory circuits with an alteration of catecholamines and glucocorticoids amongst other factors and effects (de Kloet *et al.*, 2005). Prenatal exposure to either of these hormones is known to influence the development of the HPA-axis (Kapoor *et al.*, 2006). Therefore in the fetus, the GR gene, which is a key regulator of the HPA-axis, constitutes a likely target upon which maternal IPV could be acting. Our data are consistent with observations in rat, where maternal effects evoke increased HPA-axis activity and fearfulness in response to stress, which are associated with methylation of the GR promoter in the hippocampus(Weaver *et al.*, 2004). In these experiments, the first days of postnatal life constitute a critical period for the manifestation of maternal effects, which

corresponds to late gestation in humans (Dobbing *et al.*, 1973). Although the observed increase in methylation was only very subtle, it might be sufficent to alter HPA-axis function. Subtle increases in methylation in a single CpG site of the GR promoter in chordblood were reported to correlate positively with the cortsiol stress response in neonates (Oberlander *et al.*, 2008). This suggests that IPV elicits fetal programming of the HPA-axis through methylation of the GR gene. However, although a link between GR promoter methylation in blood and cortisol stress responses has already been provided (Oberlander *et al.*, 2008), it remains inconclusive whether methylation in blood cells reflects methylation in the hippocampus or whether it impacts HPA-axis function. This emphasizes the need for follow up studies, which includes HPA-axis function and psychological function. This is of particular interest, as blood cells – as opposed to brain tissue - represents an easily sampled tissue.

The results of our study suggest that maternal stress-induced changes in intrauterine environment can have an impact on the methylation pattern of the children's GR gene, rather than a direct maternal transmission of methylation pattern via the germ line. The Composite Abuse Scale (CAS) assesses exclusively environmental factors, episodes of domestic violence in this case, thereby minimizing the influence of genetic components. However, an individual's genotype might affect others' behaviour towards them, thus their own social environment, or predispose them to seek risky or unfavourable social environments (Scarr *et al.*, 1983). As this type of indirect effect is difficult to avoid in studies with humans, we believe that our chosen methods go a long way to minimizing the influence of underlying genetic background. Hence we consider gestational IPV to be the main source of variation in our study.

The methodologies employed by our study present some limitations: we have adapted a commonly used psychological survey – the CAS, to examine the influence of past events on current methylation patterns of mothers and their children. The effectiveness of retrospective surveys relies on the accuracy of our participants' memories. We believe that our use of an event-based analysis tool is particularly robust due to its reliance on emotionally arousing events, which are known to create long-lasting memories (McGaugh, 2003). Our data represent correlative findings and thus cannot prove a causal relationship between changes in methylation and adverse experiences. For instance, it might be possible that IPV correlates with aspects of maternal diet that in turn might have affected the observed methylation patterns. Such a scenario, however, seems unlikely, as we would then predict an association between IPV and methylation in the mothers. This, however, was not observed.

## III.6. Conclusions

Our findings show that prenatal exposure to intimate partner violence (IPV) is associated with a sustained increase in methylation of the human glucocorticoid receptor (GR) promoter in the blood. Prenatal stress is known to alter HPA-axis regulatory function later in life (O'Connor *et al.*, 2005). Specifically gestational marital discord is associated with psychopathology of the offspring (Stott, 1973; Ward, 1991). This is the first demonstration that gestational exposure to psychological stressors can have a lasting impact on methylation status in human offspring. Our results provide a potential mechanism – methylation of the GR promoter – upon which prenatal stress could act, to influence psychological function. This emphasises the importance of IPV interventions to assure the wellbeing not only of the mother but also of the unborn child. This mechanism opens up many new avenues for research on the transgenerational epigenetic effects of stress and aggression on human behaviour.

## III.7. Acknowledgements

We thank I. T. Kolassa, A. Ashe, Ozden Hatirnaz and W.A. Schulz for helpful suggestions on the methods, M. Roth and H. Riedke for help during sample collection and P. M. Hull and F. Henning for helpful suggestions on the manuscript. H.M.G is supported by the Zukunftskolleg of University of Konstanz. Research was supported by the DFG.

## III.8. Conflict of interest

The authors declare no conflict of interest.

**Supplementary information**

Supplementary information is included at Translational Psychiatry's website.

# IV Epigenetic modifications of the glucocorticoid receptor gene are associated with the vulnerability to psychopathology in childhood maltreatment

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## IV.1. Abstract

Stress, particularly when experienced early in life, can have profound implications for mental health. Previous research covering various tissues such as the brain, suggests that the detrimental impact of early life stress (ELS) on mental health is mediated via epigenetic modifications including DNA methylation. Genes of the hypothalamic pituitary adrenal (HPA) axis – in particular the Glucocorticoid Receptor (hGR) gene – stand out as key targets for ELS. Even though the link between *hGR* methylation and either ELS or psychopathology is fairly well established, the mutually dependent relationships between ELS, DNA methylation and psychopathology remain to be uncovered. The specific psychopathology an individual might develop in the aftermath of stressful events can be highly variable, however most studies investigating *hGR* methylation and psychopathology suffer from being limited to a single symptom cluster of mental disorders. Here, we screened volunteers for childhood maltreatment and analyzed whether it associates with *hGR* methylation in lymphocytes and a range of measures of psychological ill-health. *hGR* methylation in lymphocytes most likely

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reflects methylation patterns found in the brain and thus provides valuable insights into the etiology of psychopathology. We find the interaction between childhood maltreatment and *hGR* methylation to be strongly correlated with an increased vulnerability to psychopathology providing evidence of epigenome x environment interactions. Furthermore, our results indicate an additive effect of childhood maltreatment and *hGR* methylation in predicting borderline personality disorder (BPD) associated symptoms, suggesting that the combination of both ELS and DNA methylation that possibly represents unfavorable events experienced even earlier in life poses the risk for BPD.

## IV.2. Introduction

Childhood maltreatment affects the development of mental health in different ways. For example, the experience of childhood adversities significantly increases the risk of developing multiple psychopathologies including major depressive disorder, borderline personality disorder (BPD), anxiety disorders and substance abuse (Crawford *et al.*, 2009; Dube *et al.*, 2003; Teicher *et al.*, 2009; Widom *et al.*, 2007). Compared to non-maltreated individuals, psychiatric patients with a history of childhood maltreatment are characterized by an earlier disease onset, greater symptom severity, more comorbidities and poorer responses to many first-line treatments(Harkness *et al.*, 2012; Nanni *et al.*, 2012; Teicher *et al.*, 2013). Teicher and others have suggested that biological determinants such as epigenetic modifications in stress-response systems, especially the hypothalamic-pituitary-adrenal (HPA) axis, may be the driving force behind the development of these childhood maltreatment-induced disorders (Teicher *et al.*, 2013).

The HPA axis plays a central role in translating early life stress into negative long-term mental health outcomes, as it is tuned by experiences occurring early in life, making it highly susceptible to early life stress (ELS) (Carpenter *et al.*, 2007; Heim *et al.*, 2001; Heim *et al.*, 2000). Its dysregulation is a key feature of a range of psychopathological symptoms (Chrousos *et al.*, 1992; de Kloet *et al.*, 2005). Both human and animal studies suggest that HPA axis function may be stably altered through aberrant epigenetic modifications resulting from early life stress. In rats, offspring that have experienced ELS show an increased HPA axis response to stress (Liu *et al.*, 1997) as well as an increased incidence of fearful behaviors (Francis *et al.*, 1999). The glucocorticoid receptor (*GR*) gene and its methylation status are of central

relevance to this phenotype (Weaver *et al.*, 2004). Genes that display high levels of DNA-methylation in their promoter regions tend to be less transcriptionally active (Razin, 1998). The *GR* initiates the feedback inhibition of the HPA-axis – after binding its ligand, cortisol, it dampens HPA-axis activity. The human *GR* (hGR) consists of eight coding (2-9) and one non-coding (1) exons (Turner *et al.*, 2005) (Fig. IV-1). The promoter region of the gene, non-coding exon 1, consists of several alternate exons (1A, 1I, 1D, 1E, 1B, 1F, 1C and 1H) that give rise to multiple transcripts that encode the same protein (Turner *et al.*, 2005). Previous research has demonstrated that the methylation of alternate exon 1F seems to be strongly influenced by ELS. Consistent with increased HPA-axis responses, alternate exon $1_7$ – the murine homologue to alternate exon 1F – is hypermethylated in the brains of stressed juvenile rats, concomitant with a decrease in *GR*-expression(Liu *et al.*, 1997; Weaver *et al.*, 2004).

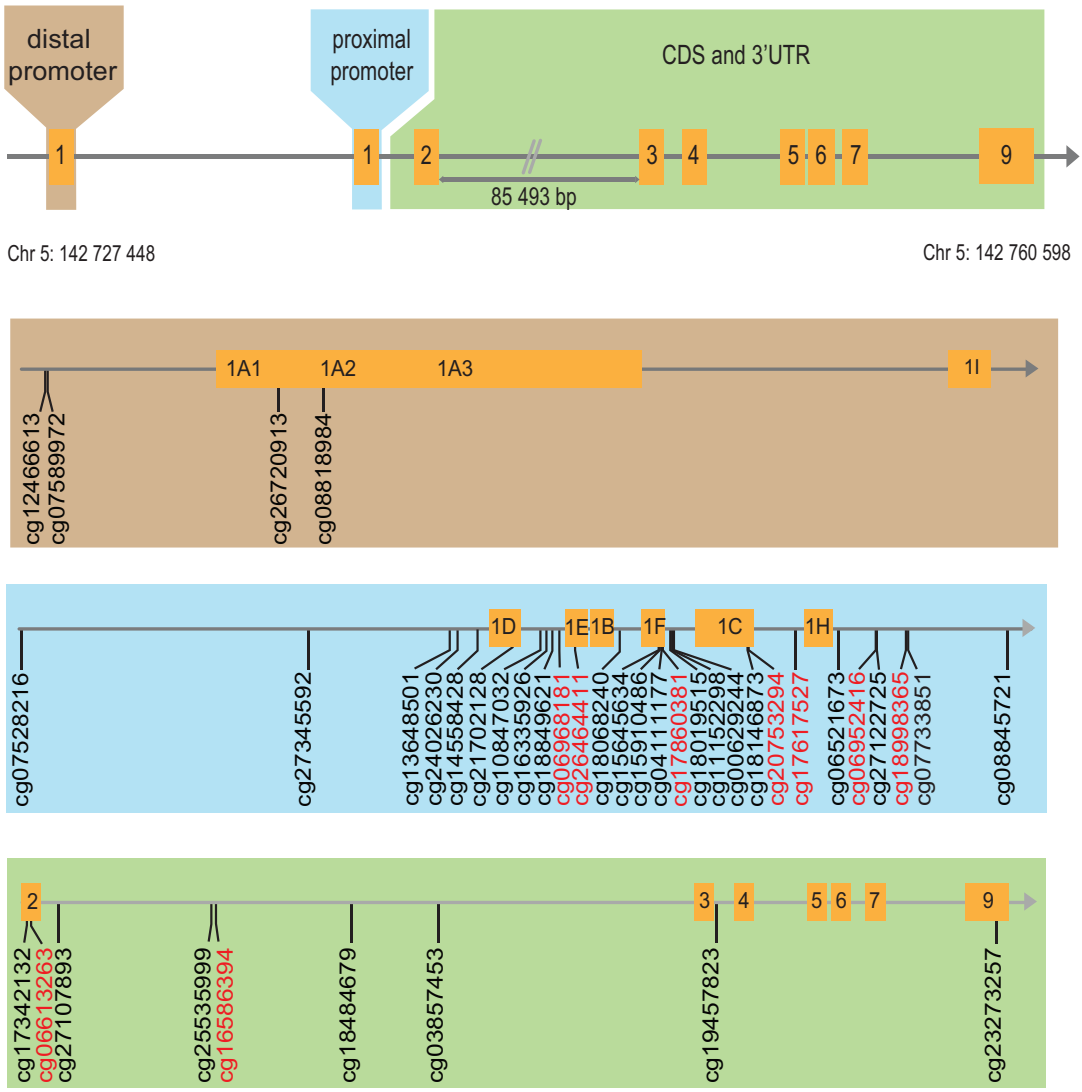


**Fig. IV-1: Summary of CpG sites in the Glucocorticoid Receptor used in our investigation**

The CpG-sites that were used in principal component analyses are highlighted in red font

Correspondingly, in humans, methylation of the *hGR* promoter is associated with both ELS (Hompes *et al.*, 2013; Mulligan *et al.*, 2012; Oberlander *et al.*, 2008; Perroud *et al.*, 2011; Radtke *et al.*, 2011; Tyrka *et al.*, 2012) and psychopathology. A history of childhood abuse in suicide victims – a group generally displaying high rates of psychopathology (Harris *et al.*, 1997) – is associated with increased *hGR* methylation in brain tissue (Labonté *et al.*, 2012b; McGowan *et al.*, 2009). Correspondingly, in the blood of patients suffering from BPD, i.e. in individuals that usually have been exposed to severe forms of abuse and neglect during development, *hGR*-methylation was observed to be increased (Dammann *et al.*, 2011) and to be positively correlated

with BPD-symptom severity (Martin-Blanco *et al.*, 2014). On the other hand, decreased *hGR*-promoter methylation in both saliva and blood seems to be related to more intrusive memories and an increased risk of developing posttraumatic stress syndrome (PTSD) when stressors are encountered in adulthood (Labonté *et al.*, 2012b; Vukojevic *et al.*, 2014). In spite of its importance to the field, none of this previous research has resolved whether epigenetic processes that alter HPA axis function expose the individual to a higher risk of developing a psychopathology, or alternatively, whether these changes are neutral or even serve to adapt the behavior towards survival in a hostile, abusive environment. All of the afore-mentioned studies that investigated the contribution of *hGR*-methylation to the development of psychopathology were limited to a single measurement of psychological health. Therefore, it is yet unknown how *hGR*-methylation contributes to psychopathology more generally.

Here, we investigate the mutually dependent relationships between ELS, epigenetic modifications and mental health. Utilizing a multivariate approach, incorporating several psychological dimensions, we aimed to investigate whether the simultaneous occurrence of ELS and increased *hGR*-methylation is accompanied by an increased vulnerability to the development of psychopathology. We predicted that childhood maltreatment would be associated with a greater range and intensity of psychopathological symptoms and that this association is modulated via DNA methylation of the *hGR* gene.

## IV.3. Materials and Methods

**Participants**

Forty-six participants ($N_{female}$=28, $N_{male}$=18), aged between 11 and 21 years (median=15) were recruited, with an emphasis on including individuals that varied in the degree of childhood adversity experienced. The study cohort represents a convenience sample from the local community with an announcement that the investigation would include investigation of potential biomarkers of ELS. A subset (N=23) had already participated in an earlier study(Radtke *et al.*, 2011). Participants received a total of 30 Euros and all participated on a voluntarily basis, i.e., free to quit the interview at any time without giving any reasons and without losing the reimbursement. The study was approved by the IRB (Ethics Committee) of the University of Konstanz.

Childhood maltreatment and psychological ill-health were assessed using structured interviews by experienced clinical psychologists. Childhood maltreatment was assessed in detail using a draft version of the KERF-I(Isele *et al.*, 2014b), a German version of the pediatric MACE(Teicher *et al.*, 2011) interview with good psychometric properties, which is designed to match the needs of children(Isele *et al.*, 2014b). It captures the lifetime occurrence of childhood maltreatment up to age 18 in eight dimensions: parental physical abuse, parental emotional abuse, sexual abuse, witnessed physical violence toward parents, witnessed violence toward siblings, peer physical violence, physical neglect and emotional neglect.

Symptoms associated with borderline personality disorder (BPD) were assessed using the Borderline Symptoms Checklist-23 (BSL-23)(Bohus *et al.*, 2007). The BSL-23 was constructed according to the Diagnostic and Statistical Manual of Mental Disorders (fourth edition, DSM-IV) criteria for BPD. It consists of 23 items that are scored from 0 (not at all) to 4 (very strong). A resulting sumscore of 64 or higher is indicative of a clinically relevant BPD-diagnosis. Symptoms of depression and anxiety dimension have been evaluated using the Hopkins Symptoms Checklist-25 (HSCL-25)(Derogatis *et al.*, 1974). Symptoms associated with oppositional defiant disorder (ODD), conduct disorder (CD) or attention deficit hyperactivity disorders (ADHD) were evaluated using the respective parts of the Mini-International Neuropsychiatric Interview (M.I.N.I.)(Sheehan *et al.*, 1998). The perceived health related life-quality has been assessed using the KIDSCREEN-53 (Rajmil *et al.*, 2006).

The Strength and Difficulties Questionnaire (SDQ)(Klasen *et al.*, 2003) was administered in order to evaluate strength and difficulties in four dimensions. Resulting scores of less than or equal to 15, greater than or equal to 16 or greater than or equal to 20, are classified as being "Normal", "Intermediate" or "Abnormal", respectively.

**DNA Methylation.**

Blood samples were collected immediately after completion of the interviews. Lymphocytes were isolated via a Ficoll (Sigma-Aldrich, Saint Louis, USA) gradient and subjected to DNA-extraction (DNeasy Blood and Tissue Kit, Qiagen, Hilden, Germany). Genome-wide analysis of DNA methylation was then conducted at the Barts and the London Genome Centre (Queen Mary University of London,

London, United Kingdom). 1µg of genomic DNA was bisulfite converted (EZ DNA Methylation Kit, Zymo) and applied to the Human Methylation 450K array (Illumina).

Samples that exhibited either abnormal methylation profiles across all CpG sites or signs of unconverted DNA based on the conversion control probes present on the array were excluded before further processing and analyses (N=0). The Human Methylation 450K array includes two different bead types associated with two different chemical assays, the Infinium I and the Infinium II assay. In order to compensate for this, the raw data were normalized using both the R package lumi and Beta Mixture Quantile Dilation (BMIQ)(Marabita *et al.*, 2013). To eliminate any potential biases that may have arisen due to differences in the labeling and scanning properties of these two bead systems, color adjustment was performed through lumi. In order to reduce any further systematic biases, quantile normalization was employed through lumi(Du *et al.*, 2008). To adjust for the probe-type bias BMIQ was performed on the quantile normalized data. After preprocessing, DNA methylation was assessed for all probes spanning the *hGR* gene, which are included on the Human Methylation 450K array, identified according to their genomic positions. This set included a total of 41 probes (Fig. IV-1).

**Statistical Analyses**

All statistical analyses were conducted in RStudio(RStudio, 2014). Depending on the distribution, either parametric Pearson-correlations or nonparametric Spearman-correlations were performed. Normality was assumed if skewness was less than an absolute value of two and kurtosis was less than an absolute value of seven. Our analysis used a false discovery rate of 0.05 to account for multiple testing across the 41 CpG-sites in the *hGR*-gene (Benjamini *et al.*, 1995). Adjusted p-values were computed using the Benjamini-Hochberg procedure. Our metric for a small effect size was $r \geq 0.10$, for a medium effect $r \geq 0.30$, and for a large effect $r \geq 0.50$(Cohen, 1992). Principal component analyses were performed using the psych-package(Revelle, 2014). In order to avoid multicollinearity the predictors were mean-scaled prior to computing the interaction term in multiple regression analyses. Furthermore, the dependent variable was log-transformed if necessary to fulfill the assumptions about the residuals in multiple regression analyses.

**Transcription factor binding sites**

In order to reveal potential functional properties associated with the CpG sites included in our study, the sequence 50 bp up- and downstream of the respective CpG sites have been submitted to the Jaspar database(Mathelier *et al.*, 2014) to predict known transcription factor binding sites (TFBS). In addition the University of California Santa Cruz (UCSC) genome browser(Kent *et al.*, 2002) was used to screen the respective genomic regions for conserved TFBSs or marks usually associated with transcriptional activity, such as H3K27 acetylation.

## IV.4. Results

**Table IV-1: Psychometric measurements**

| | n | mean (± sd) | range | max score possible | clinical cut off | clinically diagnosed / classification |
|---|---|---|---|---|---|---|
| n(childhood adversities) | 46 | 7.4 (4.9) | 1 - 20 | 35 | *n.a.* | *n.a.* |
| perceived health related life quality | 43 | 201.4 (28.8) | 129 - 239 | 260 | *n.a.* | *n.a.* |
| BPD associated symptoms | 46 | 12.1 (15.0) | 0 - 81.6 | 89 | 64 | 1 (2.1%) |
| strength and difficulties | 45 | 11.9 (5.6) | 1 - 26 | 40 | Intermediate=16, Abnormal=20 | Normal=32 (71.1%), Intermediate=8 (17.8%), Abnormal=5 (11.1%) |
| Anxiety associated symptoms | 46 | 6.0 (5.7) | 1 - 27 | 30 | 8 | 15 (32.6%) |
| Depression associated symptoms | 46 | 9.4 (8.7) | 0 - 37 | 45 | 12 | 14 (30.4%) |
| ADHD associated symptoms | 45 | 4.6 (3.8) | 0 - 18 | 18 | 12 | 1 (2.1%) |
| CD associated symptoms | 46 | 1.0 (1.7) | 0 - 8 | 15 | 3 | 4 (8.7%) |
| ODD associated symptoms | 46 | 1.8 (2.0) | 0 - 8 | 8 | 4 | 5 (11.0%) |

Abbreviations: ADHD, attention deficit hyperacitvity disorder; BPD, borderline personality disorder; CD, conduct disorder; n.a., not applicable; ODD, oppositional defiant disorder

Subjects reported that they had experienced between one and 20 different events of childhood adversities (Table IV-1). The majority of the subjects displayed sub-clinical values with respect to psychopathological symptoms with some presenting clinically relevant symptoms of anxiety and/ or depression. The psychometric

measurements obtained appeared to be randomly distributed amongst the participants, with no specific symptoms clustering within certain individuals (Fig. IV-S1).

**Relationship between hGR-methylation, childhood abuse and psychological ill-health**

We investigated DNA-methylation in 41 CpG-sites associated with the *hGR*-gene in lymphocytes (Table S-IV-1). Neither of the investigated psychometric measurements nor childhood maltreatment was associated with average methylation of all investigated CpGs that were distributed across the whole *hGR* (data not shown). However, we observed statistically significant correlations between the methylation of two specific CpG-sites located in the promoter of the hGR-gene, and stress- or psychological ill-health related measurements.

We identified a positive correlation between methylation of cg17860381 (located in the alternate exon 1F) and childhood maltreatment, as indicated by the statistically significant correlations with the number of experienced childhood adversities (Fig. IV-2, Table S-IV-2). Interestingly, methylation of the same CpG-site also displayed a highly significant positive correlation to borderline personality disorder (BPD) symptoms (Fig. IV-2, Table S-IV-2). Additionally, methylation of cg17860381 was positively correlated to depression symptoms to a degree that approached statistical significance (Fig. IV-2, Table S-IV-2).

Methylation of a second CpG-site, cg26464411, was also positively correlated with two measurements associated with psychological health. The correlation with depression symptoms was statistically significant, while the correlation with behavioral strength and difficulties approached statistical significance (Fig. IV-2, Table S-IV-2).

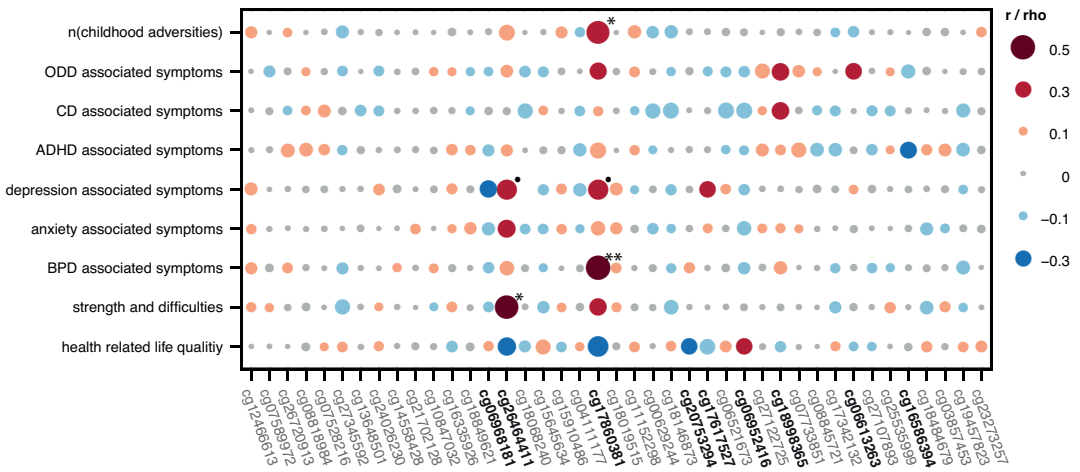


**Fig. IV-2: Correlation between individual CpG sites and stress exposure or psychological health**

The strength of the correlations are depicted by a color gradient, ranging from far blue (negatively correlated) to far red (positively regulated). The size of the dots represents the effect size, i.e. the larger the dot the greater the effect size.
The CpG-sites that were used in principal component analyses are highlighted in black font.
*Note:* ODD, oppositional defiant disorder; CD, conduct disorder; ADHD, attention deficit hyperactivity disorder; BPD, borderline personality disorder; ˙:adj p≤0.1; *: adj p≤0.05; **: adj p≤0.01; adj p: adjusted p value.

Apart from the large effect sizes displayed in the correlations between the methylation of cg17860381 with BPD-associated symptoms and between the methylation of cg26464411 and behavioral strengths and difficulties, all of the remaining aforementioned correlations displayed medium effect sizes. Especially for small samples, effect size may be the reference of choice, as the p-value heavily depends on sample size and thus true effects are prone to be missed (Cohen, 1990). While not being statistically significant by means of p-values, both methylation of cg17860381 and of cg26464411 negatively correlated with health related life quality at medium effect sizes. Furthermore, oppositional defiant disorder (ODD) associated symptoms and behavioral strength and difficulties were positively associated with cg17860381-methylation, while anxiety associated symptoms correlated with cg26464411-methylation, each with medium effect sizes. Along the same lines, considering effect size alone, further associations with medium effect sizes were observed. The methylation at seven additional CpG sites correlated with psychological health related measurements including health related life quality and symptoms associated with ODD, -conduct disorder (CD), -attention deficit hyperactivity disorder (ADHD), -depression, -anxiety, and -BPD (Fig. IV-2).

To further investigate the relationship between childhood abuse, methylation of the *hGR*-gene and psychological ill-health, we performed two principal component analyses (PCA). These comprised either variables relating to DNA methylation, or psychological health. In PCA, the first principal component (PC) accounts for the largest proportion of the variance created by the included variables. Therefore, each of the obtained first PCs was used in a subsequent multiple regression analysis. All psychological health related variables correlating with the methylation of at least one CpG site with at least medium effect size and the corresponding CpG sites were considered for PCAs. Using this threshold all of the elevated psychometric variables (n=8) and nine CpG sites were included in the PCAs. The first PCs relating to either psychological health or DNA methylation explained 61.7% or 20.5% of the variance respectively. For the PC comprising psychological ill-health, an exploratory factor analysis identified absolute factor loadings ranging between 0.68 and 0.88 with maximum loadings for depression and anxiety associated symptoms (Fig. IV-S1a). For *hGR* methylation absolute factor loadings ranged between 0.11 and 0.59 with the highest loading for cg17860381 (Fig. IV-S1c).

Next, we performed a linear model using the first PC comprising psychological ill-health as outcome and the number of experienced childhood adversities, the first PC comprising *hGR* methylation as well their interaction as predictors. The model explained 63.2% ($F(5, 36)=12.4$, $p\leq0.001$) of the variance. Both of the predictors as well their interaction impacted psychological health significantly ($\beta_{hGR\ methylation}=0.3$, $p_{hGR\ methylation}\leq0.05$, $\beta_{n\ (childhood\ adversities)}=0.3$, $p_{n\ (childhood\ adversities)}\leq0.05$, $\beta_{interaction}=0.5$, $p_{interaction}\leq0.001$, Fig. IV-3). To correct for effects potentially arising from sex or age (Terry *et al.*, 2011), these variables

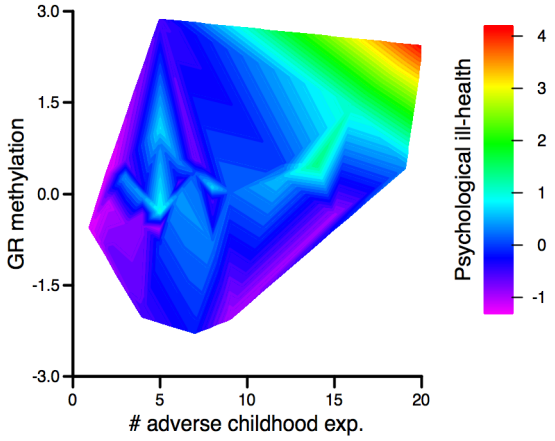


**Fig. IV-3: Relationship between psychological ill-health, childhood abuse and *hGR* methylation**

*hGR* methylation and psychological ill-health are represented by the first principal components of a principal component analyses summarizing nine CpG sites in the Glucocorticoid Receptor gene and all evaluated psychometric measurements, respectively. The color gradient ranging from purple to red represents the level of psychological ill-health, i.e. purple for healthy and red for unhealthy.

were also included in the model. Neither sex nor age seemed to affect psychological health ($\beta_{sex}$=0.1, $p_{sex}$>0.1, $\beta_{age}$=-0.1, $p_{age}$>0.1).

**Interaction of childhood adversities and methylation of cg17860381 in the development of BPD**

As both BPD-associated symptoms and childhood adversities correlated significantly with methylation at cg17860381, we hypothesized that the simultaneous occurrence of both facilitates the development of BPD-symptoms. To further investigate a potential interaction between methylation at cg17860381 and childhood adversities, influencing the subsequent development of BPD-symptoms, we performed a linear model using these two variables and their interaction as predictors for BPD-symptoms. In order to guarantee heteroscedasticity of the model residuals, BPD-associated symptoms were log-transformed prior to the analysis. The model accounted for 52.6% ($F(5, 40)=8.87$, $p\leq0.001$) of the variance in BPD-symptoms. Under this model both of the predictors impacted BPD-symptoms significantly ($\beta_{cg17860381}$=0.5, $p_{cg17860381}\leq0.01$, $\beta_{n\ (childhood\ adversities)}$=0.5, $p_{n\ (childhood\ adversities)}\leq0.01$, Fig. IV-4), while their interaction did not exert contribution to BPD-symptoms that reached statistical significance ($\beta_{interaction}$=-0.2, $p_{interaction}\leq0.1$). To correct for effects potentially arising from sex or age we also included those as variables in the model. While the former did not influence BPD-symptoms there was a statistical trend towards an influence of age on BPD-symptoms ($\beta_{sex}$=-0.2, $p_{sex}$>0.1, $\beta_{age}$=0.2, $p_{age}\leq0.1$). An investigation of the genomic location did not reveal the presence of a transcription factor binding site.

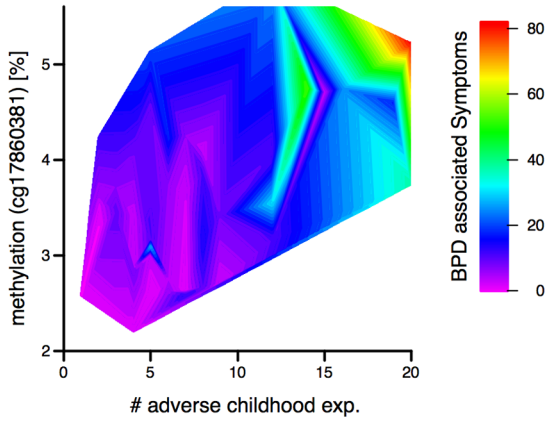


**Fig. IV-4: Relationship between methylation of cg178603981, adverse childhood experiences and BPD associtated symptoms**

Relationship between methylation of cg17860381, adverse childhood experiences and BPD associated symptoms. The color gradient ranging from purple to red represents the level of BPD associated symptoms, i.e. purple for low levels and red for high levels

## IV.5. Discussion

Early life stress (ELS) is known to exert a lifelong detrimental influence on mental health, and previous studies suggest that this is associated with methylation of the glucocorticoid receptor (*hGR*) gene in various tissues (McGowan *et al.*, 2009; Oberlander *et al.*, 2008; Weaver *et al.*, 2004). However, to date, no study has explicitly tested for a relationship between ELS and both *hGR*-methylation and psychopathology. Moreover, very few studies include analyses of psychopathology in multiple dimensions. Rather than discovering the etiology of particular psychopathological states, we aimed to unravel the threats posed by ELS to psychological wellbeing as a general concept. Therefore, in this study, we analyzed psychological ill-health from several perspectives, including testing for symptoms associated with oppositional defiant disorder, conduct disorder, attention deficit hyperactivity disorder, depression, anxiety or borderline personality disorder (BPD). In addition, we focused on the perceived life quality, functioning and strength and difficulties, as they are associated with various categories of disorders, such as the ones mentioned above. By employing a combined approach that incorporated principal component analysis (PCA) and multiple regression analysis, we identified a strong relationship between childhood maltreatment, methylation of the *hGR* gene in lymphocytes and psychological ill-health. Moreover, we identified an additive effect of childhood maltreatment and cg17860381 methylation in the *hGR* promoter on the development of BPD-associated symptoms.

In addition to an additive effect of both *hGR*-methylation and childhood maltreatment, the interaction of these two variables predicted the intensity of psychopathological symptoms. Thus, the combination of childhood maltreatment with h*GR*-methylation by means of the composite methylation of nine CpGs spanning the entire *hGR* is associated with impaired mental health, i.e., we observed an epigenome x environment effect. Given the substantial evidence for gene x environment effects on psychological wellbeing, we anticipated a similar epigenome x environment effect, even though – to the best of our knowledge – it had not been demonstrated yet. While the impact of methylation at single or few CpG sites on gene transcription is debated, in the case of the glucocorticoid receptor, site specific correlations between DNA methylation and gene expression and/or early life stress have been repeatedly reported(Labonté *et al.*, 2014; Labonté *et al.*, 2012b; McGowan *et al.*, 2009; Oberlander *et al.*, 2008). Moreover, different directions of correlations between childhood abuse

and DNA methylation have been identified for different regions of the *hGR* promoter(Labonté *et al.*, 2012b). In order to avoid canceling out of opposing effects, we chose a PCA-based approach rather than averaging several CpG's methylation to gauge *hGR* methylation. Indeed, we did not find an effect for average methylation. For BPD-associated symptoms we focused on its relationship between DNA methylation at a single CpG site – cg176860381 – and childhood maltreatment. This relationship seems to be simpler, as it shows a strong additive effect of childhood maltreatment and cg176860381 methylation while the interaction of those two predictors did not impact the intensity of BPD-associated symptoms. Interestingly, cg17860381 also seems to play an important role in the above-described relationship between childhood maltreatment, *hGR*-methylation and psychopathological symptoms, as it exerted the highest influence on the joint principal component summarizing *hGR* methylation. This CpG site is part of the alternate exon 1F, which has been previously found to be associated with early life stress or psychopathology (Dammann *et al.*, 2011; Hompes *et al.*, 2013; Mulligan *et al.*, 2012; Oberlander *et al.*, 2008; Perroud *et al.*, 2011; Radtke *et al.*, 2011; Tyrka *et al.*, 2012; Weaver *et al.*, 2004). Due to the location of cg17860381 in the proximal promoter of *hGR*, it is plausible that methylation at this site may influence the dynamics of *hGR* expression, although it is not located at the transcription factor binding site (TFBS) recently implicated in the epigenetic regulation of *hGR*-expression (Weaver *et al.*, 2004) nor could we detect another TFBS nearby. Thus the functional implications of cg17860381 methylation need to be further investigated. In addition, future studies should also evaluate other epigenetic mechanisms such as histone modifications or gene expression to gain a more cohesive picture. Our results suggest, that *hGR* methylation in lymphocytes – either evaluated at a single CpG site or as the joint profile of several CpGs – represents certain environmental conditions setting these individuals at a higher risk of developing psychopathology. Indeed, *hGR*-methylation in blood or brain tissue was shown to associate with exposure to prenatal stress(Mulligan *et al.*, 2012; Radtke *et al.*, 2011), a familial history of posttraumatic stress disorder(Yehuda *et al.*, 2014) or childhood stress (McGowan *et al.*, 2009; Perroud *et al.*, 2011; Tyrka *et al.*, 2012). However, the exact causes of *hGR*-methylation need to be further investigated. Meaney suggested that hippocampal *GR*-methylation results from a lack of tactile stimulation (Meaney *et al.*, 2005). Already Harlow proved that somatosensory deprivation can cause social-emotional disorders (Harlow *et al.*, 1962) that were later suggested to be mediated by impaired cerebellar development (Prescott, 1971). BPD-patients have frequently been

exposed to childhood neglect (Crawford *et al.*, 2009), which usually associates with somatosensory deprivation. Accordingly, BPD was found to associate with decreased cerebellar vermis size (Schauer *et al.*, in prep) and – hinting towards functional cerebellar impairment with reduced balance skills (Isele *et al.*, 2014a). Thus, the differential cg17860381 methylation in our sample might reflect childhood somatosensory deprivation. Accordingly, we suggest the course of early neglect with somatosensory deprivation and later childhood abuse as the toxic combination that promotes BPD symptoms.

Several other studies analyzed childhood maltreatment and *hGR* methylation. Perroud *et al.(Perroud et al., 2011)* showed that childhood sexual abuse correlates with *hGR*-methylation in blood drawn from patients suffering from either BPD or major depressive disorder. However, this study lacked healthy controls, thus attributing the observed differences in DNA-methylation only to the advent of childhood abuse. To the best of our knowledge, ours is the first study to provide a combined contribution of both early life stress and methylation in a regulator gene of the HPA-axis to psychological health. Three other studies have combined the analysis of early life stress, *hGR*-methylation in blood with phenotypic characterizations, showing that *hGR*-methylation is associated with both early life stress and either lower birth weight (Mulligan *et al.*, 2012) or altered cortisol levels (Oberlander *et al.*, 2008; Tyrka *et al.*, 2012), two features that are predictive for psychopathology.

The methodologies employed by our study present some limitations. Our data are correlational in nature and thus cannot prove a causal relationship between child maltreatment and methylation patterns or decreased psychological well-being. In addition, due the limited sample size, generalizations to the general population must be regarded with caution. The brain constitutes the most prominent organ into which differential DNA methylation, and hence differential gene expression, affects behavior. As epigenetic modifications occur in a tissue-specific manner, it remains unclear at this point whether DNA methylation measured in blood reflects DNA methylation patterns in the brain. However, research conducted on the effects of early life stress on the methylation of the alternate promoter 1F in both blood (Radtke *et al.*, 2011) and brain tissue (McGowan *et al.*, 2009) points in the same direction, i.e. early life stress being associated with increased DNA-methylation. In addition a recent meta-analysis reported methylation patterns in blood and brain tissue to be highly correlated (Tylee *et al.*, 2013). Accordingly, individual differences in *hGR* methylation in peripheral tissue, i.e. saliva, were found to associate with differential brain activity(Vukojevic *et al.*,

2014). Together, these findings suggest, that similar patterns of *hGR* methylation may be present across tissues and that our results are likely to reflect DNA methylation patterns present in the brain.

## IV.6. Conclusions

Our results indicate an increased vulnerability to develop a psychopathology in general and BPD in particular, if childhood maltreatment is combined with increased methylation of the *hGR* gene, as exemplified here in lymphocytes. Remarkably, rather than being exposed to extreme forms of stress our participants were exposed to only moderate levels of childhood maltreatment. Strengthened by the inclusion of epigenetic markers, we emphasize the threats of moderate stress to psychological well-being. In conclusion, we highlight a plausible molecular mechanism by which ELS might translate into undesirable consequences on mental health later in life, and in doing so, greatly strengthen the utility of *hGR* methylation in peripheral tissues such as lymphocytes as a potential diagnostic marker to identify whether victims of childhood maltreatment are at risk of developing severe psychopathological symptoms later in life.

## IV.7. Acknowledgements

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## IV.8. Conflict of interest

The authors declare no conflict of interests.

# V Associations between child abuse, mental health and epigenetic modifications in the POMC gene: A study with children in Tanzania

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## V.1. Abstract

Child abuse is associated with a number of emotional and behavioral problems. Nevertheless, it has been argued that these adverse consequences may not hold for societies in which many of the specific acts of abuse are culturally normed. Epigenetic modifications in the genes of the hypothalamic pituitary adrenal (HPA) axis may provide a potential mechanism translating abuse into altered gene expression, which subsequently results in behavioral changes. Our investigation took place in Tanzania - a society in which many forms of abuse are commonly employed as disciplinary methods. We included 35 children with high exposure and compared them to 25 children with low exposure. Extreme group comparisons revealed that children

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with high exposure reported more mental health problems. Child abuse was associated with differential methylation in the *POMC* gene, measured both in saliva and in blood. Hierarchical clustering based on the methylation of *POMC* found two distinct clusters. These corresponded with children's self-reported abuse, with two-thirds of the children allocated into their respective group. Our results emphasize the consequences of child abuse based on both molecular and behavioral grounds, providing further evidence that acts of abuse affect children, even when culturally acceptable. Furthermore, on a molecular level our findings strengthen the credibility of children's self-reports.

## V.2. Introduction

Child abuse is commonly defined as any act of commission by a parent or any other caregiver that results in harm, potential for harm, or threat of harm to a child (Leeb *et al.*, 2008). Child abuse may result in emotional and behavioral problems that begin in childhood and can persist throughout adolescence and adulthood (Carr *et al.*, 2013). For example, child abuse increases the risk of developing depression, anxiety disorders, posttraumatic stress disorder (PTSD), substance abuse, reduced self-esteem, suicidal behavior, conduct disorder, and aggressive or delinquent behavior (Catani *et al.*, 2008; Dong *et al.*, 2003; Hermenau *et al.*, 2014; Sugaya *et al.*, 2012), as confirmed by numerous longitudinal studies (Kaplan et al., 1998; Widom, DuMont, & Czaja, 2007). Most abused children have been exposed to multiple forms of abuse, and the greater the number of different forms of abuse, the higher the likelihood of subsequent psychopathologies (Teicher, Samson, Polcari, & Mcgreenery, 2006). Furthermore, abused individuals with a psychiatric disorder are characterized by earlier onset of disease, increased symptom severity, increased comorbidity, increased risk of suicide, poorer treatment response and shorter interval before recurrence than individuals with the same diagnoses who were not abused (Harkness, Bagby, & Kennedy, 2012; Nanni, Uher, & Danese, 2012; Teicher & Samson, 2013). Finally, child abuse is a major burden not only upon the affected individual but also upon the society at large due to the high costs associated with the utilization of healthcare, educational, welfare, and law enforcement services (Fang, Brown, Florence, & Mercy, 2012).

It has been argued that the aforementioned adverse consequences may not hold for societies or communities in which many of the specific acts of child abuse are culturally normed and highly prevalent. In other words, abused individuals in

communities that deem such practices to be socially acceptable and legal would find the effects to be less harmful than those living in societies in which such practices are unacceptable or illegal. Lansford et al. (2005) empirically tested this idea in six countries. They found that more frequent corporal punishment is related to more aggression and more anxiety in all six countries. However, the strength of the relation did vary by the perceived normativeness across countries. Many other studies demonstrated detrimental consequences for the psychological well-being and development of abused children, regardless of whether or not the surrounding society deems such practices acceptable (Ani & Grantham-McGregor, 1998; Hecker, Hermenau, Isele, & Elbert, 2014; Hermenau et al., 2011).

There are many countries in which many of the acts constituting child abuse are legal and socially accepted. In Tanzania, for example, a national survey with a representative sample of more than 3700 youths revealed that the great majority (almost 75%) of both girls and boys had experienced physical abuse and more than one quarter faced emotional abuse prior to the age of 18 (UNICEF, 2011). Concordantly, we and others reported the use of harmful physical acts and psychological tactics on behalf of caregivers towards children to be highly prevalent in Tanzanian families and schools (Feinstein & Mwahombela, 2010; Hecker et al., 2014). In April 2013, the Tanzanian Government reportedly confirmed that the use of corporal punishment in public schools persists (Tanzania Daily News, 2013). Given such high prevalence of child abuse, it is vital for both individuals and societies to have a better understanding of the potential effects of abuse. In particular, whether the negative consequences of physical and emotional abuse of children are diminished in societies where such acts are legal and socially accepted.

Most studies on mental health problems have been conducted in Western samples. However, findings from DR Congo, Ethiopia and Nigeria have shown that various mental health problems such as anxiety disorders, affective disorders and hyperactivity are also common phenomena in Sub-Saharan Africa (Adelekan, Ndom, Ekpo, & Oluboka, 1999; Kashala, Elgen, Sommerfelt, & Tylleskar, 2005). Adelekan et al. (1999) indicated a prevalence rate of internalizing problems of 7.3% and of externalizing problems of 8% in a representative sample from Nigeria. Kashala et al. (2005) compared their findings in a study with a representative sample in DR Congo (Goodman, Meltzer, & Bailey, 1998) with prior findings from Great Britain. They found that the mean scores on all subscales of the Strength and Difficulties Questionnaire (SDQ) were significantly higher than the British mean scores of a

comparable sample. Hence, Cortina, Sodha, Fazel, and Ramchandani (2012) concluded that child and adolescent mental health problems are also common in Sub-Saharan Africa.

**Child abuse and the HPA axis**

The hypothalamic pituitary adrenal (HPA) axis, when functioning properly, helps us to deal with crises. It describes a set of interactions between the hypothalamus, the pituitary and the adrenal gland, which results in the release of its effector cortisol (Chrousos & Gold, 1992; de Kloet, Joëls, & Holsboer, 2005). Upon stress perception, cortiocotropin-releasing hormone (CRH) and arginine-vasopressin (AVP) are released from the hypothalamic paraventricular nucleus to activate the synthesis of pro-opiomelanocortin (POMC) in the anterior pituitary. POMC is processed into several peptides including adrenocorticotropic hormone (ACTH). Finally, ACTH is released into the blood stream and triggers the secretion of cortisol from the adrenal cortex. At each organizational level, the HPA-axis is tightly regulated by negative feedback loops meditated by glucocorticoid receptors. After binding their ligand, cortisol, glucocorticoid receptors dampen HPA-axis activity.

Child abuse is translated into negative long-term mental health outcomes via the HPA axis. It plays a central role, as it is tuned to experiences occurring early in life, making it highly susceptible to early childhood adversities (Heim & Nemeroff, 2001). For example, adults with a history of childhood maltreatment displayed altered ACTH- and cortisol-responses following exposure to an acute stressor (Carpenter et al., 2007; Heim et al., 2000). HPA-axis dysregulation is a key feature of a range of psychopathological symptoms (Chrousos & Gold, 1992; de Kloet et al., 2005). Both human and animal studies show that unremitting threat or stress weakens the immune response, increases abdominal fat, mental ill-health, and depression via alterations of HPA functioning (McEwen & Lasley, 2002). HPA axis function, and with it behavioral changes, may be stably altered through aberrant epigenetic modifications, established as the result of child abuse.

**Epigenetic modifications of HPA axis genes**

Of the various and complex mechanisms leading to epigenetic modification, DNA methylation is currently being studied most extensively. In humans, the relationship between early life adversities and the methylation of the

glucocorticoid receptor *(GR)* has been extensively studied. *GR* promoter methylation is associated with both child abuse and psychopathology (Dammann *et al.*, 2011; Hompes *et al.*, 2013; Labonté *et al.*, 2014; McGowan *et al.*, 2009). Suicide victims with a history of childhood abuse displayed increased *GR* methylation in brain tissue (Labonte et al., 2012; McGowan et al., 2009). Higher *GR* methylation in peripheral blood mononuclear cells has been observed in patients suffering from borderline personality disorder, i.e., in individuals who have usually been exposed to severe forms of abuse during development. Disruption or lack of adequate nurturing, as measured by child maltreatment and inadequate parental care, was also associated with increased *GR* promoter methylation (Perroud et al., 2011; Tyrka, Price, Marsit, Walters, & Carpenter, 2012). In addition, epigenetic changes in the *proopiomelanocortin* (*POMC*) gene may promote HPA axis dysfunction. Recent studies suggest epigenetic programming of *POMC* operates through nutritional cues, such as being underweight (Ehrlich et al., 2010), while other research suggests an association with alcohol abuse and dependence via increased craving (Muschler et al., 2010). Animal models demonstrate epigenetic programming of additional HPA-axis genes such as *CRH* (Mueller & Bale, 2008) and *AVP* (Murgatroyd et al., 2009). Thus, current research has highlighted epigenetic modifications in genes associated with the HPA axis as being a possible driving force producing child abuse-induced disorders.

In the present study we investigated associations of child abuse with both the phenotype and the methylation status of genes related to the HPA axis in Tanzanian children. We limited our analyses of DNA methylation to the genes coding for the main components of the HPA axis. That is, the genes coding for arginine-vasopressin (*AVP*), corticotrophin-releasing hormone (*CRH*) and pro-opiomelanocortion (*POMC*), from which adrenocorticotropic hormone (ACTH) is cleaved. In addition we included the gene encoding the glucocorticoid receptor (*NR3C1*), as several studies demonstrated its methylation status as being predictive for childhood abuse (Labonte et al., 2012; McGowan et al., 2009; Perroud et al., 2011; Tyrka et al., 2012). We hypothesized that (a) exposed children report more emotional and behavioral problems and (b) display altered epigenetic modifications in the genes related to HPA axis functioning.

## V.3. Methods

**Procedure**

In the context of a larger research project, a team of Tanzanian and German psychologists conducted structured interviews with a sample of Tanzanian school children (N = 409). Interviewers were taught in interview skills during a two-week training session. Furthermore, the Tanzanian interviewers were trained to translate from English to Swahili and back in order to assist the German researchers. All instruments were translated in written form to Swahili. A valid and accurate translation into English was ensured through the use of a written, blind, back-translation. In the total sample, 33 interviews were rated by two independent assessors to determine high inter-rater reliability. Prior to the interviews we sent a written informed consent form to all parents or caregivers of the children from class 2 to 7 (age: 6 – 15) explaining the purpose of the study.

Based on these structured interviews, we selected children who had been exposed to high levels of physical and emotional abuse in their homes and those who had been exposed to only low levels of physical and emotional abuse. An *a priori* power analysis ($\alpha$ =.05, power =.80, $d$ = .80) using G*Power software (Faul, Erdfelder, Lang, & Buchner, 2007) indicated a required sample size of n = 26 per group to detect significant group differences. Therefore, we aimed for two groups from the extreme ends of the abuse continuum (no abuse vs. high levels of abuse) of 30 children each. As many children, particularly younger children, reported a strong fear of drawing blood, due to harmful experiences in the Tanzanian health system, we decided not to include children of 8 years or younger. We sent an invitation and informed consent form to 96 parents and caregivers of the selected children clarifying that donating blood and saliva samples would be entirely voluntary and no monetary compensation would be offered. In total, 64% (n = 61 of 96) of the parents and caregivers signed the informed consent. We were unable to recruit enough children who had never been exposed to any type of abuse. This is not too surprising, given that several acts of child abuse are culturally normed and highly prevalent in Tanzania. In fact almost 75% reported exposure to physical abuse in a nationally representative sample (UNICEF, 2011). Nevertheless, our sampling approach resulted in two extreme groups; one group (n = 35) reporting high levels of child abuse (i.e., 6 or more different types) and one group (n = 25) reporting low levels of child abuse (i.e., 4 or less different types). In the total sample, 173 (42%) children reported low levels of child abuse with only 8

(2%) reporting no exposure to any form of child abuse. On the other hand, 175 (43%) children of the total sample reported high levels of child abuse. Only children with an informed consent signed by their caregivers and who also assented themselves orally were included in the study (only one child refused to participate despite parents informed consent). A trained nurse from the University of Konstanz with extensive work experience in East Africa collected the blood and saliva samples. The Ethical Review Board of the University of Konstanz approved the study. Other, nonepigenomic parts of the data gathered for the total sample are presented in reports by Hecker et al. (2014), Hermenau et al. (2014), and Hermenau, Eggert, Landolt and Hecker (2015).

**Participants**

The children participating in this study were enrolled at a primary school in a city of approximately 150,000 inhabitants in southern Tanzania. The high exposure group consisted of n = 35 children (60% girls) who were on average M = 11.31 years old (SD = 1.47; range: 9 – 15). The low exposure group consisted of n = 25 children (56% girls) who were on average M = 11.76 years old (SD = 1.20; range: 10 – 14).

**Measures**

All instruments were applied as a structured interview in Swahili. The first part of the interview consisted of socio-demographic information, including age, grade and gender.

*Child abuse:* We assessed exposure to abuse at home using the Maltreatment and Abuse Chronology of Exposure - Pediatric Version (pediMACE; Isele et al., 2015; Teicher & Parigger, 2015). The pediMACE is a structured interview for children consisting of 45 dichotomous (yes/no) questions, measuring witnessed or self-experienced forms of child maltreatment throughout the lifetime. In this study, we only used the 14 items covering possible forms of physical and emotional abuse (see Table V-1) by an adult person living in the same household (e.g. parent, relative or caregiver) or by a minor living in the same household (e.g. housemaid or sibling). In Tanzania many children not only grow up with their parents in one household, but also with other members of their extended families. We also focused on minors in the

household as in urban Tanzania many children are raised by an under-aged housemaid (12-17) as primary caregiver, while both parents have to work. Using an event checklist we assessed the presence of different types of abuse but not the frequency. We calculated an abuse score by totaling up all of the question responses. The possible score ranges from 0 – 14. Cohen's Kappa coefficient measuring the inter-rater reliability was > .99 (.99 – 1).

**Table V-1: Occurrence of physical and emotional abuse during the children's lifetime**

| | High exposure % (*n*) | Low exposure % (*n*) |
|---|---|---|
| *Physical abuse* | | |
| 1) Has any adult intentionally pinched, slapped, punched or kicked you? | 80 (28) | 48 (12) |
| 2) Has any adult spanked you with the palm of his/her hand on buttocks, arms or legs? | 74 (26) | 24 (6) |
| 3) Has any adult spanked you with an object such as a belt, stick, tube, wooden spoon? | 89 (31) | 60 (15) |
| 4) Has any adult hit you so hard that you were injured? | 40 (14) | 4 (1) |
| 5) Has any minor intentionally pinched, slapped, punched or kicked you? | 74 (26) | 24 (6) |
| 6) Has any minor spanked you with the palm of his/her hand on buttocks, arms or legs? | 51 (18) | 4 (1) |
| 7) Has any minor spanked you with an object such as a belt, stick, tube, wooden spoon? | 31 (11) | 0 (0) |
| 8) Has any minor hit you so hard that you were injured? | 46 (16) | 0 (0) |
| *Emotional abuse* | | |
| 9) Has any adult called you names or said hurtful things (e.g. fat, ugly, stupid)? | 51 (18) | 32 (8) |
| 10) Has any adult yelled or screamed at you? | 86 (30) | 68 (17) |
| 11) Has any adult called you locked you in a dark & narrow place (e.g. basement, closet)? | 20 (7) | 0 (0) |
| 12) Has any minor called you names or said hurtful things (e.g. fat, ugly, stupid)? | 77 (27) | 4 (1) |
| 13) Has any minor yelled or screamed at you? | 60 (21) | 8 (2) |
| 14) Has any minor called you locked you in a dark & narrow place (e.g. basement, closet)? | 3 (1) | 0 (0) |

Note. Adult = person living in the same household (e.g. parent, relative or caregiver); minor = person under the age of 18 living in the same household (e.g. housemaid or sibling).

*Mental health:* The self-evaluation of internalizing and externalizing problems was assessed with the Strengths and Difficulties Questionnaire (SDQ; Goodman, Ford, Simmons, Gatward, & Meltzer, 2000; Goodman et al., 1998). We

used the self-report version for children in interview form, which consists of 25 statements. The total difficulties score is generated by summing the scores of all items, except the items for prosocial behavior, and ranges from 0 to 40. A score over 16 indicates an enhanced level of internalizing and externalizing problems. In the present sample the Cronbach's Alpha coefficient was .71 and the Cohen's Kappa coefficient was .99 (.94 – 1).

The UCLA PTSD Index for Children DSM-IV (Steinberg, Brymer, Decker, & Pynoos, 2004) was used to screen for symptoms of PTSD, again in interview form. For each DSM-IV symptom, the frequency of occurrence within the last month is scored. The PTSD severity score ranges from 0 - 68. In the present sample Cronbach's Alpha was .92 and the Cohen's Kappa .98 (.82 – 1).

The severity of depressive symptoms was assessed by means of the Children's Depression Inventory (CDI; Kovacs, 2001; Sitarenios & Kovacs, 1999), which has already been successfully implemented and validated in Tanzanian settings (Traube, Dukay, Kaaya, Reyes, & Mellins, 2010; Wallis & Dukay, 2009). For each of its 27 items, the children were offered three statements and asked to choose the one which best describes their situation. The maximum score possible is 54. A threshold of 12 has been established as being ideal for identifying children at risk of depression (Kovacs, Goldstein, & Gastonis, 1993; Kovacs, 2001; Traube et al., 2010). In the present sample the Cronbach's Alpha was .81 and the Cohen's Kappa was .99 (.92 – 1).

*DNA Methylation:* Lymphocytes from blood were isolated via a Ficoll gradient and stored in a preservation solution (DNAgard® Tissues & Cells, Biomatrica, San Diego, USA) in order to ensure recovery of high quality DNA. In addition, saliva samples were collected and stored using the Oragene•DISCOVER (OGR-500) saliva collection kit (DNA Genotek Inc., Ontario, Canada). The tissue samples were subjected to DNA-extraction (DNeasy® Blood & Tissue Kit, Qiagen, Hilden, Germany). Genome-wide analysis of DNA methylation was then conducted at the Barts and the London Genome Centre (Queen Mary University of London, London, United Kingdom). 1µg of genomic DNA was bisulfite converted (EZ DNA Methylation Kit, Zymo) and applied to the Human Methylation 450K array (Illumina). The raw data were preprocessed using both the R package lumi (Du, Kibbe, & Lin, 2008) and Beta Mixture Quantile Dilation as suggested elsewhere (Marabita et al., 2013). After preprocessing, DNA methylation was assessed for all of the 41, 26, 14

and 14 CpG sites associated with the GR gene (NR3C1), the POMC gene, the CRH gene or the AVP gene, respectively.

*Transcription Factor Binding Sites:* To reveal potential functional properties associated with the CpG sites included in our study, the respective sequences were submitted to the Jaspar database (Mathelier et al., 2014) in order to predict known transcription factor binding sites (TFBSs). A conservative threshold of 90% sequence identity was applied.

**Data analysis**

For the analyses regarding either mental health or exposure to abuse, parametric Welch's t-tests were performed. For DNA methylation, individual 2 (abuse) X 2 (gender) ANOVAs for each CpG site were performed using exposure to abuse and gender as between group factors. We included gender in these analyses in order to account for potential effects arising from gender on DNA methylation. For three CpGs in blood (cg27107893, cg02079741, cg09916783) and one in saliva (cg23035419), the models did not fulfill the requirement of homogeneity of variances, as indicated by a significant Levene's test (Fox & Weisberg, 2011) and are thus not reported. Non-parametric tests could not be performed as these would not control for the potential influence of gender. In addition, we computed individual 2 (tissue) X 2 (gender) ANOVAs for each CpG site using tissue and gender as between group factors. Due to heterogeneity of variances, 25 probes were excluded from the analyses (*NR3C1*: cg06613263, cg08818984, cg08845721, cg10847032, cg18998365, cg19457823, cg26720913, cg27107893; *POMC:* cg02079741, cg03560973, cg08030082, cg09527270, cg09672383, cg09916783, cg13025668, cg16302441, cg20387815, cg20807790; *CRH*: cg00603617 cg23027580; AVP: cg03279206, cg04360210, cg14065127, cg23035419, cg24257309). Non-parametric tests could not be performed as these would not control for the potential influence of gender.

All analyses used a two-tailed $\alpha = .05$. Our metric for a small effect size was $d \geq .20$ or $\eta^2 \geq .01$, for a medium effect $d \geq .50$ or $\eta^2 \geq .06$, and for a large effect $d \geq .80$ or $\eta^2 \geq .13$. To adjust for multiple testing (for three mental health variables and across the CpG-sites for each gene), p-values were computed according to Benjamin-Hochberg (Benjamini *et al.*, 1995) applying a false discovery rate of 0.05. In an exploratory approach, we also considered the unadjusted p-values. All statistical

analyses were performed using IBM SPSS Statistics version 21 for Mac or R for Mac version 3.0.3.

## V.4. Results

### Mental health

Table V-2 displays the descriptive statistics for both groups. In concordance with the sample selection, the high exposure group reported a substantially higher number of different abuse types than the low exposure group. The differences between the two groups are especially notable for the items indicating that a minor in the household was the perpetrator of the abuse (see Table V-1). All mental health variables (SDQ, UCLA, CDI) differed significantly between groups with medium to large effects (see Table V-2). In total, $n$ = 11 (31%) children in the high exposure group showed an enhanced level of internalizing and externalizing problems compared to $n$ = 1 (4%) in the low exposure group. Accordingly, $n$ = 9 (26%) children in the high exposure group fulfilled the clinical diagnosis for PTSD compared to $n$ = 2 (8%) in the low exposure group. Additionally, $n$ = 10 (29%) children in the high exposure group were at risk of suffering from depression compared to $n$ = 1 (4%) in the low exposure group.

**Table V-2: Demographic characteristics of children with high and low exposure to child abuse**

| | High exposure ($n$ = 35) | | Low exposure (n = 25) | | | | |
|---|---|---|---|---|---|---|---|
| | *M* | *SD* | *M* | *SD* | *t* | *Adj. p* | *d* |
| Abuse types | 7.80 | 1.26 | 2.64 | 1.29 | 15.47 | < .001 | 4.05 |
| SDQ score | 12.31 | 5.83 | 7.48 | 4.83 | 3.50 | < .001 | 0.81 |
| UCLA score | 9.77 | 11.47 | 2.04 | 5.04 | 3.55 | < .001 | 0.77 |
| CDI score | 9.14 | 5.59 | 3.64 | 3.33 | 4.76 | < .001 | 1.00 |

*Note*. *M*: Mean, *SD*: standard deviation, *t*: test statistics based on Welch-*t*-test, *Adj. p*: adjusted p-value based on Welch-*t*-test corrected for alpha-inflation due to multiple testing, d: Cohen's d effect size.

### DNA-methylation of genes associated with the HPA axis

We found a group difference between the high exposure and low exposure group in *POMC* with higher DNA methylation in children with high exposure. This effect was particularly evident in saliva. In the saliva of the high exposure group, one CpG site was significantly hypermethylated in one-tailed tests at an adjusted

significance level of .05 and three additional CpG sites would be significantly hypermethylated in one-tailed tests at an adjusted significance level of .10 (Fig. V-1, Fig. V-2, Table V-3). Considering unadjusted p-values as well, three additional CpG sites belonging to *POMC* were differentially methylated in the saliva of the high exposure group. All of the aforementioned CpG sites displayed medium to large effect sizes. In saliva, two more CpG sites in *POMC* displayed moderate effect sizes, although unadjusted p-values exceeded the significance level of .05. In blood, six CpG sites in *POMC* were differentially methylated if unadjusted p-values are considered. These six CpG sites displayed medium to large effect sizes.

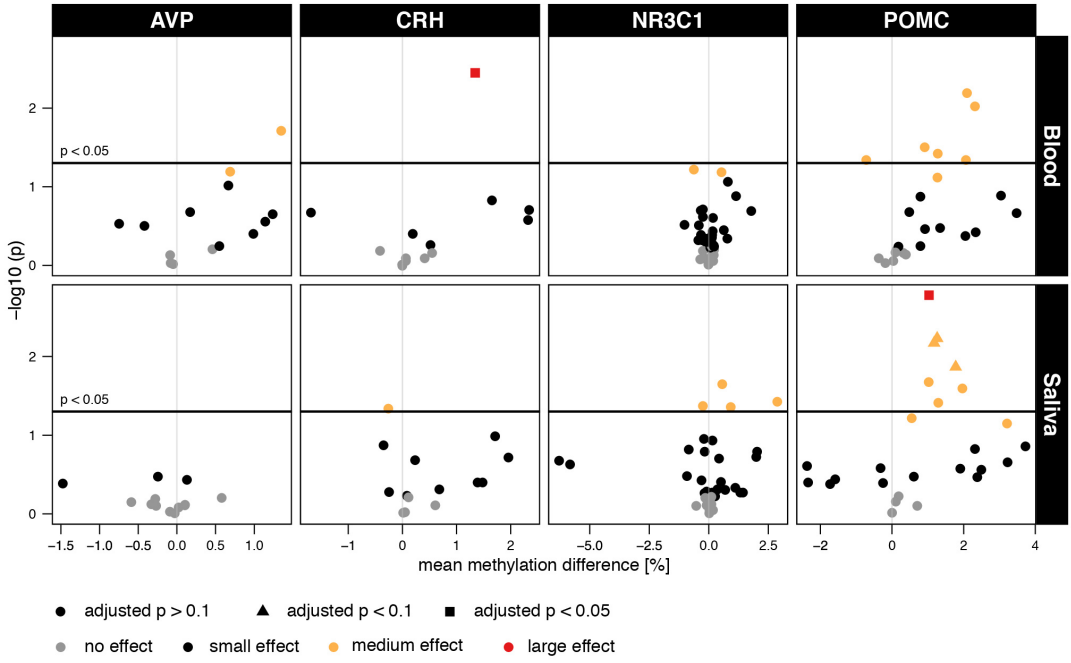


**Fig. V-1: Mean methylation differences in high and low exposure groups.**
The effect size and the level of significance are color-coded or depicted by the shape, respectively. *AVP = arginine-vasopressin* gene, *CRH = corticotropin-releasing hormone* gene, *NR3C1 = glucocorticoid receptor gene*, *POMC = proopiomelanocortin* gene.

For the remaining HPA axis genes investigated we did not find a clear group difference in DNA methylation. In saliva, four CpG sites were differently methylated in *GR* and one in *CRH* displaying moderate effect sizes and unadjusted p-values below .05. In the blood of the high exposure group, one CpG was hypermethylated in *CRH* at an adjusted significance level of .05 displaying a large effect. If uncorrected p-values were considered, one additional differentially methylated CpG could be found in *AVP* displaying a medium effect. If only effect sizes were considered, two additional CpG sites associated with *GR* differed between

the groups in blood displaying moderate effect sizes, but no significant p-values were obtained.

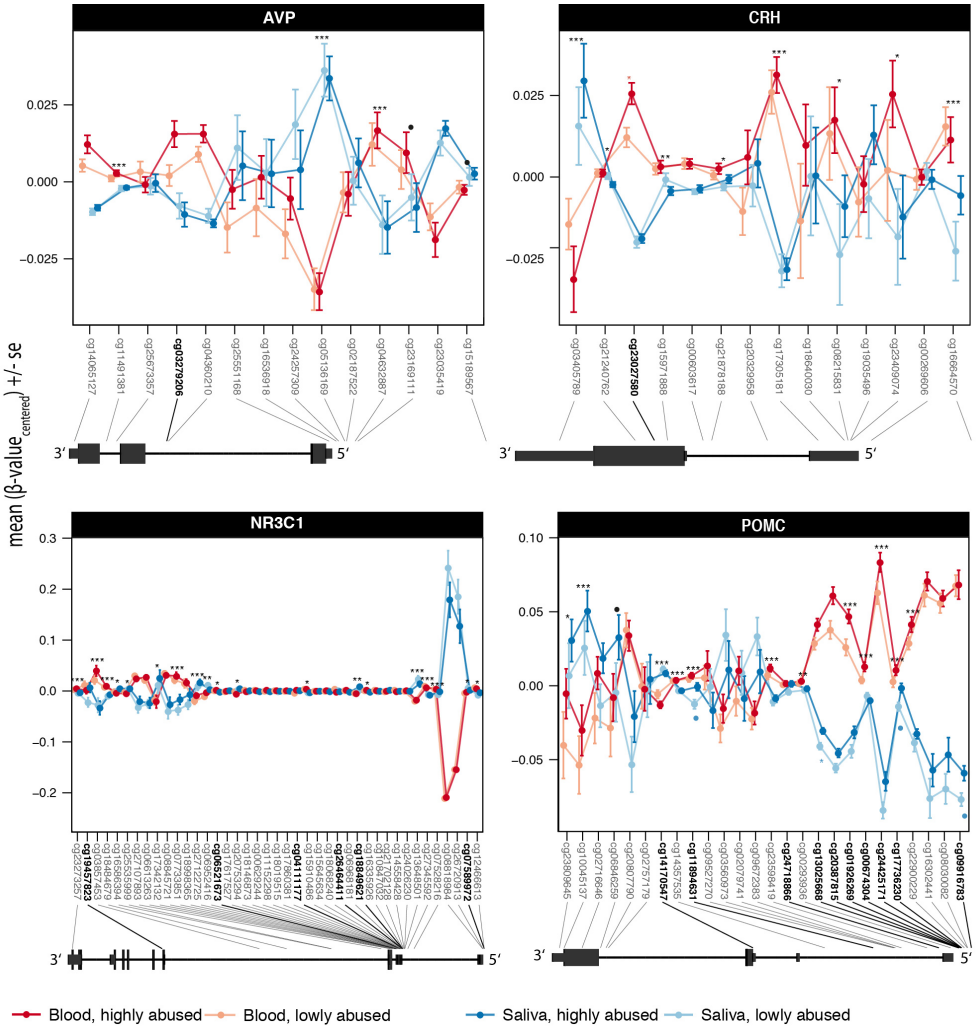


**Fig. V-2: DNA methylation of HPA axis genes.**
Mean methylation of all analyzed CpG sites. CpG sites are ordered according to their genomic location (not drawn to scale). For visual purposes, the data were mean centered. Beneath the scatterplots, the respective CpG sites and their positions in the gene model are displayed. CpG sites, which revealed at least moderate effect sizes comparing the high and low exposure groups are highlighted in black and bold font. *AVP = arginine-vasopressin gene, CRH = corticotropin-releasing hormone gene, NR3C1 = glucocorticoid receptor gene, POMC = proopiomelanocortin gene, se = standard error.*
•: adjusted $p < 0.1$, *: adjusted $p < 0.05$; **: adjusted $p < 0.01$; ***: adjusted $p < 0.001$
black asterisks/ dots depict tissue comparisons, red asterisks/ dots depict comparisons in relation to child abuse in blood, blue asterisks/ dots depict comparisons in relation to child abuse in saliva.

As we found the most pronounced effects in *POMC*, we inspected the seven and nine CpGs, which differed between the groups with at least moderate effect size in blood and saliva, respectively, in more detail. A comparison with the Jaspar-database (Fox & Weisberg, 2011) revealed that five and six of these CpGs are either located in or directly flanking a potential transcription factor binding site (TFBS). The potential TFBSs included TFAP2A, ZEB1, THAP1, YY1, BRCA1, E2F, ZNF354C,

MZF1 and SPIB. Interestingly, all of these CpGs are located in the 5'promoter, whose methylation status has been shown to modulate transcriptional activity of the *POMC* gene (Newell-Price, King, & Clark, 2001). Our analyses covered eleven and 12 CpGs in this region in blood and saliva, respectively. In blood, one CpG-site was excluded from the analyses due heterogeneity of variances. Thus, about one half of the CpGs in this region differed in their methylation by means of child abuse, and are associated with TFBSs.

**Table V-3: ANOVAs analyzing the effect of childhood abuse on DNA methylation in CpGs associated with the *AVP*, *POMC*, *NR3C1* and *CRH* gene**

| Gene | CpG | Blood | | | | Saliva | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | *F* | $\eta^2$ | *p* | *adj. p* | *F* | $\eta^2$ | *p* | *adj. p* |
| *AVP* | cg03279206 | 5.82 | **.09** | **<.05** | >.10 | 0.22 | .00 | >.10 | >.10 |
| *AVP* | cg14065127 | 3.56 | **.06** | **<.10** | <.10 | 0.82 | .01 | >.10 | >.10 |
| *CRH* | cg21240762 | 0.00 | .00 | >.10 | >.10 | 4.19 | **.07** | **<.05** | >.10 |
| *CRH* | cg23027580 | 9.29 | **.14** | **<.01** | **<.05** | 0.25 | .00 | >.10 | >.10 |
| *NR3C1* | cg04111177 | 3.56 | **.06** | <.10 | >.10 | 0.23 | .00 | >.10 | >.10 |
| *NR3C1* | cg06521673 | 0.37 | .01 | >.10 | >.10 | 4.32 | **.07** | **<.05** | >.10 |
| *NR3C1* | cg07528216 | 0.03 | .00 | >.10 | >.10 | 5.53 | **.09** | **<.05** | >.10 |
| *NR3C1* | cg18849621 | 0.05 | .00 | >.10 | >.10 | 4.25 | **.07** | **<.05** | >.10 |
| *NR3C1* | cg19457823 | 1.09 | .02 | >.10 | >.10 | 4.54 | **.08** | **<.05** | >.10 |
| *NR3C1* | cg26464411 | 3.68 | **.06** | <.10 | >.10 | 0.25 | .00 | >.10 | >.10 |
| *POMC* | cg00674304 | 4.88 | **.08** | **<.05** | >.10 | 1.30 | .02 | >.10 | >.10 |
| *POMC* | cg01926269 | 8.04 | **.13** | **<.01** | >.10 | 4.49 | **.07** | **<.05** | >.10 |
| *POMC* | cg02716646 | 2.38 | .04 | >.10 | >.10 | 3.40 | **.06** | <.10 | >.10 |
| *POMC* | cg11894631 | 0.32 | .01 | >.10 | >.10 | 7.93 | **.13** | **<.01** | **<.10** |
| *POMC* | cg13025668 | 4.55 | **.08** | **<.05** | >.10 | 10.94 | **.16** | **<.01** | **<.05** |
| *POMC* | cg14170547 | 4.18 | **.07** | **<.05** | >.10 | 0.71 | .01 | >.10 | >.10 |
| *POMC* | cg17736230 | 2.32 | .04 | >.10 | >.10 | 8.22 | **.13** | **<.01** | **<.10** |
| *POMC* | cg20387815 | 7.23 | **.12** | **<.01** | >.10 | 5.65 | **.09** | **<.05** | >.10 |
| *POMC* | cg22900229 | 3.26 | **.06** | <.10 | >.10 | 0.94 | .01 | >.01 | >.10 |
| *POMC* | cg24425171 | 4.20 | **.07** | **<.05** | >.10 | 5.28 | **.08** | **<.05** | >.10 |
| *POMC* | cg24718866 | 0.18 | .00 | >.10 | >.10 | 3.68 | **.06** | <.10 | >.10 |
| *POMC* | cg09916783 | NA | NA | NA | NA | 6.51 | **.10** | **<.05** | **<.10** |

*Note*. F: F statistic for abuse; Adj. p: adjusted p-value ; $\eta^2$ = eta square effect size; NA = not available;
*p*-values below .05, *adj. p*-values below .10 and effect sizes above .06 are highlighted in bold; *AVP = arginine-vasopressin gene; CRH = corticotropin-releasing hormone gene; NR3C1 = glucocorticoid receptor gene, POMC = proopiomelanocortin gene*. Only CpGs, which are differentially either in blood or saliva are displayed.

**DNA-methylation of the *POMC* gene strengthens children's self-reports**

Post-hoc we hypothesized that we could replicate, on the molecular level, the group allocation that was originally based on children's self-reports. We performed unsupervised hierarchical clustering on methylation of the 26 CpG sites representing the *POMC* gene using the Euclidean distance metric and the ward clustering method in the *hclust* package in R. To account for the dispersion differences across the methylation of the CpG sites, data were z-standardized prior to cluster analysis. Both in blood and saliva, two distinct clusters reflecting the high exposure and low exposure group could be detected (Fig. V-3). In blood, the analysis allocated $n$ = 39 (68%) children into their respective group and in saliva $n$ = 35 (60%). A chi-square test confirmed the significant concordance between the group allocation based on children's self-report and based on methylation value in blood ($\chi^2$= 5.95, df = 1, $p$ = .015) and showed a trend in saliva ($\chi^2$= 3.49, df = 1, $p$ = .062).

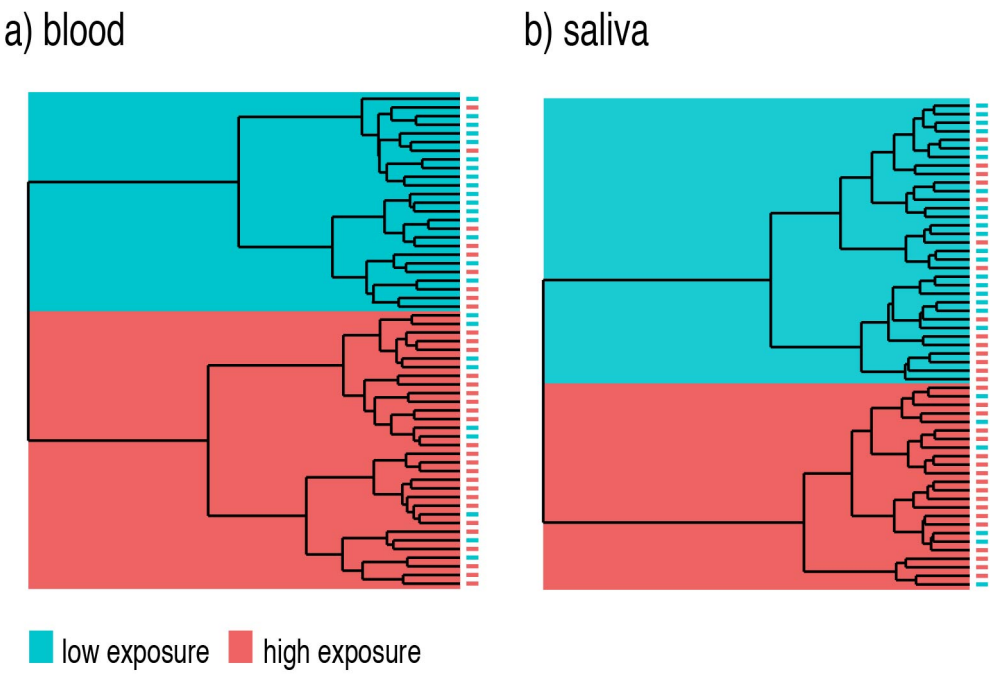


**Fig. V-3: Hierarchical clustering dendrogram**

Based on the methylation of 26 CpGs present in *POMC* a hierarchical cluster analysis has been performed. Two distinct clusters were formed in both blood (a) and saliva (b) significantly replicating the two groups that are based on children's self-reports regarding exposure to child abuse. The parts of the dendrograms highlighted in red represent the clusters containing mainly children exposed to high levels of child abuse while the turquoise highlighted segments denote the clusters containing mainly children with low exposure. The colored boxes next to the final branches denote the exposure to childhood abuse based on the self-reports (red ≙ high exposure, turquoise ≙ low exposure)

**DNA methylation of HPA axis genes**

We additionally compared DNA methylation in the four HPA-axis genes between the two tissues. Generally, blood tended to show stronger signals of DNA methylated than saliva (Fig. V-2). The only exception was seen in *AVP*, in which the pattern was reversed and saliva was characterized by elevated DNA methylation levels compared to blood. This tendency was also revealed in the ANOVAs, as we found three, eight, eighteen and eleven CpG sites in *AVP*, *CRH*, *POMC* and *NR3C1*, respectively, which displayed differential methylation between the tissues (Table S-V-1).

## V.5. Discussion

Child abuse is known to impair mental health across the entire lifespan (Carr et al., 2013). However, it has been claimed that the effects of specific forms of child abuse are not as harmful when they take place in societies or cultural groups in which such practices are common, socially accepted and legal. Lansford et al. (2005), for example, demonstrated that the relation between corporal punishment and mental health problems varied with the perceived normativeness of corporal punishment in the respective country. We and others have, however, already demonstrated the detrimental effects of child abuse in such societies (Ani & Grantham-McGregor, 1998; Hecker et al., 2014). Concordantly, in the present study children with high exposure to child abuse showed decreased psychological well-being. Furthermore, we demonstrated that this link manifests itself on a molecular level that cannot be manipulated by the subject: child abuse was strongly associated with the methylation of the *POMC* gene in both blood and saliva. To date, research incorporating child abuse and the methylation of HPA axis genes has focused mainly on the *GR* gene (de Kloet et al., 2005; Labonte et al., 2014; McGowan et al., 2009; Perroud et al., 2011). Little is known about the physiological and phenotypic consequences of *POMC* methylation. The *POMC* gene is characterized by a 5' CpG-islands, located at exon 1 and the promoter region, and a 3'CpG-island more downstream around the intron 2 and exon 3 boundary (Gardiner-Garden & Frommer, 1994). Research investigating various disease traits or stress exposure have mainly reported differential methylation at the 5' CpG-island (Mizoguchi et al., 2007; Muschler et al., 2010; Newell-Price et al., 2001; Stevens et al., 2010), but effects on the 3' CpG island (Kuehnen et al., 2012) have also been reported. In cancer tissue that did not belong to the piturity gland that

caused Cushing's syndrome (hypercortisolism), differential *POMC* methylation at the 5' CpG-island and increased ACTH levels were reported, suggesting HPA axis dysregulation, a key feature of many mental diseases (Mizoguchi et al., 2007; Newell-Price et al., 2001). Our research supports these previous findings, as the majority of differentially methylated CpGs in our study were located in the 5' CpG-island. Moreover, the respective CpGs colocate with transcription factor binding sites (TFBS), suggesting transcriptional regulation. These TFBS include an E2F response element, methylation of which has been shown to suppress POMC promoter activity *in vitro* (Newell-Price et al., 2001).

In addition to ACTH, the functionally relevant peptides β-endorphin and α-melanocyte stimulating hormone (αMSH) are cleaved from the prohormone pro-opiomelanocortin. Thus, the possible impairment of other systems than the HPA axis through *POMC* methylation has to be considered. β-endorphin has anti-nociceptive effects that are essential for stress, in particular the fight-flight situations. It also was reported to have rewarding properties and is considered as a factor in stress-related psychiatric disorders (Merenlender-Wagner, Dikshtein, & Yadid, 2009) and drug abuse (Roth-Deri, Green-Sadan, & Yadid, 2008). Indeed, *POMC* methylation was associated with alcohol craving in patients suffering from alcohol dependence (Muschler et al., 2010). Thus differential *POMC* methylation by means of child abuse, as found in our study, may heighten the risk of the development of abuse-related mental illness (Carr et al., 2013), including drug abuse (Dube et al., 2003). Abuse seems to affect the methylation of the *POMC* gene and may lead to increased emotional and behavioral problems in the children, which then increase the likelihood for further abuse. In short, settings of frequent abuse would generate a vicious cycle of further abuse and behavioral problems. Due to the nature of our study, it was not possible to test this idea statistically. Future studies using larger samples and ideally longitudinal designs should test this hypothesis empirically. Nevertheless, our findings are congruent with prior findings that child abuse is related to worse child mental health, even in a society in which specific acts of child abuse are common practice.

DNA methylation profiles appear to be tissue-specific (Ollikainen et al., 2010) and several studies indicated a clear separation of samples derived from saliva and blood (Smith et al., 2014; Thompson et al., 2013; Wu et al., 2014). Accordingly, we found significantly different methylation profiles between saliva and blood. Moreover, there was a general trend of hypermethylation in saliva, which has previously been demonstrated. However, despite tissue-specific methylation, we demonstrate that

childhood abuse is associated with DNA methylation in both saliva and blood. Thus methylation evoked by adverse experiences seems to be preserved across tissues.

Moreover, parents and caregivers often argue that children tend to over-report the exposure to abuse and the resulting harm. Thus the children's perception of their experiences is often ignored, as children are not regarded as being mature enough to accurately gauge their situation (Qvortrup, Bardy, Sgritta, & Wintersberger, 1994). Hierarchical clustering based on the methylation of *POMC*, however, allocated two-thirds of children into their respective group and a subsequent chi-square test confirmed the significant concordance between the group allocations based on children's self-report and based on methylation value. Therefore, our results strengthen the credibility of children's self-reports on a molecular level and support the conclusion that children are indeed capable of accurately reporting their exposure to abuse. In the school context of our data assessment, we were unable to include parents' reports for logistical reasons. Furthermore, we deliberately focused on the credibility of children's reports, as their view has been often neglected in research thus far. While it is possible that the inclusion of parents' reports could have further strengthened our findings, previous studies in resource-poor countries cast doubt on the validity of parents' knowledge about their children's suffering (Elbert et al., 2009).

The methodologies employed by our study present some limitations. Our data are correlational in nature and thus cannot prove a causal relationship between child abuse and methylation patterns or decreased psychological well-being. But even if certain methylation patterns might increase the likelihood of child abuse, the data still confirm the credibility of children's subjective reports and with it a wealth of data showing that abused children are more likely to suffer. However, the sample size and our study design using extreme group comparisons limit the generalizability of our findings. In the school context of our data assessment we were unable to include parents' reports for logistical reasons. Therefore, we could not gather information regarding the socio-economic status (SES) of our sample. It remains to be tested whether SES can impact DNA methylation through other pathways than abuse. Furthermore, it has been suggested that probes containing single nucleotide polymorphisms (SNPs) might result in a biased signal (Price et al., 2013). Based on the *1000 Genomes* project's database (The 1000 Genomes Project Consortium, 2012) eight SNPs colocalize with the target sequence of probes associated with *POMC*. However,

the majority of those are very rare in African populations with minor allele frequencies (MAF) below 0.2% and are thus considered not relevant to our sample. Excluding one CpGs, whose respective probe contained a SNP in their target sequence at higher MAF (i.e., 1.0%), did not markedly change the results (data not shown). Furthermore, this SNP was located more than ten base pairs away from its target CpG, which does not seem to evoke biased signals (Price et al., 2013). Therefore, we consider our findings to reflect the epigenome of the participants and not as artifacts of their genotype.

In summary, we provide further evidence that in societies or cultural groups in which many specific acts of child abuse are common, legal, and socially accepted, child abuse is nevertheless detrimental for the psychological well-being of affected children. Our evidence for such a link is strengthened by the inclusion of epigenetic information from both blood and saliva. This is the first study reporting the link between child abuse and modifications of DNA-methylation of *POMC*. Epigenetic modifications provide a promising mechanism through which child abuse could act to influence psychological well-being. In addition, on a molecular level our study strengthens the credibility of children's self-reports evaluating their exposure to abuse. All in all, our findings underscore the need to inform the population at large about the adverse consequences associated with various forms of child abuse, both those societally accepted and those not. This holds especially true in societies in which such practices are commonly employed and generally regarded as effective.

## V.6. Competing interests

The authors declare that they have no competing interests.

# VI General Discussion

## VI.1. Discussion of the results

In the preceding chapters, I described how early life adversities, by means of DNA methylation of genes associated to the HPA-axis, become persistently embedded in the DNA and how this might relate to impaired psychological wellbeing. Chapter III demonstrated increased methylation of the *glucocorticoid receptor* (*GR*) gene after prenatal exposure to maternal intimate partner violence (IPV) 10 to 19 years after birth. Although in neonates, others present similar findings after prenatal exposure to maternal depression (Oberlander *et al.*, 2008) or maternal war experience (Mulligan *et al.*, 2012), this has been the first demonstration that prenatal psychological stress can result in sustained alterations in DNA methylation. Four years later, these results have been replicated on a genome-wide basis as pregnant women's cognitive appraisal of a natural disaster (the Québec ice storm) associated with patterns of DNA methylation in the offspring 13 years later (Cao-Lei *et al.*, 2015). Evidenced in chapter IV, such alterations in DNA methylation might underlie the reliably reported decrease of psychological wellbeing seen in the aftermath of early adversities (Heim *et al.*, 2001; Talge *et al.*, 2007).

In chapter IV, I demonstrate an effect of the interaction between the environment and the epigenome on the vulnerability to develop psychopathology. In this study, the co-occurrence of methylation at the *GR* gene and childhood adversities increased the vulnerability to developing borderline personality disorder (BPD) in particular and psychopathology in general as evidenced by heightened rates of both symptoms related to psychopathological conditions and behavioral and emotional problems. Although many groups report associations between *GR* methylation and either psychopathology or adversities in early life (for reviews see Palma-Gudiel *et al.*, 2015; Turecki *et al.*, 2016), the combined contribution of *GR* methylation and early life adversities on psychological wellbeing has never been tested explicitly. This study harbors several more key findings. Rather than being limited to a single diagnosis as in previous studies, this study regards psychopathology as a general concept defined as the occurrence of several features associated with mental disease such as BPD and depression or behavioral and emotional problems. I regard this holistic approach to more accurately reflect the threats to the wellbeing of the participants even though

they might appear asymptomatic in respect to singular measurements of mental illness. Furthermore, rather than being exposed to extreme forms of stress, the children participating in this study experienced relatively moderate levels of childhood maltreatment. Substantiated by the inclusion of molecular markers, this study highlights the detrimental consequences of even moderate stress on mental health.

Particularly consequences of child abuse have been argued to be less pronounced in societies or cultural groups in which such practices are common, socially accepted and legal (Lansford *et al.*, 2005). Tanzania represents such a country in which harmful physical acts and psychological tactics towards children are highly prevalent in families and schools (Feinstein *et al.*, 2010). In accordance with previous research (Ani *et al.*, 1998; Hecker *et al.*, 2014), in chapter V, I report decreased psychological well-being in Tanzanian children exposed to high levels of child abuse. That finding was accompanied by differential methylation of HPA-axis genes, especially of the *POMC* gene, hinting towards a dysregulation of the HPA-axis and thus representing a risk factor for mental health (for a review see Heim *et al.*, 2001). These results strongly suggest that child abuse perpetrated in societies in which it is a common practice affects mental health to the same extent as in societies in which it is less frequent and accepted, although this study did not include data of the latter and thus cannot infer direct comparisons. However, the study does justify the conclusion that – in the given societal context of frequent and socially accepted child abuse – child abuse relates to a worsening of mental health.

## VI.2. Implications

In summary, all of the original research articles included in this thesis evidence persistent epigenetic changes of genes related to the HPA-axis in individuals exposed to adversities early in life and thus highlight a mechanism by which these might translate into mental illness. All analyses were conducted in peripheral tissue, blood and saliva, and not in the brain, where epigenetic modifications are most likely to affect psychological function. Therefore the results need to be interpreted cautiously. However, as the glucocorticoid receptor (GR) is ubiquitously expressed across tissues, stress-released glucocorticoids could elicit similar epigenetic modifications in the brain and in the periphery (Klengel *et al*, 2014; Szyf, 2012, 2013). Accordingly, in chapter V, I found similar associations between childhood abuse and DNA methylation in both blood and saliva. Such preservation across tissues favors the idea of a system-wide

epigenetic response to early adversities and highlights the potential of peripheral epigenetic modifications as biomarkers for identifying individuals both with a history of early adversities (as shown in chapters III-V) and at a heightened risk to develop psychopathological symptoms (as shown in chapter V). Such diagnostic potential is strengthened by studies reporting increased *GR* gene methylation in blood concomitant with altered HPA-axis activity in individuals with a history of either prenatal (Oberlander *et al.*, 2008) or childhood stress (Tyrka *et al.*, 2012). Moreover, epigenetic modifications in peripheral tissues correlate with those in the brain (Davies *et al.*, 2012; Tylee *et al.*, 2013) and with brain function (Vukojevic *et al.*, 2014; Wang *et al.*, 2012). First pilot studies in persons suffering from PTSD (Yehuda *et al.*, 2013) or BPD (Perroud *et al.*, 2013) show epigenetic modifications in blood in response to therapeutic intervention in genes related to the HPA-axis (*FKBP5*) or neuroplasticity (*BDNF*). Although these studies are in need for replication, they highlight the potential of epigenetic modifications in peripheral tissues as biomarkers for therapeutic success.

These findings point at the possibility to assist psychotherapeutic therapies by pharmacological interventions modifying the epigenome. In animal experiments hippocampal application of HDAC inhibitors (HDACi) were shown to reverse epigenetic modifications and behavioral patterns induced by low levels of maternal care (Weaver *et al.*, 2004) and to enhance fear extinction memory concomitant with histone acetylation in memory related genes (Matsumoto *et al.*, 2013). Also in humans, similar mechanisms are conceivable. Narrative Exposure Therapy (NET) for example aims at contextualizing intrusive memory representations in trauma patients i.a accomplished through basic mechanisms of learning (Schauer *et al.*, 2011). HDACis could serve as cognitive enhancers facilitating this process (for a review see Whittle *et al.*, 2014). A major challenge for pharmacological approaches acting on epigenetic modifications lies in targeting systematically administrated agents towards the relevant brain regions. This might be overcome by epigenetic priming, i.e. a more pronounced effects of HDACis in already activated cell populations and genes (for a review see Gräff *et al.*, 2013). Learning triggers epigenetic modifications favoring an open chromatin structure in neuroplasticity genes for example in the prefrontal cortex (Bredy *et al.*, 2007) and in the hippocampus (Levenson *et al.*, 2004) thus rendering these genes more accessible to HDACis.

# VII Final Conclusion

This thesis shows that early adversities in life associate with sustained epigenetic modifications in genes associated with the HPA-axis – the *POMC* gene and the *GR* gene – and thus highlights a potential mechanism by which these might translate into impaired psychological wellbeing. For the first time, I demonstrate that prenatal psychological stress leads to such persistent epigenetic modifications. The association of early adversities with *GR* methylation in blood and similar effects of childhood abuse on *POMC* methylation in both blood and saliva strongly highlight the potential of peripheral epigenetic modifications as biomarkers for early adversities and associated risks on mental health. Moreover, I demonstrate epigenome x environment interactions – as manifested in the joint occurrence of *GR* methylation and childhood maltreatment – in predicting the vulnerability to develop psychopathology. The finding that child abuse associates with both impaired psychological well-being and *POMC* methylation in Tanzanian children, substantiates the notion that child abuse harbors detrimental consequences regardless of the societal and cultural embedding. Finally this thesis emphasizes the importance of interventions targeting intimate partner violence (IPV) and childhood maltreatment. The latter might be of particular importance in societies in which practices of child abuse are commonly employed and generally regarded as effective disciplinary measures.

# Record of Achievements

**Chapter II: Epigenetic biomarkers of maternal stress**

**My Contributions:**

- Literature research
- Drafting of the manuscript in collaboration with Fernanda Serpeloni-Henning

**Chapter III: Transgenerational impact of intimate partner violence on methylation in the promoter of the glucocorticoid receptor**

**My Contributions:**

- Participation in the design of the study
- Molecular analyses
- Statistical analyses
- Drafting of the manuscript

**Chapter IV: Epigenetic modifications of the glucocorticoid receptor gene are associated with the vulnerability to psychopathology in childhood maltreatment**

**My Contributions:**

- Participation in the design of the study
- Participation in the molecular analyses, i.e. isolation of lymphocytes from whole blood and DNA extraction
- Statistical analyses
- Drafting of the manuscript

**Chapter V: Associations between child abuse, mental health and epigenetic modifications in the POMC gene: A study with children in Tanzania**

**My Contributions:**

- Participation in the design of the study
- Participation in the molecular analyses, i.e DNA extraction
- Statistical analyses
- Drafting of the manuscript in collaboration with Tobias Hecker

# References


Andersen, S. L., & Teicher, M. H. (2004). Delayed effects of early stress on hippocampal development. *Neuropsychopharmacology, 29*(11), 1988-1993.

Andersen, S. L., & Teicher, M. H. (2008a). Stress, sensitive periods and maturational events in adolescent depression. *Trends Neurosci, 31*(4), 183-191.

Andersen, S. L., Tomada, A., Vincow, E. S., Valente, E., Polcari, A., & Teicher, M. H. (2008b). Preliminary evidence for sensitive periods in the effect of childhood sexual abuse on regional brain development. *The Journal of neuropsychiatry and clinical neurosciences, 20*(3), 292-301.

Ani, C. C., & Grantham-McGregor, S. (1998). Family and personal characteristics of aggressive Nigerian boys: Differences from and similarities with Western findings. *J Adolesc Health, 23*(5), 311-317.

Austin, M.-P., Colton, J., Priest, S., Reilly, N., & Hadzi-Pavlovic, D. (2013). The Antenatal Risk Questionnaire (ANRQ): acceptability and use for psychosocial risk assessment in the maternity setting. *Women and Birth, 26*(1), 17-25.

Barker, D. J. (2004). The developmental origins of adult disease. *J Am Coll Nutr, 23*(6 Suppl).

Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society Series B (Methodological), 57*(1), 289-300.

Bergman, K., Sarkar, P., Glover, V., & O'Connor, T. G. (2010). Maternal prenatal cortisol and infant cognitive development: moderation by infant-mother attachment. *Biol Psychiatry, 67*(11), 1026-1032.

Betts, K. S., Williams, G. M., Najman, J. M., & Alati, R. (2015). The relationship between maternal depressive, anxious, and stress symptoms during pregnancy and adult offspring behavioral and emotional problems. *Depress Anxiety, 32*(2), 82-90.

Beversdorf, D. Q., Manning, S., Hillier, A., Anderson, S., Nordgren, R., Walters, S., . . . Bauman, M. (2005). Timing of prenatal stressors and autism. *J Autism Dev Disord, 35*(4), 471-478.

Blackmore, E., Moynihan, J. A., Rubinow, D. R., Pressman, E. K., Gilchrist, M., & O'Connor, T. G. (2011). Psychiatric Symptoms and Proinflammatory Cytokines in Pregnancy. *Psychosom Med, 73*(8), 656-663.

Blechert, J., Michael, T., Vriends, N., Margraf, J., & Wilhelm, F. H. (2007). Fear conditioning in posttraumatic stress disorder: evidence for delayed extinction of autonomic, experiential, and behavioural responses. *Behav Res Ther, 45*(9), 2019-2033.

Bohus, M., Limberger, M., Frank, U., Chapmanc, A., Kühler, T., & Stieglitze, R. (2007). Psychometric properties of the borderline symptom list (BSL). *Psychopathology, 40*(2), 126-132.

Borghol, N., Suderman, M., McArdle, W., Racine, A., Hallett, M., Pembrey, M., . . . Szyf, M. (2012). Associations with early-life socio-economic position in adult DNA methylation. *Int J Epidemiol, 41*(1), 62-74.

Boyle, J. S., & Lew, A. M. (1995). An inexpensive alternative to glassmilk for DNA purification. *Trends Genet, 11*(1).

Bredy, T. W., Wu, H., Crego, C., Zellhoefer, J., Sun, Y. E., & Barad, M. (2007). Histone modifications around individual BDNF gene promoters in prefrontal cortex are associated with extinction of conditioned fear. *Learn Mem, 14*(4), 268-276.

Brown, A. S., Schaefer, C. A., Wyatt, R. J., Goetz, R., Begg, M. D., Gorman, J. M., & Susser, E. S. (2000). Maternal exposure to respiratory infections and adult schizophrenia spectrum disorders: a prospective birth cohort study. *Schizophr Bull, 26*(2), 287-295.

Brown, A. S., Susser, E. S., Lin, S. P., Neugebauer, R., & Gorman, J. M. (1995). Increased risk of affective disorders in males after second trimester prenatal exposure to the Dutch hunger winter of 1944-45. *Br J Psychiatry, 166*(5), 601-606.

Brown, D. W., Anda, R. F., Felitti, V. J., Edwards, V. J., Malarcher, A. M., & Gill, W. H. (2010). Adverse childhood experiences are associated with the risk of lung cancer: a prospective cohort study. *BMC Public Health, 10*(311).

Brunton, P. J., & Russell, J. A. (2010). Prenatal Social Stress in the Rat Programmes Neuroendocrine and Behavioural Responses to Stress in the Adult Offspring: Sex - Specific Effects. *J Neuroendocrinol, 22*(4).

Buchmann, A. F., Zohsel, K., Blomeyer, D., Hohm, E., Hohmann, S., Jennen-Steinmetz, C., . . . Laucht, M. (2014). Interaction between prenatal stress and dopamine D4 receptor genotype in predicting aggression and cortisol levels in young adults. *Psychopharmacology (Berl), 231*(16), 3089-3097.

Buitelaar, J. K., Huizink, A. C., Mulder, E. J., de Medina, P. G., & Visser, G. H. (2003). Prenatal stress and cognitive development and temperament in infants. *Neurobiol Aging, 24*, S53-S60.

Buss, C., Davis, E. P., Muftuler, L. T., Head, K., & Sandman, C. A. (2010). High pregnancy anxiety during mid-gestation is associated with decreased gray matter density in 6–9-year-old children. *Psychoneuroendocrinology, 35*(1), 141-153.

Caldji, C., Tannenbaum, B., Sharma, S., Francis, D., Plotsky, P. M., & Meaney, M. J. (1998). Maternal care during infancy regulates the development of neural systems mediating the expression of fearfulness in the rat. *Proc Natl Acad Sci U S A, 95*(9), 5335-5340.

Cao-Lei, L., Elgbeili, G., Massart, R., Laplante, D., Szyf, M., & King, S. (2015). Pregnant women's cognitive appraisal of a natural disaster affects DNA methylation in their children 13 years later: Project Ice Storm. *Transl Psychiatry, 5*(2), e515.

Cao-Lei, L., Massart, R., Suderman, M. J., Machnes, Z., Elgbeili, G., Laplante, D. P., . . . King, S. (2014). DNA methylation signatures triggered by prenatal maternal stress exposure to a natural disaster: Project Ice Storm. *PLoS One, 9*(9).

Carpenter, L. L., Carvalho, J. P., Tyrka, A. R., Wier, L. M., Mello, A. F., Mello, M. F., . . . Price, L. H. (2007). Decreased adrenocorticotropic hormone and cortisol responses to stress in healthy adults reporting significant childhood maltreatment. *Biol Psychiatry*(62).

Carr, C. P., Martins, C. M., Stingel, A. M., Lemgruber, V. B., & Juruena, M. F. (2013). The role of early life stress in adult psychiatric disorders: a systematic review according to childhood trauma subtypes. *The Journal of nervous and mental disease, 201*(12), 1007-1020.

Carrion, V. G., Weems, C. F., & Reiss, A. L. (2007). Stress predicts brain changes in children: a pilot longitudinal study on youth stress, posttraumatic stress disorder, and the hippocampus. *Pediatrics, 119*(3), 509-516.

Catani, C., Jacob, N., Schauer, E., Kohila, M., & Neuner, F. (2008). Family violence, war, and natural disasters: A study of the effect of extreme stress on children's mental health in Sri Lanka. *BMC Psychiatry, 8*(1), 33.

Champagne, D. L., Bagot, R. C., van Hasselt, F., Ramakers, G., Meaney, M. J., de Kloet, E. R., . . . Krugers, H. (2008). Maternal care and hippocampal plasticity: evidence for experience-dependent structural plasticity, altered synaptic functioning, and differential responsiveness to glucocorticoids and stress. *J Neurosci, 28*(23), 6037-6045.

Champagne, F. A. (2008). Epigenetic mechanisms and the transgenerational effects of maternal care. *Front Neuroendocrinol, 29*(3), 386-397.

Champagne, F. A., & Curley, J. P. (2011). Epigenetic influence of the social environment *Brain, behavior and epigenetics* (pp. 185-208): Springer.

Chen, J., Li, Q., Rialdi, A., Mystal, E., Ly, J., Finik, J., . . . Nomura, Y. (2014). Influences of Maternal Stress during Pregnancy on the Epi/genome: Comparison of Placenta and Umbilical Cord Blood. *J Depress Anxiety, 3*(152), 2167-1044.

Chrousos, G. P., & Gold, P. W. (1992). The concepts of stress and stress system disorders. Overview of physical and behavioral homeostasis. *JAMA, 267*(9), 1244-1252.

Clark, S. J., Harrison, J., Paul, C. L., & Frommer, M. (1994). High-sensitivity mapping of methylated cytosines. *Nucleic Acids Res, 22*(15), 2990-2997.

Cohen, J. (1990). Things I have learned (so far). *Am Psychol, 45*(12), 1304-1312.

Cohen, J. (1992). A power primer. *Psychol Bull, 112*(1), 155-159.

Conradt, E., Lester, B. M., Appleton, A. A., Armstrong, D. A., & Marsit, C. J. (2013). The roles of DNA methylation of NR3C1 and 11 $\beta$ -HSD2 and exposure to maternal mood disorder in utero on newborn neurobehavior. *Epigenetics, 8*(12), 1321-1329.

Cornelius, M. D., Goldschmidt, L., Genna, D. N. M., & Larkby, C. (2012). Long-term effects of prenatal cigarette smoke exposure on behavior dysregulation among 14-year-old offspring of teenage mothers. *Association of Maternal and Child Health Programs, 16*(3), 694-705.

Coussons-Read, M. E., Okun, M. L., & Nettles, C. D. (2007). Psychosocial stress increases inflammatory markers and alters cytokine production across pregnancy. *Brain Behav Immun, 21*(3), 343-350.

Crawford, T. N., Cohen, P. R., Chen, H., Anglin, D. M., & Ehrensaft, M. (2009). Early maternal separation and the trajectory of borderline personality disorder symptoms. *Dev Psychopathol, 21*(3), 1013-1030.

Dammann, G., Teschler, S., Haag, T., Altmuller, F., Tuczek, F., & Dammann, R. H. (2011). Increased DNA methylation of neuropsychiatric genes occurs in borderline personality disorder. *Epigenetics, 6*(12), 1454-1462.

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. *Front Neurosci, 8*.

Davies, M. N., Volta, M., Pidsley, R., Lunnon, K., Dixit, A., Lovestone, S., . . . Troakes, C. (2012). Functional annotation of the human brain methylome identifies tissue-specific epigenetic variation across brain and blood. *Genome Biol, 13*(6), R43.

De Bellis, M. D., Keshavan, M. S., Clark, D. B., Casey, B., Giedd, J. N., Boring, A. M., . . . Ryan, N. D. (1999). Developmental traumatology part II: brain development. *Biol Psychiatry, 45*(10), 1271-1284.

de Kloet, E. R., Joels, M., & Holsboer, F. (2005). Stress and the brain: from adaptation to disease. *Nat Rev Neurosci, 6*(6), 463-475.

Demyttenaere, K., Bruffaerts, R., Posada-Villa, J., Gasquet, I., Kovess, V., Lepine, J. P., . . . Consortium, W. H. O. (2004). Prevalence, severity, and unmet need for treatment of mental disorders in the World Health Organization World Mental Health Surveys. *JAMA, 291*(21), 2581-2590.

Derogatis, L. R., Lipman, R. S., Rickels, K., Uhlenhuth, E. H., & Covi, L. (1974). The Hopkins Symptom Checklist (HSCL): A self - report symptom inventory. *Behav Sci, 19*(1), 1-15.

Devlin, A. M., Brain, U., Austin, J., & Oberlander, T. F. (2010). Prenatal exposure to maternal depressed mood and the MTHFR C677T variant affect SLC6A4 methylation in infants at birth. *PLoS One*.

Diego, M. A., Field, T., Hernandez-Reif, M., Cullen, C., Schanberg, S., & Kuhn, C. (2004). Prepartum, postpartum, and chronic depression effects on newborns. *Psychiatry, 67*(1), 63-80.

Dobbing, J., & Sands, J. (1973). Quantitative growth and development of human brain. *Arch Dis Child, 48*(10), 757-767.

Dobbing, J., & Sands, J. (1979). Comparative aspects of the brain growth spurt. *Early Hum Dev, 3*(1), 79-83.

Dong, M., Dube, S. R., Felitti, V. J., Giles, W. H., & Anda, R. F. (2003). Adverse childhood experiences and self-reported liver disease: new insights into the causal pathway. *Arch Intern Med, 163*.

Dong, M., Giles, W. H., Felitti, V. J., Dube, S. R., Williams, J. E., Chapman, D. P., & Anda, R. F. (2004). Insights into causal pathways for ischemic heart disease adverse childhood experiences study. *Circulation, 110*(13).

Du, P., Kibbe, W. A., & Lin, S. M. (2008). lumi: a pipeline for processing Illumina microarray. *Bioinformatics, 24*(13), 1547-1548.

Dube, S. R., Felitti, V. J., Dong, M., Chapman, D. P., Giles, W. H., & Anda, R. F. (2003). Childhood abuse, neglect, and household dysfunction and the risk of illicit drug use: The adverse childhood experiences study. *Pediatrics, 111*(3), 564-572.

Ehrlich, S., Weiss, D., Burghardt, R., Infante-Duarte, C., Brockhaus, S., Muschler, M. A., . . . Frieling, H. (2010). Promoter specific DNA methylation and gene expression of POMC in acutely underweight and recovered patients with anorexia nervosa. *J Psychiatr Res, 44*(13), 827-833.

Elbert, T., & Rockstroh, B. (2003). Stress factors. The science of our flexible responses to an unpredictable world. *Nature, 421*(6922), 477-478.

Elbert, T., Schauer, M., Ruf, M., Weierstall, R., Neuner, F., Rockstroh, B., & Junghöfer, M. (2011). The tortured brain: Imaging neural representations of traumatic stress experiences using RSVP with affective pictorial stimuli. *Zeitschrift für Psychologie/Journal of Psychology, 219*(3), 167.

Entringer, S., Buss, C., Kumsta, R., Hellhammer, D. H., Wadhwa, P. D., & Wüst, S. (2009a). Prenatal psychosocial stress exposure is associated with subsequent working memory performance in young women. *Behav Neurosci, 123*(4), 886.

Entringer, S., Kumsta, R., Hellhammer, D. H., Wadhwa, P. D., & Wüst, S. (2009b). Prenatal exposure to maternal psychosocial stress and HPA axis regulation in young adults. *Horm Behav, 55*(2), 292-298.

Entringer, S., Wüst, S., Kumsta, R., Layes, I. M., Nelson, E. L., Hellhammer, D. H., & Wadhwa, P. D. (2008). Prenatal psychosocial stress exposure is associated with insulin resistance in young adults. *Am J Obstet Gynecol, 199*(5), 498. e491-498. e497.

Essex, M. J., Thomas Boyce, W., Hertzman, C., Lam, L. L., Armstrong, J. M., Neumann, S., & Kobor, M. S. (2013). Epigenetic vestiges of early developmental adversity: childhood stress exposure and DNA methylation in adolescence. *Child Dev, 84*(1), 58-75.

Feinstein, S., & Mwahombela, L. (2010). Corporal punishment in Tanzania's schools. *International Review of Education, 56*(4), 399-410.

Felitti, V. J., Anda, R. F., Nordenberg, D., Williamson, D. F., Spitz, A. M., Edwards, V., . . . Marks, J. S. (1998). Relationship of childhood abuse and household dysfunction to many of the leading causes of death in adults: The Adverse Childhood Experiences (ACE) Study. *Am J Prev Med, 14*(4), 245-258.

Ferrari, A. J., Norman, R. E., Freedman, G., Baxter, A. J., Pirkis, J. E., Harris, M. G., . . . Vos, T. (2014). The burden attributable to mental and substance use disorders as risk factors for suicide: findings from the Global Burden of Disease Study 2010. *PLoS One, 9*(4).

Field, T., Diego, M., Hernandez-Reif, M., Salman, F., Schanberg, S., Kuhn, C., . . . Bendell, D. (2002). Prenatal anger effects on the fetus and neonate. *J Obstet Gynaecol, 22*(3), 260-266.

Francis, D., Diorio, J., Liu, D., & Meaney, M. J. (1999). Nongenomic transmission across generations of maternal behavior and stress responses in the rat. *Science, 286*(5442), 1155-1158.

Frommer, M., McDonald, L. E., Millar, D. S., Collis, C. M., Watt, F., Grigg, G. W., . . . Paul, C. L. (1992). A genomic sequencing protocol that yields a positive display of 5-methylcytosine residues in individual. *Proc Natl Acad Sci U S A, 89*(5), 1827-1831.

Fuchikami, M., Morinobu, S., Segawa, M., Okamoto, Y., Yamawaki, S., Ozaki, N., . . . Tsuchiyama, K. (2011). DNA methylation profiles of the brain-derived neurotrophic factor (BDNF) gene as a potent diagnostic biomarker in major depression. *PLoS One, 6*(8), e23881.

Gapp, K., von Ziegler, L., Tweedie‐Cullen, R. Y., & Mansuy, I. M. (2014). Early life epigenetic programming and transmission of stress‐induced traits in mammals. *Bioessays, 36*(5), 491-502.

Gitau, R., Cameron, A., Fisk, N. M., & Glover, V. (1998). Fetal exposure to maternal cortisol. *Lancet, 352*, 707-708.

Glover, V. (2011). Annual research review: prenatal stress and the origins of psychopathology: an evolutionary perspective. *J Child Psychol Psychiatry*.

Glover, V. (2014). Maternal depression, anxiety and stress during pregnancy and child outcome; what needs to be done. *Best practice & research Clinical obstetrics & gynaecology, 28*(1), 25-35.

Glover, V., O'Connor, T. G., Heron, J., Golding, J., & Team, A. S. (2004). Antenatal maternal anxiety is linked with atypical handedness in the child. *Early Hum Dev, 79*(2), 107-118.

Gluckman, P. D., & Hanson, M. A. (2004a). Living with the past: evolution, development, and patterns of disease. *Science, 305*(5691).

Gluckman, P. D., & Hanson, M. A. (2004b). Maternal constraint of fetal growth and its consequences. *Seminars in fetal and neonatal medicine*.

Gluckman, P. D., Hanson, M. A., & Spencer, H. G. (2005). Predictive adaptive responses and human evolution. *Trends in ecology and evolution, 20*(10).

Gould, E., Beylin, A., Tanapat, P., Reeves, A., & Shors, T. J. (1999). Learning enhances adult neurogenesis in the hippocampal formation. *Nat Neurosci, 2*(3), 260-265.

Gräff, J., & Tsai, L.-H. (2013). The potential of HDAC inhibitors as cognitive enhancers. *Annu Rev Pharmacol Toxicol, 53*, 311-330.

Gregory, S. G., Connelly, J. J., Towers, A. J., Johnson, J., Biscocho, D., Markunas, C. A., . . . Pericak-Vance, M. A. (2009). Genomic and epigenetic evidence for oxytocin receptor deficiency in autism. *BMC Med, 7*.

Grizenko, N., Fortier, M.-E., Zadorozny, C., Thakur, G., Schmitz, N., Duval, R., & Joober, R. (2012). Maternal stress during pregnancy, ADHD symptomatology in children and genotype: gene-environment interaction. *Journal of the Canadian Academy of Child and Adolescent Psychiatry, 21*(1), 9.

Hackett, J. A., Reddington, J. P., Nestor, C. E., Dunican, D. S., Branco, M. R., Reichmann, J., . . . Meehan, R. R. (2012). Promoter DNA methylation couples genome-defence mechanisms to epigenetic reprogramming in the mouse germline. *Development, 139*(19), 3623-3632.

Hales, C. N., & Barker, D. J. P. (2001). The thrifty phenotype hypothesis Type 2 diabetes. *Br Med Bull*.

Harkness, K. L., Bagby, R. M., & Kennedy, S. H. (2012). Childhood maltreatment and differential treatment response and recurrence in adult major depressive disorder. *J Consult Clin Psychol, 80*(3), 342-353.

Harlow, H. F., & Harlow, M. (1962). Social deprivation in monkeys. *Sci Am, 207*, 136-146.

Harlow, H. F., & Suomi, S. J. (1974). Induced depression in monkeys. *Behav Biol, 12*(3), 273-296.

Harris, E. C., & Barraclough, B. (1997). Suicide as an outcome for mental disorders - A meta-analysis. *Br J Psychiatry, 170*, 205-228.

Harrison, P. A., & Sidebottom, A. C. (2008). Systematic prenatal screening for psychosocial risks. *J Health Care Poor Underserved, 19*(1), 258-276.

Hecker, T., Hermenau, K., Isele, D., & Elbert, T. (2014). Corporal punishment and children's externalizing problems: a cross-sectional study of Tanzanian primary school aged children. *Child Abuse Negl, 38*(5), 884-892.

Hecker, T., Radtke, K. M., Hermenau, K., Papassotiropoulos, A., & Elbert, T. (2016). Associations between child abuse, mental health and epigenetic modifications in the POMC gene: A study with children in Tanzania. *Dev Psychopathol*, 1-16.

Hegarty, K., Fracgp, Bush, R., & Sheehan, M. (2005). The composite abuse scale: further development and assessment of reliability and validity of a multidimensional partner abuse measure in clinical settings. *Violence Vict, 20*(5), 529-547.

Hegarty, K., Sheehan, M., & Schonfeld, C. (1999). A Multidimensional Definition of Partner Abuse: Development and Preliminary Validation of the Composite Abuse Scale. *Journal of Family Violence, 14*(4), 399-415.

Heijmans, B. T., Tobi, E. W., & Stein, A. D. (2008). Persistent epigenetic differences associated with prenatal exposure to famine in humans. *Proceedings of the ….*

Heim, C., & Nemeroff, C. B. (2001). The role of childhood trauma in the neurobiology of mood and anxiety disorders: preclinical and clinical studies. *Biol Psychiatry, 49*(12), 1023-1039.

Heim, C., Newport, D. J., Heit, S., Graham, Y. P., Wilcox, M., Bonsall, R., . . . Nemeroff, C. B. (2000). Pituitary-adrenal and autonomic responses to stress in women after sexual and physical abuse in childhood. *JAMA, 284*(5), 592-597.

Hermenau, K., Hecker, T., Elbert, T., & Ruf-Leuschner, M. (2014). Maltreatment and mental health in institutional care - comparing early and late institutionalized children in Tanzania. *Infant mental health journal, 35*(2).

Hompes, T., Izzi, B., Gellens, E., Morreels, M., Fieuws, S., Pexsters, A., . . . Claes, S. (2013). Investigating the influence of maternal cortisol and emotional state during pregnancy on the DNA methylation status of the glucocorticoid receptor gene (NR3C1) promoter region in cord blood. *J Psychiatr Res, 47*(7), 880-891.

Huizink, A. C., Bartels, M., Rose, R. J., Pulkkinen, L., Eriksson, C. J., & Kaprio, J. (2008). Chernobyl exposure as stressor during pregnancy and behaviour in adolescent offspring. *J Epidemiol Community Health, 62*(e5), 438-446.

Huizink, A. C., de Medina, R. P. G., Mulder, E. J., Visser, H. A., & Buitlelaar, J. K. (2003). Stress during pregnancy is associated with developmental outcome in infancy. *J Child Psychol Psychiatry, 44*(6), 810-818.

Isele, D., Elbert, T., Ruf-Leuschner, M., Kraus, B., Gruber, M., & Schauer, M. (2014a). Borderline symptoms in relation to adverse childhood experiences and early sensorimotor-vestibular deprivation. *submitted for publication*.

Isele, D., Teicher, M. H., Ruf-Leuschner, M., Elbert, T., Kolassa, I.-T., Schury, K., & Schauer, M. (2014b). KERF–Ein Instrument zur umfassenden Ermittlung belastender Kindheitserfahrungen: Erstellung und psychometrische Beurteilung der deutschsprachigen MACE (Maltreatment and Abuse Chronology of Exposure) Scale. *Z Klin Psychol Psychother, 43*(2), 121-130.

Jenuwein, T., & Allis, C. D. (2001). Translating the histone code. *Science, 293*(5532), 1074-1080.

Jones, P. A. (1999). The DNA methylation paradox. *Trends Genet, 15*(1), 34-37.

Jones, P. A. (2012). Functions of DNA methylation: islands, start sites, gene bodies and beyond. *Nat Rev Genet, 13*(7), 484-492.

Joubert, B. R., Håberg, S. E., Nilsen, R. M., Wang, X., Vollset, S. E., Murphy, S. K., . . . London, S. J. (2012). 450K epigenome-wide scan identifies differential DNA methylation in newborns related to maternal smoking during pregnancy. *Environ Health Perspect, 120*(10), 1425-1431.

Kang, H.-J., Kim, J.-M., Lee, J.-Y., Kim, S.-Y., Bae, K.-Y., Kim, S.-W., . . . Yoon, J.-S. (2013). BDNF promoter methylation and suicidal behavior in depressive patients. *J Affect Disord, 151*(2), 679-685.

Kaplan, S. J., Pelcovitz, D., Salzinger, S., Weiner, M., Mandel, F. S., Lesser, M. L., & Labruna, V. E. (2014). Adolescent physical abuse: risk for adolescent psychiatric disorders. *Am J Psychiatry*.

Kapoor, A., Dunn, E., Kostaki, A., Andrews, M. H., & Matthews, S. G. (2006). Fetal programming of hypothalamo-pituitary-adrenal function: prenatal stress and glucocorticoids. *J Physiol, 572*(1), 31-44.

Kent, W. J., Sugnet, C. W., Furey, T. S., Roskin, K. M., Pringle, T. H., Zahler, A. M., & Haussler, D. (2002). The human genome browser at UCSC. *Genome Res, 12*(6), 996-1006.

Kessler, R. C., McLaughlin, K. A., Green, J. G., Gruber, M. J., Sampson, N. A., Zaslavsky, A. M., . . . Angermeyer, M. (2010). Childhood adversities and adult psychopathology in the WHO World Mental Health Surveys. *Br J Psychiatry, 197*(5), 378-385.

Khashan, A. S., Abel, K. M., McNamee, R., Pedersen, M. G., Webb, R. T., Baker, P. N., . . . Mortensen, P. (2008). Higher risk of offspring schizophrenia following antenatal maternal exposure to severe adverse life events. *Arch Gen Psychiatry, 65*(2), 146-152.

Kim, J.-M., Stewart, R., Kang, H.-J., Kim, S.-W., Shin, I.-S., Kim, H.-R., . . . Cho, K.-H. (2013). A longitudinal study of SLC6A4 DNA promoter methylation and poststroke depression. *J Psychiatr Res, 47*(9), 1222-1227.

King, S., & Laplante, D. P. (2005). The effects of prenatal maternal stress on children's cognitive development: Project Ice Storm. *Stress, 8*(1), 35-45.

King, S., Mancini-Marïe, A., Brunet, A., Walker, E., Meaney, M. J., & Laplante, D. P. (2009). Prenatal maternal stress from a natural disaster predicts dermatoglyphic asymmetry in humans. *Dev Psychopathol, 21*(02), 343-353.

Kinney, D. K., Miller, A. M., Crowley, D. J., & Gerber, E. (2008). Autism prevalence following prenatal exposure to hurricanes and tropical storms in Louisiana. *J Autism Dev Disord, 38*(3), 481-488.

Kirkham, C., Harris, S., & Grzybowski, S. (2005). Evidence-Based Prenatal Care: Part I. General Prenatal Care and Counseling Issues. *Am Fam Physician, 71*(7).

Klasen, H., Woerner, W., Rothenberger, A., & Goodman, R. (2003). German version of the Strength and Difficulties Questionnaire (SDQ-German) - overview and evaluation of initial validation and normative results. *Prax Kinderpsychol Kinderpsychiatr, 52*(7), 491-502.

Kleinhaus, K., Harlap, S., Perrin, M., Manor, O., Margalit-Calderon, R., Opler, M., . . . Malaspina, D. (2013). Prenatal stress and affective disorders in a population birth cohort. *Bipolar Disord, 15*, 92-99.

Klengel, T., & Binder, E. B. (2015). Epigenetics of Stress-Related Psychiatric Disorders and Gene × Environment Interactions. *Neuron, 86*(6), 1343-1357.

Klengel, T., Mehta, D., Anacker, C., Rex-Haffner, M., Pruessner, J., Pariante, C. M., . . . Binder, E. B. (2013). Allele-specific FKBP5 DNA demethylation mediates gene-childhood trauma interactions. *Nat Neurosci, 16*(1).

Klengel, T., Pape, J., Binder, E. B., & Mehta, D. (2014). The role of DNA methylation in stress-related psychiatric disorders. *Neuropharmacology, 80*, 115-132.

Kouzarides, T. (2007). Chromatin modifications and their function. *Cell, 128*(4), 693-705.

Krug, E. G., World Health, O., Dahlberg, L. L., Mercy, J. A., Zwi, A. B., & Lozano, R. (2002). *World report on violence and health*. Geneva: 2002: World Health Organization.

Labonté, B., Azoulay, N., Yerko, V., Turecki, G., & Brunet, A. (2014). Epigenetic modulation of glucocorticoid receptors in posttraumatic stress disorder. *Transl Psychiatry, 4*(3), e368.

Labonté, B., Suderman, M., Maussion, G., Lopez, J. P., Navarro-Sánchez, L., Yerko, V., . . . Turecki, G. (2013). Genome-wide methylation changes in the brains of suicide completers. *Genome, 170*(5).

Labonté, B., Suderman, M., Maussion, G., Navaro, L., Yerko, V., Mahar, I., . . . Meaney, M. J. (2012a). Genome-wide epigenetic regulation by early-life trauma. *Arch Gen Psychiatry, 69*(7), 722-731.

Labonté, B., Yerko, V., Gross, J., Mechawar, N., Meaney, M. J., Szyf, M., & Turecki, G. (2012b). Differential glucocorticoid receptor exon 1(B), 1(C), and 1(H) expression and methylation in suicide completers with a history of childhood abuse. *Biol Psychiatry, 72*(1), 41-48.

Lane, M., Robker, R. L., & Robertson, S. A. (2014). Parenting from before conception. *Science, 345*(6198), 756-760.

Lansford, J. E., Chang, L., Dodge, K. A., Malone, P. S., Oburu, P., Palmérus, K., . . . Zelli, A. (2005). Physical discipline and children's adjustment: Cultural normativeness as a moderator. *Child Dev, 76*(6), 1234-1246.

Laplante, D. P., Brunet, A., Schmitz, N., & Ciampi, A. (2008). Project Ice Storm: prenatal maternal stress affects cognitive and linguistic functioning in 5½-year-old children. *J Am Acad Child Adolesc Psychiatry, 47*(9), 1063-1072.

Leeb, R. T., Paulozzi, L. J., Melanson, C., Simon, T. R., & Arias, I. (2008). Child maltreatment surveillance: Uniform definitions for public health and recommended data elements. *Centers for Disease Control and Prevention, National Center for Injury Prevention and Control, 1.0*.

Levenson, J. M., O'Riordan, K. J., Brown, K. D., Trinh, M. A., Molfese, D. L., & Sweatt, J. D. (2004). Regulation of histone acetylation during memory formation in the hippocampus. *J Biol Chem, 279*(39), 40545-40559.

Lister, R., Pelizzola, M., Dowen, R. H., Hawkins, R. D., Hon, G., Tonti-Filippini, J., . . . Ngo, Q.-M. (2009). Human DNA methylomes at base resolution show widespread epigenomic differences. *Nature, 462*(7271), 315-322.

Liu, D., Diorio, J., Tannenbaum, B., Caldji, C., Francis, D., Freedman, A., . . . Meaney, M. J. (1997). Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. *Science, 277*(5332), 1659-1662.

Liu, Y., Murphy, S. K., Murtha, A. P., & Fuemmeler, B. F. (2012). Depression in pregnancy, infant birth weight and DNA methylation of imprint regulatory elements. *Epigenetics*.

Lopez-Serra, L., & Esteller, M. (2008). Proteins that bind methylated DNA and human cancer: reading the wrong words. *Br J Cancer, 98*(12), 1881-1885.

Lupien, S. J., McEwen, B. S., Gunnar, M. R., & Heim, C. (2009). Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nat Rev Neurosci, 10*(6), 434-445.

Maccari, S., Krugers, H. J., Morley‐Fletcher, S., Szyf, M., & Brunton, P. (2014). The Consequences of Early‐Life Adversity: Neurobiological, Behavioural and Epigenetic Adaptations. *J Neuroendocrinol, 26*(10), 707-723.

MacMillan, H. L., Wathen, C. N., Jamieson, E., Boyle, M. H., Shannon, H. S., Ford-Gilboe, M., . . . McNutt, L.-A. (2009). Screening for intimate partner violence in health care settings: a randomized trial. *JAMA, 302*(5), 493-501.

Marabita, F., Almgren, M., Lindholm, M. E., Ruhrmann, S., Fagerstrom-Billai, F., Jagodic, M., . . . Gomez-Cabrero, D. (2013). An evaluation of analysis pipelines for DNA methylation profiling using the Illumina HumanMethylation450 BeadChip platform. *Epigenetics, 8*(3), 333-346.

Marsit, C. J., Maccani, M. A., Padbury, J. F., & Lester, B. M. (2012). Placental 11-beta hydroxysteroid dehydrogenase methylation is associated with newborn growth and a measure of neurobehavioral outcome. *PLoS One, 7*(3).

Martin-Blanco, A., Ferrer, M., Soler, J., Salazar, J., Vega, D., Andion, O., . . . Pascual, J. C. (2014). Association between methylation of the glucocorticoid receptor gene, childhood maltreatment, and clinical severity in borderline personality disorder. *J Psychiatr Res, 57*, 34-40.

Mathelier, A., Zhao, X., Zhang, A. W., Parcy, F., Worsley-Hunt, R., Arenillas, D. J., . . . Wasserman, W. W. (2014). JASPAR 2014: an extensively expanded and updated open-access database of transcription factor binding profiles. *Nucleic Acids Res, 42*(Database issue), D142-147.

Matsumoto, Y., Morinobu, S., Yamamoto, S., Matsumoto, T., Takei, S., Fujita, Y., & Yamawaki, S. (2013). Vorinostat ameliorates impaired fear extinction possibly via the hippocampal NMDA-CaMKII pathway in an animal model of posttraumatic stress disorder. *Psychopharmacology (Berl), 229*(1).

McCormick, J. A., Lyons, V., Jacobson, M. D., Noble, J., Diorio, J., Nyirenda, M., . . . Chapman, K. E. (2000). 5 '-heterogeneity of glucocorticoid receptor messenger RNA is tissue specific: Differential regulation of variant transcripts by early-life events. *Mol Endocrinol, 14*(4), 506-517.

McCrory, E., De Brito, S. A., & Viding, E. (2010). Research review: the neurobiology and genetics of maltreatment and adversity. *J Child Psychol Psychiatry, 51*(10), 1079-1095.

McEwen, B. S., & Gianaros, P. J. (2011). Stress- and allostasis-induced brain plasticity. *Annu Rev Med, 62*, 431-445.

McEwen, B. S., & Lasley, E. N. (2002). *The end of stress as we know it*: Joseph Henry Press.

McGaugh, J. L. (2003). *Memory and emotion: the making of lasting memories*: Columbia University Press.

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. *Nat Neurosci, 12*(3), 342-348.

McGowan, P. O., Sasaki, A., Huang, T. C., Unterberger, A., Suderman, M., Ernst, C., . . . Szyf, M. (2008). Promoter-wide hypermethylation of the ribosomal RNA gene promoter in the suicide brain. *PLoS One, 3*(5), e2085.

Meaney, M. J., & Szyf, M. (2005). Maternal care as a model for experience-dependent chromatin plasticity? *Trends Neurosc, 28*(9), 456-463.

Mendelson, T., Dipietro, J. A., Costigan, K. A., Chen, P., & Henderson, J. (2011). Associations of maternal psychological factors with umbilical and uterine blood flow. *Journal of Psychosomatic Obstetrics & Gynecology, 32*(1), 3-9.

Mennes, M., Stiers, P., Lagae, L., & Van den Bergh, B. (2006). Long-term cognitive sequelae of antenatal maternal anxiety: involvement of the orbitofrontal cortex. *Neurosci Biobehav Rev, 30*(8), 1078-1086.

Milad, M. R., Pitman, R. K., Ellis, C. B., Gold, A. L., Shin, L. M., Lasko, N. B., . . . Rauch, S. L. (2009). Neurobiological basis of failure to recall extinction memory in posttraumatic stress disorder. *Biol Psychiatry, 66*(12), 1075-1082.

Moisiadis, V. G., & Matthews, S. G. (2014). Glucocorticoids and fetal programming part 2: mechanisms. *Nat Rev Endocrinol, 10*(7), 403-411.

Monk, C., Newport, D. J., Korotkin, J. H., Long, Q., Knight, B., & Stowe, Z. N. (2012). Uterine blood flow in a psychiatric population: impact of maternal depression, anxiety, and psychotropic medication. *Biol Psychiatry, 72*(6), 483-490.

Morley-Fletcher, S., Darnaudery, M., Koehl, M., Casolini, P., Van Reeth, O., & Maccari, S. (2003a). Prenatal stress in rats predicts immobility behavior in the forced swim test. Effects of a chronic treatment with tianeptine. *Brain Res, 989*(2), 246-251.

Morley-Fletcher, S., Rea, M., Maccari, S., & Laviola, G. (2003b). Environmental enrichment during adolescence reverses the effects of prenatal stress on play behaviour and HPA axis reactivity in rats. *Eur J Neurosci, 18*(12), 3367-3374.

Morris, K. V., Chan, S. W., Jacobsen, S. E., & Looney, D. J. (2004). Small interfering RNA-induced transcriptional gene silencing in human cells. *Science, 305*(5688), 1289-1292.

Morris, K. V., & Mattick, J. S. (2014). The rise of regulatory RNA. *Nat Rev Genet, 15*(6), 423-437.

Moser, D., Molitor, A., Kumsta, R., Tatschner, T., Riederer, P., & Meyer, J. (2007). The glucocorticoid receptor gene exon 1-F promoter is not methylated at the NGFI-A binding site in human hippocampus. *World J Biol Psychiatry, 8*(4), 262-268.

Mueller, B. R., & Bale, T. L. (2008). Sex-specific programming of offspring emotionality after stress early in pregnancy. *J Neurosci, 28*(36), 9055-9065.

Mulligan, C., D'Errico, N., Stees, J., & Hughes, D. (2012). Methylation changes at NR3C1 in newborns associate with maternal prenatal stress exposure and newborn birth weight. *Epigenetics, 7*(8), 853-857.

Murgatroyd, C., Patchev, A. V., Wu, Y., Micale, V., Bockmuhl, Y., Fischer, D., . . . Spengler, D. (2009). Dynamic DNA methylation programs persistent adverse effects of early-life stress. *Nat Neurosci, 12*(12), 1559-1566.

Murgatroyd, C., Quinn, J. P., Sharp, H. M., Pickles, A., & Hill, J. (2015). Effects of prenatal and postnatal depression, and maternal stroking, at the glucocorticoid receptor gene. *Transl Psychiatry, 5*(5), e560.

Murphy, S. K., Adigun, A., Huang, Z., Overcash, F., Wang, F., Jirtle, R. L., . . . Hoyo, C. (2012). Gender-specific methylation differences in relation to prenatal exposure to cigarette smoke. *Gene, 494*(1), 36-43.

Murphy, T. M., Mullins, N., Ryan, M., Foster, T., Kelly, C., McClelland, R., . . . Reilly, M. (2013). Genetic variation in DNMT3B and increased global DNA methylation is associated with suicide attempts in psychiatric patients. *Genes Brain Behav, 12*(1), 125-132.

Nanni, V., Uher, R., & Danese, A. (2012). Childhood maltreatment predicts unfavorable course of illness and treatment outcome in depression: a meta-analysis. *Am J Psychiatry, 169*(2), 141-151.

Nemoda, Z., Massart, R., Suderman, M., Hallett, M., Li, T., Coote, M., . . . Szyf, M. (2015). Maternal depression is associated with DNA methylation changes in cord blood T lymphocytes and adult hippocampi. *Transl Psychiatry, 5*.

Neugebauer, R., Hoek, H. W., & Susser, E. S. (1999). Prenatal exposure to wartime famine and development of antisocial personality disorder in early adulthood. *JAMA, 282*(5), 455-462.

Niederhofer, H., & Reiter, A. (2004). Prenatal maternal stress, prenatal fetal movements and perinatal temperament factors influence behavior and school marks at the age of 6 years. *Fetal Diagn Ther, 19*(2), 160-162.

Non, A. L., Binder, A. M., Kubzansky, L. D., & Michels, K. B. (2014). Genome-wide DNA methylation in neonates exposed to maternal depression, anxiety, or SSRI medication during pregnancy. *Epigenetics*.

Novina, C. D., & Sharp, P. A. (2004). The rnai revolution. *Nature, 430*(6996), 161-164.

O'Connor, T. G., Ben-Shlomo, Y., Heron, J., Golding, J., Adams, D., & Glover, V. (2005). Prenatal anxiety predicts individual differences in cortisol in pre-adolescent children. *Biol Psychiatry, 58*(3), 211-217.

O'Connor, T. G., Bergman, K., Sarkar, P., & Glover, V. (2013). Prenatal cortisol exposure predicts infant cortisol response to acute stress. *Dev Psychobiol, 55*(2), 145-155.

O'Connor, T. G., Caprariello, P., Blackmore, E. R., Gregory, A. M., Glover, V., Fleming, P., & Team, A. S. (2007). Prenatal mood disturbance predicts sleep problems in infancy and toddlerhood. *Early Hum Dev, 83*(7), 451-458.

O'Connor, T. G., Heron, J., Golding, J., Beveridge, M., & Glover, V. (2002). Maternal antenatal anxiety and children's behavioural/emotional problems at 4 years. Report from the Avon Longitudinal Study of Parents and Children. *Br J Psychiatry, 180*, 502-508.

O'Connor, T. G., Heron, J., Golding, J., & Glover, V. (2003). Maternal antenatal anxiety and behavioural/emotional problems in children: a test of a programming hypothesis. *J Child Psychol Psychiatry, 44*(7), 1025-1036.

O'Connor, T. G., Monk, C., & Fitelson, E. M. (2014). Practitioner review: maternal mood in pregnancy and child development–implications for child psychology and psychiatry. *J Child Psychol Psychiatry, 55*(2), 99-111.

O'Donnell, K. J., Glover, V., Jenkins, J., Browne, D., Ben-Schlomo, Y., & O'Connor, T. G. (2013). Prenatal maternal mood is associated with altered diurnal cortisol in adolescence. *Psychoneuroendocrinology, 38*(9), 1630-1638.

O'Donnell, K. J., Jensen, A. B., Freeman, L., Khalife, N., & Glover, V. (2012). Maternal prenatal anxiety and downregulation of placental 11 $\beta$ -HSD2. *Psychoneuroendocrinology, 37*(6), 818-826.

O'Donnell, K. J., O'Connor, T. G., & Glover, V. (2009). Prenatal stress and neurodevelopment of the child: focus on the HPA axis and role of the placenta. *Dev Neurosci, 31*(4), 285-292.

Oberlander, T. F., Weinberg, J., Papsdorf, M., Grunau, R., Misri, S., & Devlin, A. M. (2008). Prenatal exposure to maternal depression, neonatal methylation of human glucocorticoid receptor gene (NR3C1) and infant cortisol stress responses. *Epigenetics, 3*(2), 97106.

Ochsner, K. N., & Gross, J. J. (2005). The cognitive control of emotion. *Trends Cogn Sci, 9*(5), 242-249.

Onishchenko, N., Karpova, N., Sabri, F., Castrén, E., & Ceccatelli, S. (2008). Long ‐ lasting depression ‐ like behavior and epigenetic changes of BDNF gene expression induced by perinatal exposure to methylmercury. *J Neurochem, 106*(3), 1378-1387.

Ouellet-Morin, I., Wong, C., Danese, A., Pariante, C., Papadopoulos, A., Mill, J., & Arseneault, L. (2013). Increased serotonin transporter gene (SERT) DNA methylation is associated with bullying victimization and blunted cortisol response to stress in childhood: a longitudinal study of discordant monozygotic twins. *Psychol Med, 43*(09), 1813-1823.

Painter, R. C., de Rooij, S. R., Bossuyt, P. M., Phillips, D. I., Osmond, C., Barker, D. J., . . . Roseboom, T. J. (2006). Blood pressure response to psychological stressors in adults after prenatal exposure to the Dutch famine. *J Hypertens, 24*(9), 1771-1778.

Palma-Gudiel, H., Córdova-Palomera, A., Leza, J. C., & Fañanás, L. (2015). Glucocorticoid receptor gene (NR3C1) methylation processes as mediators of early adversity in stress-related disorders causality: A critical review. *Neurosci Biobehav Rev, 55*, 520-535.

Park, H., & Poo, M. m. (2013). Neurotrophin regulation of neural circuit development and function. *Nature Reviews Neuroscience, 14*.

Perroud, N., Paoloni-Giacobino, A., Prada, P., Olie, 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. *Transl Psychiatry, 1*(12), e59.

Perroud, N., Salzmann, A., Prada, P., & Nicastro, R. (2013). Response to psychotherapy in borderline personality disorder and methylation status of the BDNF gene. *Transl Psychiatry, 13*(1), 2207.

Phelps, E. A., & LeDoux, J. E. (2005). Contributions of the amygdala to emotion processing: from animal models to human behavior. *Neuron, 48*(2), 175-187.

Pirini, F., Guida, E., Lawson, F., Mancinelli, A., & Guerrero-Preston, R. (2015). Nuclear and Mitochondrial DNA Alterations in Newborns with Prenatal Exposure to Cigarette Smoke. *Int J Environ Res Public Health, 12*(2), 1135-1155.

Prescott, J. W. (1971). Early somatosensory deprivation as an ontogenetic process in the abnormal development of the brain and behavior *Medical primatology 1970* (pp. 356-375): S. Karger, Basel, New York.

Provencal, N., & Binder, E. B. (2015). The neurobiological effects of stress as contributors to psychiatric disorders: focus on epigenetics. *Curr Opin Neurobiol, 30*, 31-37.

Provençal, N., & Binder, E. B. (2015). The effects of early life stress on the epigenome: From the womb to adulthood and even before. *Exp Neurol, 268*, 10-20.

Provençal, N., Suderman, M. J., Guillemin, C., Massart, R., Ruggiero, A., Wang, D., . . . Szyf, M. (2012). The signature of maternal rearing in the methylome in rhesus macaque prefrontal cortex and T cells. *J Neurosci, 32*(44), 15626-15642.

Radtke, K. M., Ruf, M., Gunter, H. M., Dohrmann, K., Schauer, M., Meyer, A., & Elbert, T. (2011). Transgenerational impact of intimate partner violence on methylation in the promoter of the glucocorticoid receptor. *Transl Psychiatry, 1*(7), e21.

Rajmil, L., Alonso, J., Berra, S., Ravens-Sieberer, U., Gosch, A., Simeoni, M.-C., & Auquier, P. (2006). Use of a children questionnaire of health-related quality of life (KIDSCREEN) as a measure of needs for health care services. *J Adolesc Health, 38*(5), 511-518.

Ravelli, A. C., van der Meulen, J. H., Michels, R., Osmond, C., Barker, D. J., Hales, C., & Bleker, O. P. (1998). Glucose tolerance in adults after prenatal exposure to famine. *Lancet, 351*(9097), 173-177.

Ravelli, A. C., van der Meulen, J. H., Osmond, C., Barker, D. J., & Bleker, O. P. (1999). Obesity at the age of 50 y in men and women exposed to famine prenatally. *Am J Clin Nutr, 70*(5), 811-816.

Razin, A. (1998). CpG methylation, chromatin structure and gene silencing - a three-way connection. *EMBO J, 17*(17), 4905-4908.

Reid, A. J., Biringer, A., Carroll, J. D., Midmer, D., Wilson, L., Chalmers, B., & Stewart, D. (1998). Using the ALPHA form in practice to assess antenatal psychosocial health. *Can Med Assoc J, 159*(6), 677-684.

Resnik, R., Brink, G. W., & Wilkes, M. (1979). Catecholamine-mediated reduction in uterine blood flow after nicotine infusion in the pregnant ewe. *J Clin Invest, 63*(5), 1133-1136.

Revelle, W. (2014). psych: Procedures for personality and psychological research. *Northwestern University, Evanston R package version, 1*(1).

Rice, F., Harold, G. T., Boivin, J., van den Bree, M., Hay, D. F., & Thapar, A. (2010). The links between prenatal stress and offspring development and psychopathology: disentangling environmental and inherited influences. *Psychol Med, 40*(2), 335-345.

Roth, M., Schauer, M., & Ruf, M. (2011a). Generationsübergreifende Folgen von Trauma und Posttraumatischer Belastungsstörung bei Flüchtlingen in Deutschland. *Z Klin Psychol Psychother, submitted*.

Roth, T. L., & David Sweatt, J. (2011b). Annual Research Review: Epigenetic mechanisms and environmental shaping of the brain during sensitive periods of development. *J Child Psychol Psychiatry, 52*(4), 398-408.

RStudio. (2014). RStudio: Integrated development environment for R (Version 0.96.122) (Version 0.98.932).

Rusiecki, J. A., Byrne, C., Galdzicki, Z., Srikantan, V., Chen, L., Poulin, M., . . . Baccarelli, A. (2013). PTSD and DNA methylation in select immune function gene promoter regions: a repeated measures case-control study of US military service members. *Front Psychiatry, 4*, 56.

Rusiecki, J. A., Chen, L., Srikantan, V., Zhang, L., Yan, L., Polin, M. L., & Baccarelli, A. (2012). DNA methylation in repetitive elements and post-traumatic stress disorder: a case-control study of US military service members. *Epigenomics, 4*(1), 29-40.

Russo, V. A., Martienssen, R. A., & Riggs, A. D. (1996). Epigenetic mechanisms of gene regulation. *Cold Spring Harbor Laboratory Press*.

Sambrook, J., & Russell, D. W. (2001). *Molecular cloning*: CSHL Press.

Sandman, C. A., Davis, E. P., Buss, C., & Glynn, L. M. (2011). Prenatal programming of human neurological function. *Int J Pept, 2011*, 837596.

Sandman, C. A., Davis, E. P., & Glynn, L. M. (2012). Prescient human fetuses thrive. *Psychol Sci, 23*(1), 93-100.

Sapolsky, R. M., Krey, L. C., & McEwen, B. S. (1986). The neuroendocrinology of stress and aging: the glucocorticoid cascade hypothesis. *Endocr Rev, 7*(3), 284-301.

Sarkar, S., Craig, M. C., Dell'Acqua, F., O'Connor, T. G., Catani, M., Deeley, Q., . . . Murphy, D. G. (2014). Prenatal stress and limbic-prefrontal white matter microstructure in children aged 6–9 years: a preliminary diffusion tensor imaging study. *The World Journal of Biological Psychiatry, 15*(4), 346-352.

Sato, T., Sakado, K., Uehara, T., Narita, T., Hirano, S., Nishioka, K., & Kasahara, Y. (1998). Dysfunctional parenting as a risk factor to lifetime depression in a sample of employed Japanese adults: evidence for the 'affectionless control' hypothesis. *Psychol Med, 28*(03), 737-742.

Scarr, S., & McCartney, K. (1983). How people make their own environments: a theory of genotype greater than environment effects. *Child Dev, 54*(2), 424-435.

Schalinski, I., Elbert, T., Steudte-Schmiedgen, S., & Kirschbaum, C. (2015). The Cortisol Paradox of Trauma-Related Disorders: Lower Phasic Responses but Higher Tonic Levels of Cortisol Are Associated with Sexual Abuse in Childhood. *PLoS One, 10*(8), e0136921.

Schauer, M., Eckart, C., Schmahl, C., Bohus, M., & Elbert, T. (in prep). Reduced volume of the cerebellar vermis in individuals with borderline personality disorder.

Seckl, J. R. (2004). Prenatal glucocorticoids and long-term programming. *Eur J Endocrinol, 151*, U49-62.

Seckl, J. R., & Meaney, M. J. (2006). Glucocorticoid "programming" and PTSD risk. *Ann N Y Acad Sci, 1071*, 351-378.

Sheehan, D. V., Lecrubier, Y., Sheehan, K. H., Amorim, P., Janavs, J., Weiller, E., . . . Dunbar, G. C. (1998). The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *J Clin Psychiatry, 59 Suppl 20*, 22-33;quiz 34-57.

Sommershof, A., Aichinger, H., Engler, H., Adenauer, H., Catani, C., Boneberg, E.-M., . . . Kolassa, I.-T. (2009). Substantial reduction of naive and regulatory T cells following traumatic stress. *Brain Behav Immun, 23*(8), 1117-1124.

Soni, A. (2009). The five most costly conditions, 1996 and 2006: Estimates for the US civilian noninstitutionalized population. Statistical brief# 248. July 2009. Agency for Healthcare Research and Quality, Rockville, MD: Rockville, MD: Agency for Healthcare Research and Quality.

Spyridou, A., Schauer, M., & Ruf-Leuschner, M. (2014). Obstetric care providers assessing psychosocial risk factors during pregnancy: validation of a short screening tool–the KINDEX Spanish Version. *Child and adolescent psychiatry and mental health, 8*(1), 1-15.

Spyridou, A., Schauer, M., & Ruf-Leuschner, M. (2015). Obstetric care providers are able to assess psychosocial risks, identify and refer high-risk pregnant women: validation of a short assessment tool–the KINDEX Greek version. *BMC Pregnancy Childbirth, 15*(1), 41.

St Clair, D., Xu, M., Wang, P., Yu, Y., Fang, Y., Zhang, F., . . . He, L. (2005). Rates of adult schizophrenia following prenatal exposure to the Chinese famine of 1959-1961. *JAMA, 294*(5), 557-562.

Stanner, S. A., Bulmer, K., Andres, C., Lantseva, O. E., Borodina, V., Poteen, V. V., & Yudkin, J. A. (1997). Does malnutrition in utero determine diabetes and coronary heart disease in adulthood? Results from the Leningrad siege study, a cross sectional study. *BMJ, 315*(7119), 1342-1348.

Stein, A., Pearson, R. M., Goodman, S. H., & Rapa, E. (2014). Effects of perinatal mental disorders on the fetus and child. *Lancet, 384*(9956), 1800-1819.

Stott, D. H. (1973). Follow-up study from birth of the effects of prenatal stresses. *Dev Med Child Neurol, 15*(6), 770-787.

Suderman, M., McGowan, P. O., Sasaki, A., Huang, T. C. T., Hallett, M. T., Meaney, M. J., . . . Szyf, M. (2012). Conserved epigenetic sensitivity to early life experience in the rat and human hippocampus. *Proc Natl Acad Sci U S A, 109*(Supplement 2), 17266-17272.

Sugaya, L., Hasin, D. S., Olfson, M., Lin, K. H., Grant, B. F., & Blanco, C. (2012). Child physical abuse and adult mental health: a national study. *J Trauma Stress, 25*(4), 384-392.

Susser, E. S., & Lin, S. P. (1992). Schizophrenia after prenatal exposure to the Dutch Hunger Winter of 1944-1945. *Arch Gen Psychiatry, 49*(12), 983-988.

Susser, E. S., Neugebauer, R., Hoek, H. W., Brown, A. S., Lin, S., Labovitz, D., & Gorman, J. M. (1996). Schizophrenia after prenatal famine: further evidence. *Arch Gen Psychiatry, 53*(1), 25-31.

Szyf, M. (2011). The early life social environment and DNA methylation: DNA methylation mediating the long-term impact of social environments early in life. *Epigenetics, 6*(8), 971-978.

Szyf, M. (2012). The early-life social environment and DNA methylation. *Clin Genet, 81*(4), 341-349.

Szyf, M., Weaver, I. C. G., Champagne, F. A., Diorio, J., & Meaney, M. J. (2005). Maternal programming of steroid receptor expression and phenotype through DNA methylation in the rat. *Front Neuroendocrinol, 26*(3-4), 139-162.

Taborelli, E., Krug, I., Karwautz, A., Wagner, G., Haidvogl, M., Fernandez-Aranda, F., . . . Collier, D. (2013). Maternal anxiety, overprotection and anxious personality as risk factors for eating disorder: A sister pair study. *Cognit Ther Res, 37*(4), 820-828.

Taft, A., Small, R., Hegarty, K., Lumley, J., Watson, L., & Gold, L. (2009). MOSAIC (MOthers' Advocates In the Community): protocol and sample description of a cluster randomised trial of mentor mother support to reduce intimate partner violence among pregnant or recent mothers. *BMC Public Health, 9*, 159.

Talge, N. M., Neal, C., & Glover, V. (2007). Antenatal maternal stress and long-term effects on child neurodevelopment: how and why? *J Child Psychol Psychiatry, 48*(3-4), 245-261.

Teicher, M. H., & Andersen, S. L. (2014a). Childhood maltreatment and psychopathology: a case for ecophenotypic variants as clinically and neurobiologically distinct subtypes. *Am J Psychiatry*.

Teicher, M. H., & Parigger, A. (2011). Modified Adverse Childhood Experience Scale, version 0.9; inspired by the ACE Scale. In F. Neuner & T. Elbert (Eds.), *A Short-Term Intervention for Traumatic Stress* (Vol. 2). Cambridge/Göttingen: Huber Publishers & Hogrefe.

Teicher, M. H., & Samson, J. A. (2013). Childhood Maltreatment and Psychopathology: A Case for Ecophenotypic Variants as Clinically and Neurobiologically Distinct Subtypes. *Am J Psychiatry, 170*(10), 1114-1133.

Teicher, M. H., & Samson, J. A. (2014b). Childhood maltreatment and psychopathology: a case for ecophenotypic variants as clinically and neurobiologically distinct subtypes. *Am J Psychiatry, 170*(10), 1114-1133.

Teicher, M. H., Samson, J. A., Polcari, A., & Andersen, S. L. (2009). Length of time between onset of childhood sexual abuse and emergence of depression in a young adult sample: a retrospective clinical report. *J Clin Psychiatry, 70*(5), 684-691.

Teixeira, J. M., Fisk, N. M., & Glover, V. (1999). Association between maternal anxiety in pregnancy and increased uterine artery resistance index: cohort based study. *BMJ, 318*(7177), 153.

Terry, M. B., Delgado-Cruzata, L., Vin-Raviv, N., Wu, H. C., & Santella, R. M. (2011). DNA methylation in white blood cells: association with risk factors in epidemiologic studies. *Epigenetics, 6*(7), 828-837.

Tobi, E. W., Goeman, J. J., Monajemi, R., Gu, H., Putter, H., Zhang, Y., . . . Heijmans, B. T. (2014). DNA methylation signatures link prenatal famine exposure to growth and metabolism. *Nature Communications, 5*.

Tobi, E. W., Lumey, L., Talens, R. P., Kremer, D., Putter, H., Stein, A. D., . . . Heijmans, B. T. (2009). DNA methylation differences after exposure to prenatal famine are common and timing-and sex-specific. *Hum Mol Genet, 18*(21), 4046-4053.

Tsai, P.-C., Spector, T. D., & Bell, J. T. (2012). Using epigenome-wide association scans of DNA methylation in age-related complex human traits. *Epigenomics, 4*(5), 511-526.

Tsigos, C., & Chrousos, G. P. (2002). Hypothalamic–pituitary–adrenal axis, neuroendocrine factors and stress. *J Psychosom Res, 53*(4), 865-871.

Tung, J., Barreiro, L. B., Johnson, Z. P., Hansen, K. D., Michopoulos, V., Toufexis, D., . . . Gilad, Y. (2012). Social environment is associated with gene regulatory variation in the rhesus macaque immune system. *Proc Natl Acad Sci U S A, 109*(17), 6490-6495.

Tupler, L. A., & De Bellis, M. D. (2006). Segmented hippocampal volume in children and adolescents with posttraumatic stress disorder. *Biol Psychiatry, 59*(6), 523-529.

Turecki, G., & Meaney, M. J. (2016). Effects of the Social Environment and Stress on Glucocorticoid Receptor Gene Methylation: A Systematic Review. *Biol Psychiatry, 79*(2), 87-96.

Turner, J. D., & Muller, C. P. (2005). Structure of the glucocorticoid receptor (NR3C1) gene 5 ' untranslated region: identification, and tissue distribution of multiple new human exon 1. *J Mol Endocrinol, 35*(2), 283-292.

Tylee, D. S., Kawaguchi, D. M., & Glatt, S. J. (2013). On the outside, looking in: a review and evaluation of the comparability of blood and brain "-omes". *Am J Med Genet B Neuropsychiatr Genet, 162B*(7), 595-603.

Tyrka, A. R., Price, L. H., Marsit, C., Walters, O. C., & Carpenter, L. L. (2012). Childhood Adversity and Epigenetic Modulation of the Leukocyte Glucocorticoid Receptor: Preliminary Findings in Healthy Adults. *PLoS One, 7*(1), e30148.

Uddin, M., Aiello, A. E., Wildman, D. E., Koenen, K. C., Pawelec, G., de Los Santos, R., . . . Galea, S. (2010). Epigenetic and immune function profiles associated with posttraumatic stress disorder. *Proc Natl Acad Sci U S A, 107*(20), 9470-9475.

Uddin, M., Koenen, K., Aiello, A., Wildman, D., de Los Santos, R., & Galea, S. (2011). Epigenetic and inflammatory marker profiles associated with depression in a community-based epidemiologic sample. *Psychol Med, 41*(05), 997-1007.

Van den Bergh, B. R., Mennes, M., Oosterlaan, J., Stevens, V., Stiers, P., Marcoen, A., & Lagae, L. (2005). High antenatal maternal anxiety is related to impulsivity during performance on cognitive tasks in 14-and 15-year-olds. *Neurosci Biobehav Rev, 29*(2), 259-269.

Van den Bergh, B. R., Van Calster, B., Smits, T., Van Huffel, S., & Lagae, L. (2008). Antenatal maternal anxiety is related to HPA-axis dysregulation and self-reported depressive symptoms in adolescence: a prospective study on the fetal origins of depressed mood. *Neuropsychopharmacology, 33*(3), 536-545.

van der Knaap, L. J., Riese, H., Hudziak, J. J., Verbiest, M., Verhulst, F. C., Oldehinkel, A. J., & van Oort, F. V. A. (2014). Glucocorticoid receptor gene (NR3C1) methylation following stressful events between birth and adolescence. The TRAILS study. *Transl Psychiatry, 4*(4).

van Os, J., & Selten, J.-P. (1998). Prenatal exposure to maternal stress and subsequent schizophrenia. The May 1940 invasion of The Netherlands. *Br J Psychiatry, 172*(4), 324-326.

Veru, F., Laplante, D. P., Luheshi, G., & King, S. (2014). Prenatal maternal stress exposure and immune function in the offspring. *Stress, 17*(2), 133-148.

Vidal, A. C., Neelon, S. E., Liu, Y., Tuli, A. M., Fuemmeler, B. F., Hoyo, C., . . . Overcash, F. (2014). Maternal Stress, Preterm Birth, and DNA Methylation at Imprint Regulatory Sequences in Humans. *Genetics & epigenetics, 6*, 37.

Volpe, T., & Martienssen, R. A. (2011). RNA interference and heterochromatin assembly. *Cold Spring Harb Perspect Biol, 3*(9), a003731.

Vucetic, Z., Kimmel, J., Totoki, K., Hollenbeck, E., & Reyes, T. M. (2010). Maternal high-fat diet alters methylation and gene expression of dopamine and opioid-related genes. *Endocrinology, 151*(10), 4756-4764.

Vukojevic, V., Kolassa, I.-T., Fastenrath, M., Gschwind, L., Spalek, K., Milnik, A., . . . Demougin, P. (2014). Epigenetic modification of the glucocorticoid receptor gene is linked to traumatic memory and post-traumatic stress disorder risk in genocide survivors. *J Neurosci, 34*(31), 10274-10284.

Waddington, C. H. (1939). An introduction to modern genetics. *The Macmillan Company*.

Waddington, C. H. (1942). The Epigenotype. *Endeavour, 1*, 18-20.

Waddington, C. H. (1968). Towards a theoretical biology. *Nature, 218*(5141), 525-527.

Wakschlag, L. S., Kistner, E. O., Pine, D. S., Biesecker, G., Picket, K. E., Skol, A. D., . . . Cook, E. H. J. (2010). Interaction of prenatal exposure to cigarettes and MAOA genotype in pathways to youth antisocial behavior. *Mol Psychiatry, 15*(9), 928-937.

Wang, D., Szyf, M., Benkelfat, C., Provençal, N., Turecki, G., Caramaschi, D., . . . Booij, L. (2012). Peripheral SLC6A4 DNA methylation is associated with in vivo measures of human brain serotonin synthesis and childhood physical aggression. *PLoS One, 7*(6), e39501.

Ward, A. J. (1991). Prenatal stress and childhood psychopathology. *Child Psychiatry Hum Dev, 22*(2), 97-110.

Watson, J. B., Mednick, S. A., Huttunen, M., & Wang, X. (1999). Prenatal teratogens and the development of adult mental illness. *Dev Psychopathol, 11*(3), 457-466.

Weaver, I. C. G., Cervoni, N., Champagne, F. A., D'Alesio, A. C., Sharma, S., Seckl, J. R., . . . Meaney, M. J. (2004). Epigenetic programming by maternal behavior. *Nat Neurosci, 7*(8), 847-854.

Weaver, I. C. G., D'Alessio, A. C., Brown, S. E., Hellstrom, I. C., Dymov, S., Sharma, S., . . . Meaney, M. J. (2007). The transcription factor nerve growth factor-inducible protein a mediates epigenetic programming: altering epigenetic marks by immediate-early genes. *J Neurosci, 27*(7), 1756-1768.

Weaver, I. C. G., Meaney, M. J., & Szyf, M. (2006). Maternal care effects on the hippocampal transcriptome and anxiety-mediated behaviors in the offspring that are reversible in adulthood. *Proc Natl Acad Sci U S A, 103*(9), 3480-3485.

Whiteford, H. A., Degenhardt, L., Rehm, J., & Baxter, A. J. (2013). Global burden of disease attributable to mental and substance use disorders: findings from the Global Burden of Disease Study 2010. *Lancet, 382*(9904), 1575-1586.

Whittle, N., & Singewald, N. (2014). HDAC inhibitors as cognitive enhancers in fear, anxiety and trauma therapy: where do we stand? *Biochem Soc Trans, 42*(2), 569-581.

WHO. (2003). *Investing in mental health*. Retrieved from http://www.who.int/iris/handle/10665/42823

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. *Arch Gen Psychiatry, 64*(1), 49-56.

Wright, R. J., Visness, C. M., Calatroni, A., Grayson, M. H., Gold, D. R., Sandel, M. T., . . . Bloomberg, G. R. (2010). Prenatal maternal stress and cord blood innate and adaptive cytokine responses in an inner-city cohort. *Am J Respir Crit Care Med, 182*(1), 25-33.

Yehuda, R., Daskalakis, N. P., Desarnaud, F., Makotkine, I., Lehrner, A., Koch, E., . . . Bierer, L. M. (2013). Epigenetic biomarkers as predictors and correlates of symptom improvement following psychotherapy in combat veterans with PTSD. *Front Psychiatry, 4*, 118.

Yehuda, R., Daskalakis, N. P., Lehrner, A., Desarnaud, F., Bader, H. N., Makotkine, I., . . . Meaney, M. J. (2014). Influences of Maternal and Paternal PTSD on Epigenetic Regulation of the Glucocorticoid Receptor Gene in Holocaust Survivor Offspring. *Am J Psychiatry, 171*(8), 872-880.

Yehuda, R., Engel, S. M., Brand, S. R., Seckl, J., Marcus, S. M., & Berkowitz, G. S. (2005). Transgenerational effects of posttraumatic stress disorder in babies of mothers exposed to the World Trade Center attacks during pregnancy. *J Clin Endocrinol Metab, 90*(7), 4115-4118.

Zhao, J., Goldberg, J., Bremner, J. D., & Vaccarino, V. (2013). Association between promoter methylation of serotonin transporter gene and depressive symptoms: a monozygotic twin study. *Psychosom Med, 75*(6).

Zohsel, K., Buchmann, A. F., Blomeyer, D., Hohm, E., Schmidt, M. H., Esser, G., . . . Laucht, M. (2014). Mothers' prenatal stress and their children's antisocial outcomes–a moderating role for the Dopamine D4 Receptor (DRD4) gene. *J Child Psychol Psychiatry, 55*(1).

# Supplements

## III Transgenerational impact of intimate partner violence

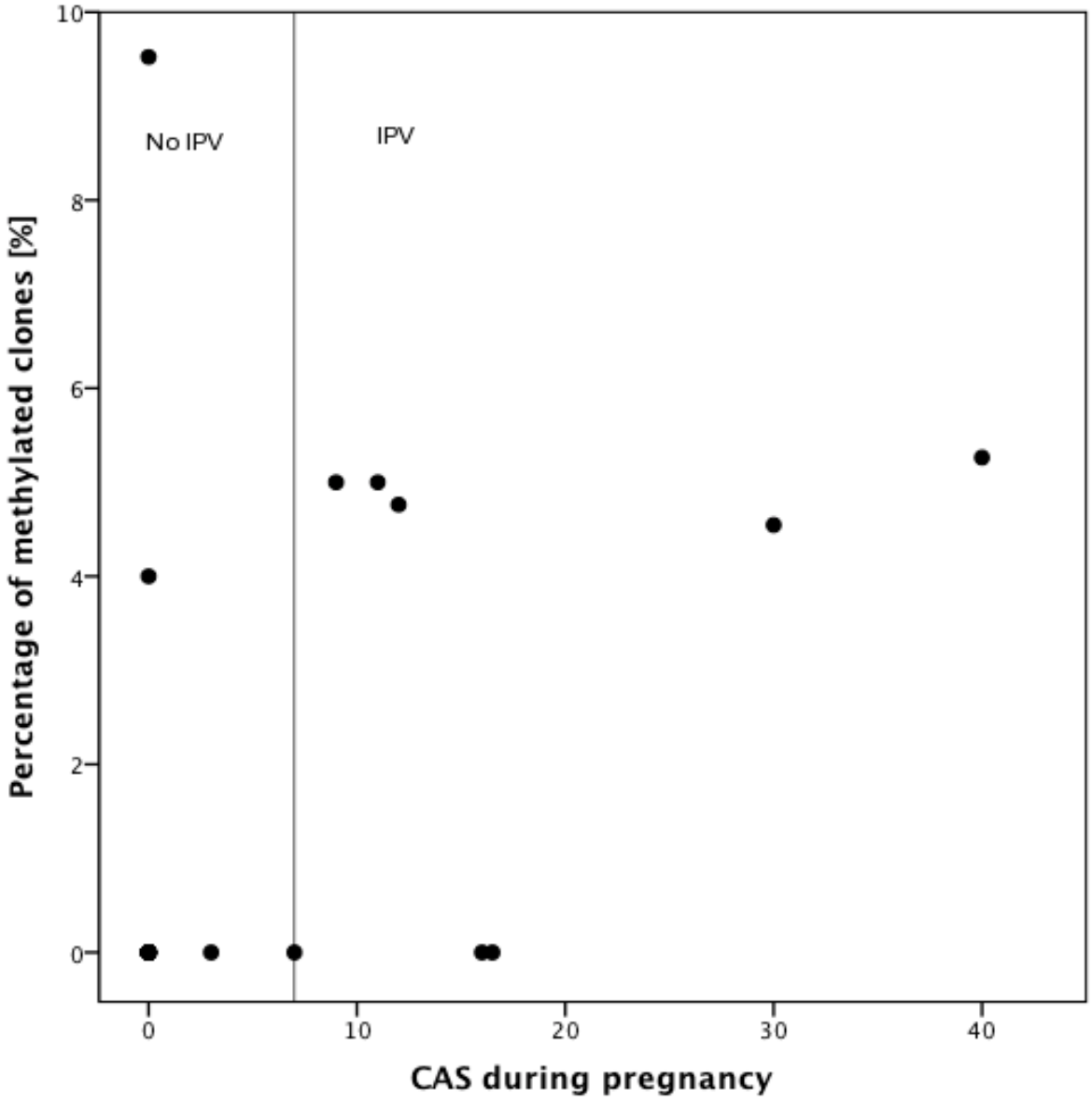


**Fig. S-III-1: Percentage of methylated clones vs CAS-score during pregnancy**

Relationship between the percentage of methylated clones of the children and maternal exposure to partner violence during pregnancy. Partner violence was evaluated by means of the CAS-score. A CAS-score of seven or higher was used as indicator for IPV exposure.

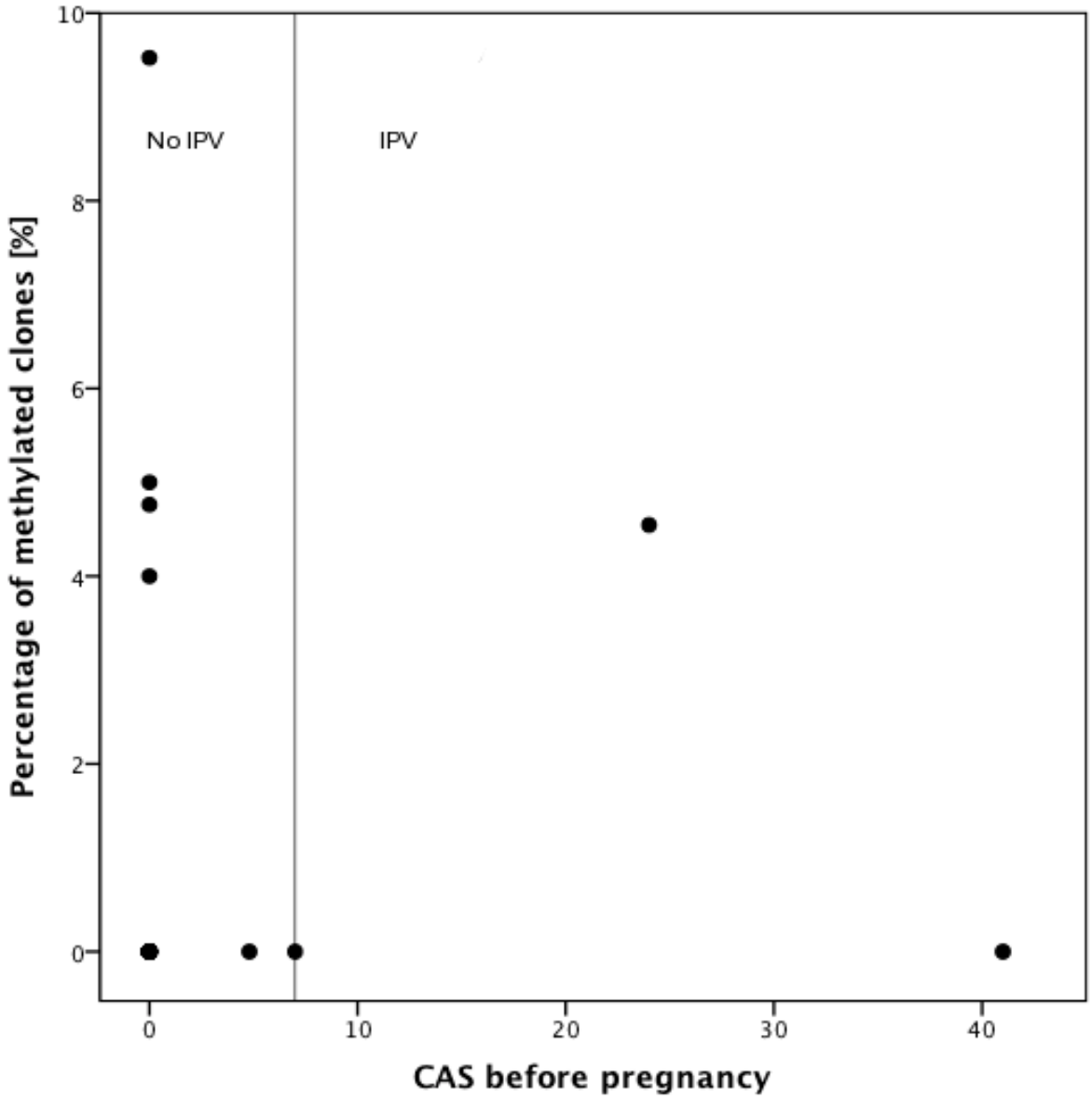


**Fig. S-III-2. Percentage of methylated clones vs CAS-score before pregnancy**

Relationship between the percentage of methylated clones of the children and maternal exposure to partner violence before pregnancy. Partner violence was evaluated by means of the CAS-score. A CAS-score of seven or higher was used as indicator for IPV exposure.

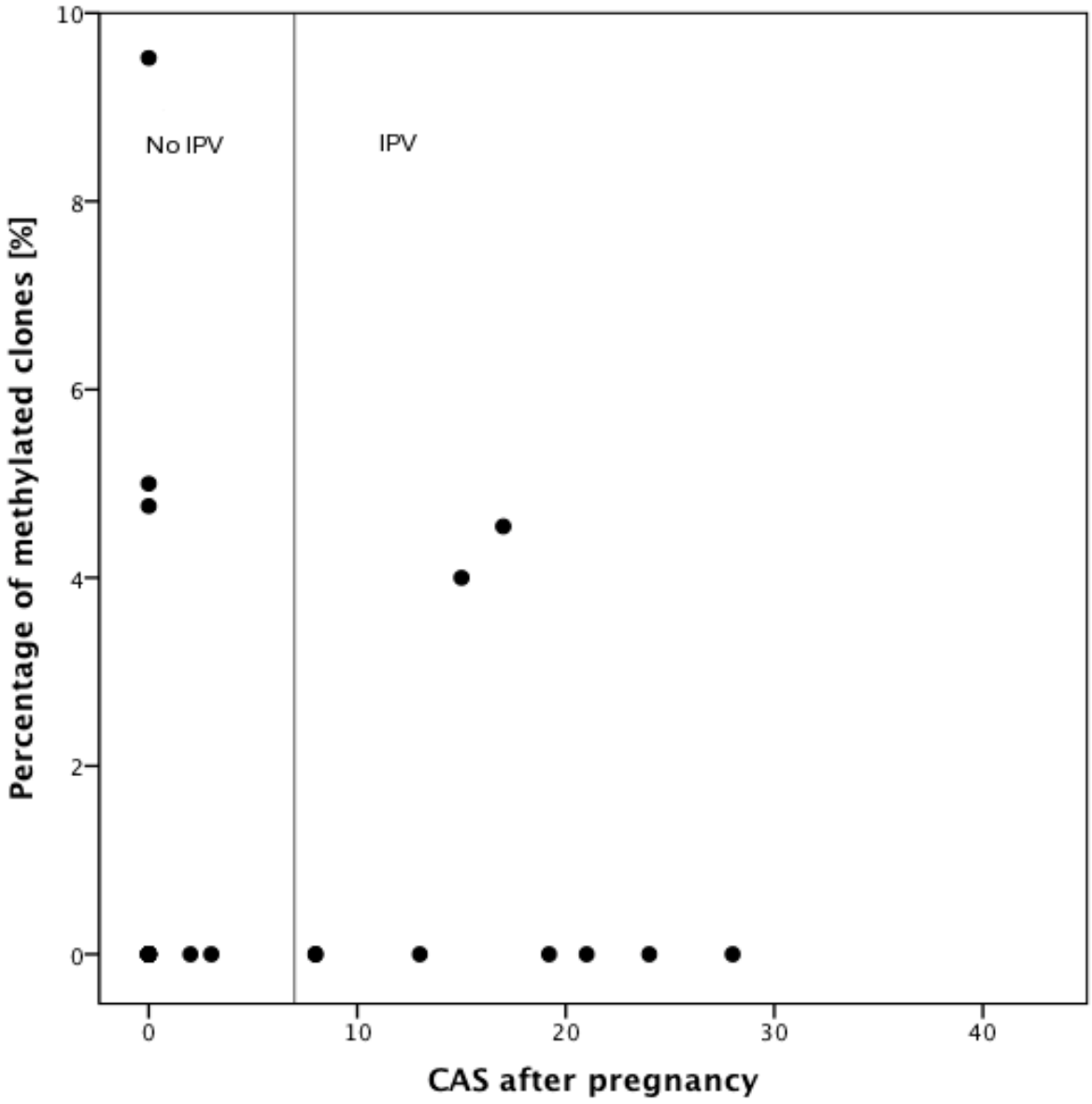


**Fig. S-III-3: Percentage of methylated clones vs CAS-score before pregnancy**

Relationship between the percentage of methylated clones of the children and maternal exposure to partner violence after pregnancy. Partner violence was evaluated by means of the CAS-score. A CAS-score of seven or higher was used as indicator for IPV exposure.

**Table S-III-1: Methylation Status vs. Third Variables**

| | | Mother | | Children | |
|---|---|---|---|---|---|
| | | n | p | n | p |
| 1 | country of origin (mother) | 25 | 1.00 | 24 | 1.00 |
| 2 | maternal age* | 25 | 1.00 | 24 | 1.00 |
| 3 | age of children** | | | 24 | 1.00 |
| 4 | marital status (mother) | 25 | 1.00 | 24 | 1.00 |
| 5 | graduation from high school, completion of vocational training or academic degree (mother) | 25 | 0.19 | 24 | 0.37 |
| 6 | pregnancy problems | 25 | 1.00 | 24 | 0.39 |
| 7 | smoking, alcohol or drug consume during pregnancy | 25 | 0.67 | 24 | 0.37 |
| 8 | use of painkillers during birth | 24 | 0.68 | 23 | 1.00 |
| 9 | anxiety, panic or helplessness during birth | 23 | 0.67 | 22 | 0.65 |
| 10 | skin contact after birth | 24 | 0.42 | 23 | 0.66 |
| 11 | severe disease of children | 18 | 0.29 | 18 | 1.00 |
| 12 | birth weight (children) | 23 | 1.00 | 23 | 0.53 |
| 13 | gestational age at birth (children) | 25 | 0.50 | 24 | 1.00 |
| 14 | mode of birth (children) | 25 | 1.00 | 24 | 0.45 |

The relationship between a variety of third variables and the methylation status of both the women and their children has been tested. Based on statistical analysis using Fisher's exact test, none of these variables were found to associate with the methylation status of women or their children.

* In order to perform Fisher's exact test maternal age has been categorized into 1) 29-36 years, 2) 37-44 years and 3) 44-51 years of age.

** In order to perform Fisher's exact test age of children has been categorized into 1) 10-12 years, 2) 13-15 years and 3) 16-19 years of age.

**Table S-III-2: Distribution of Third Variables**

| | | Mother | | Children | |
|---|---|---|---|---|---|
| | | n | Distribution | n | Distribution |
| 1 | country of origin (mother) | 25 | Ger: 3, Rus: 4, Pl: 1, Irq: 3, Rc: 1, Tr: 4, Ir: 1, Cs: 1, Srb: 6, Scg: 1 | 24 | Ger: 3, Rus: 4, Pl: 1, Irq: 3, RC: 1, Tr: 4, Ir: 1, Cs: 1, Srb: 6 |
| 2 | maternal age [years] | 25 | 29-36: 7, 37-44: 15, 45-51: 3 | 24 | 29-36: 6, 37-44: 15, 45-51: 3 |
| 3 | age of children [years] | | | 24 | 10-12: 6, 13-15: 10, 16-19: 8 |
| 4 | marital status (mother) | 25 | single: 2, married: 19, divorced or separated: 4 | 24 | single: 2, married: 18, divorced or separated: 4 |
| 5 | graduation from high school, completion of vocational training or academic degree (mother) | 25 | yes: 17, no: 8 | 24 | yes: 17, no: 7 |
| 6 | pregnancy problems | 25 | yes: 11, no: 14 | 24 | yes: 11, no: 13 |
| 7 | smoking, alcohol or drug consume during pregnancy | 25 | yes: 8, no: 17 | 24 | yes: 7, no: 17 |
| 8 | use of painkillers during birth | 24 | yes: 10, no: 14 | 23 | yes: 10, no: 13 |
| 9 | anxiety, panic or helplessness during birth | 23 | yes: 11, no: 12 | 22 | yes: 10, no: 12 |
| 10 | skin contact after birth | 24 | yes: 13, no: 11 | 23 | yes: 13, no: 10 |
| 11 | severe disease of children | 18 | yes: 9, no: 9 | 18 | yes: 9, no: 9 |
| 12 | birth weight (children) | 23 | low: 4, normal: 19 | 23 | low: 4, normal: 19 |
| 13 | gestational age at birth (children) | 25 | preterm: 2, at term: 23 | 24 | preterm: 1, at term: 23 |
| 14 | mode of birth (children) | 25 | spont.: 18, emerg. C-sect.: 3, elect. C-sect.: 3 other: 1 | 24 | spont.: 17, emerg. C-sect.: 3, elect.C-sect.: 3 other: 1 |

In the case of one mother, we could not obtain a blood sample from any of her children. Therefore sample sizes between mothers and children differs in statistical analysis.

Abbreviations: spont., spontaneous; emerg C.-sect, emergency caesarean section; elect. C-sect, elective caesarean section; Cs., Czechoslovakia; Ger., Germany; IPV, intimate partner violence; Ir., Iran; Irq., Iraq; Pl., Poland; Rus., Russia; Scg., Serbia and Montenegro; Sos., Kosovo; Tw., Taiwan; Tr., Turkey.

## IV Childhood maltreatment and *hGR* methylation

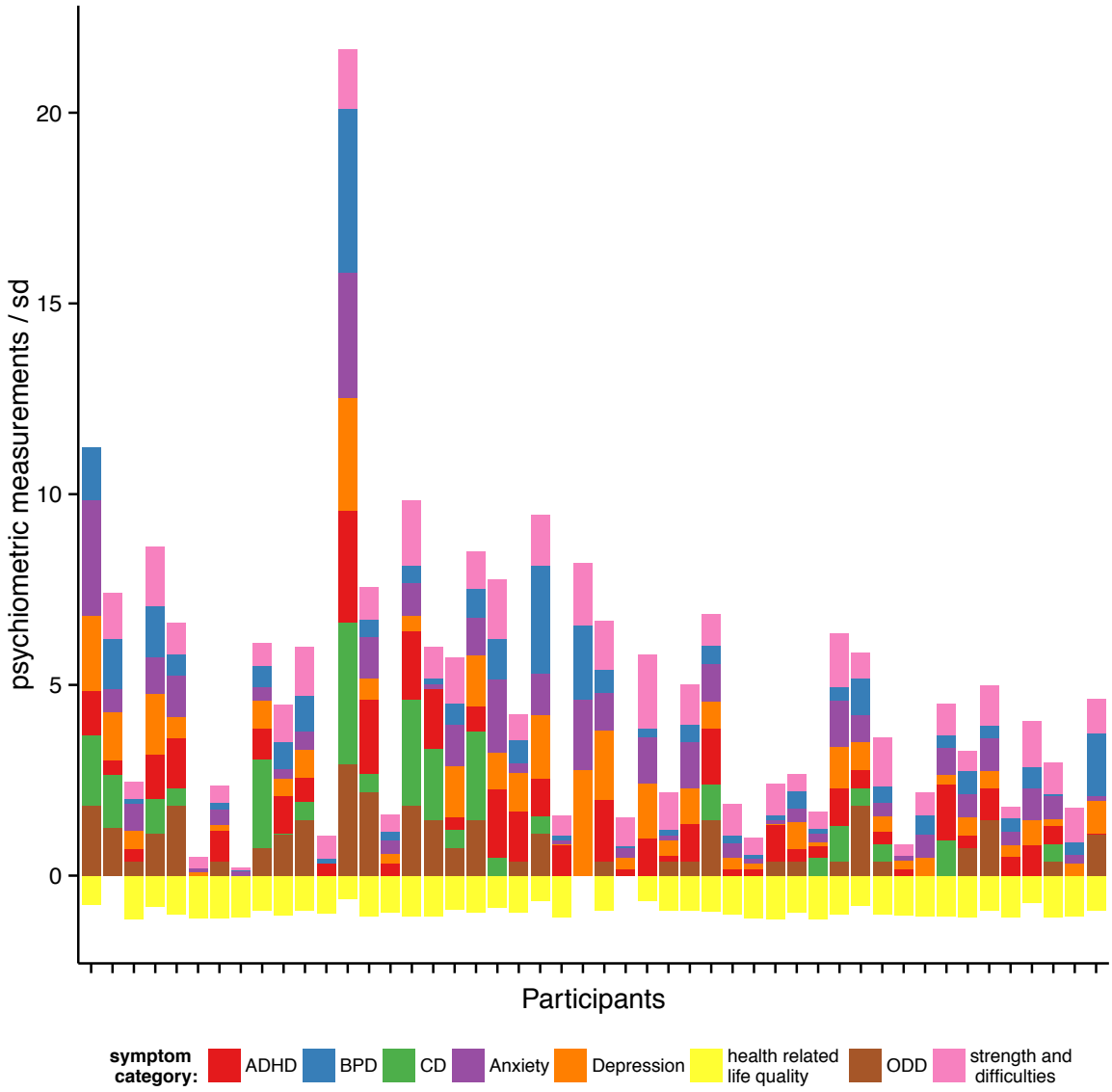


**Fig. S-IV-1**Fehler! Kein Text mit angegebener Formatvorlage im Dokument.**: Distribution of psychological health associated measurements**

All variables have been standardized by dividing through their standard deviation in order to allow for a comparison between different scales. As “heath related life quality” represents rather a measurement for psychological health than ill-health we multiplied it with minus one prior standardizing.

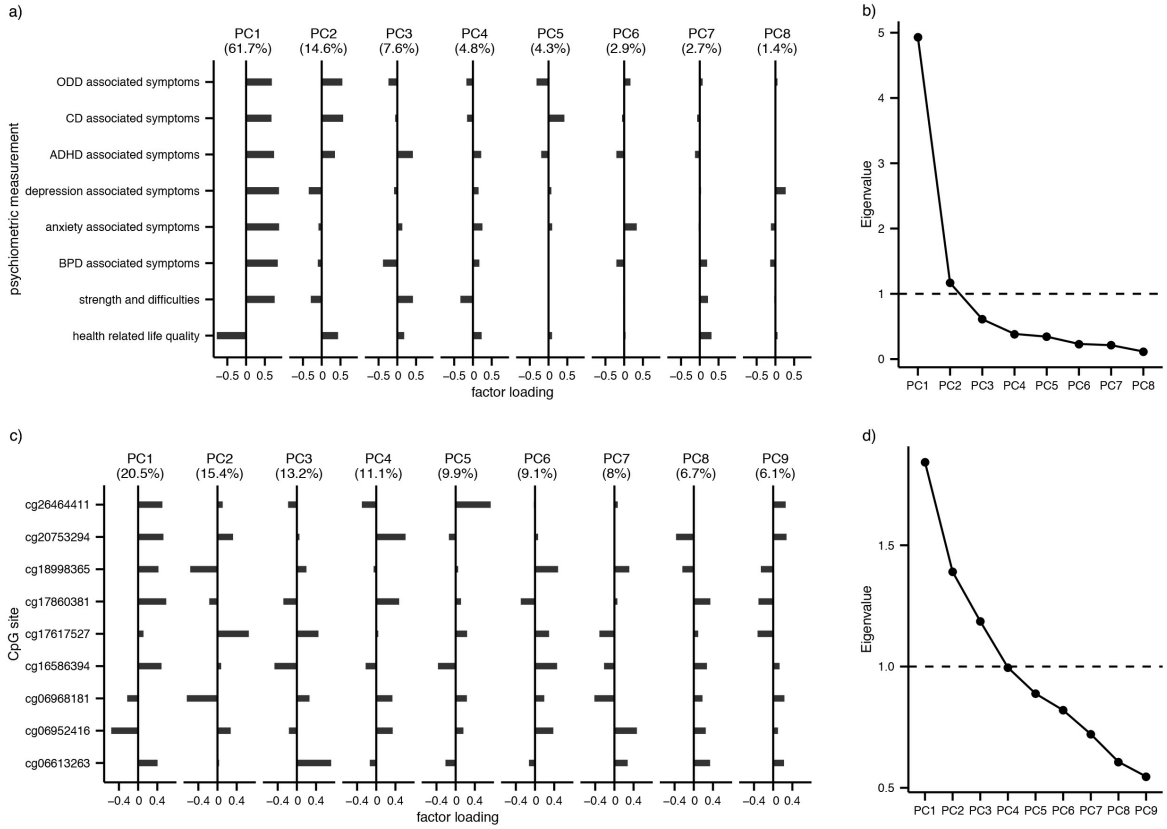


**Fig. S-IV-2: Principal component analyses (PCA) analyzing psychometric measurements or *hGR* methylation**

**a)** Factor loadings of PCA analyzing psychometric measurements; **b)** Eigenvalues of PCA analyzing psychometric measurements; **c)** factor loadings of PCA analyzing *hGR* methylation; **d)** Eigenvalues of PCA analyzing *hGR* methylation.
Abbr.: PC, principal component; Numbers in brackets in a) and b) denote the amount of explained variance

**Table S-IV-1: Mean methylation of individual CpG sites**

| CpG site | mean (methylation) [%] | sd (methylation) [%] |
|---|---|---|
| cg00629244 | 3.33 | 0.58 |
| cg03857453 | 72.82 | 6.65 |
| cg04111177 | 3.82 | 0.69 |
| cg06521673 | 2.45 | 0.56 |
| cg06613263 | 90.08 | 2.25 |
| cg06952416 | 9.00 | 1.79 |
| cg06968181 | 5.98 | 0.82 |
| cg07528216 | 96.82 | 1.20 |
| cg07589972 | 94.55 | 1.09 |
| cg07733851 | 36.29 | 3.32 |
| cg08818984 | 5.34 | 1.59 |
| cg08845721 | 94.63 | 2.33 |
| cg10847032 | 2.97 | 0.48 |
| cg11152298 | 4.95 | 0.67 |
| cg12466613 | 93.41 | 2.00 |
| cg13648501 | 9.68 | 2.33 |
| cg14558428 | 4.15 | 0.76 |
| cg15645634 | 3.61 | 0.65 |
| cg15910486 | 7.98 | 1.35 |
| cg16335926 | 2.58 | 0.36 |
| cg16586394 | 94.96 | 1.07 |
| cg17342132 | 91.17 | 5.30 |
| cg17617527 | 2.01 | 0.39 |
| cg17860381 | 3.60 | 0.83 |
| cg18019515 | 2.19 | 0.39 |
| cg18068240 | 2.42 | 0.58 |
| cg18146873 | 2.48 | 0.61 |
| cg18484679 | 93.09 | 1.92 |
| cg18849621 | 5.99 | 1.50 |
| cg18998365 | 63.97 | 3.17 |
| cg19457823 | 90.83 | 3.58 |
| cg20753294 | 7.93 | 1.77 |
| cg21702128 | 5.30 | 0.69 |
| cg23273257 | 97.57 | 0.75 |
| cg24026230 | 3.64 | 0.68 |
| cg25535999 | 93.06 | 1.38 |
| cg26464411 | 4.47 | 0.92 |
| cg26720913 | 1.84 | 0.68 |
| cg27107893 | 91.90 | 4.33 |
| cg27122725 | 14.17 | 2.66 |
| cg27345592 | 96.10 | 0.89 |

**Table S-IV-2: Correlations between DNA methylation and psychometric measurements**

| | n | r / rho | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | cg06968181 | cg26464411 | cg17860381 | cg20753294 | cg17617527 | cg06952416 | cg18998365 | cg06613263 | cg16586394 |
| n(childhood adversities) | 46 | -0.05 | 0.29 | **0.49*** | -0.05 | 0.05 | -0.08 | 0.08 | -0.17 | 0 |
| ODD associated symptoms | 46 | -0.12 | 0.21 | **0.33** | 0.08 | -0.13 | -0.17 | **0.34** | **0.31** | -0.24 |
| CD associated symptoms | 46 | -0.09 | 0.08 | 0.14 | -0.02 | -0.05 | -0.29 | **0.34** | 0.03 | -0.05 |
| ADHD associated symptoms | 45 | -0.19 | 0.19 | **0.3** | 0.06 | 0.05 | -0.13 | 0.16 | 0.07 | **-0.32** |
| Anxiety associated symptoms | 46 | -0.22 | **0.35** | 0.25 | 0.06 | 0.13 | -0.25 | 0.14 | 0.08 | -0.06 |
| Depression associated symptoms | 46 | **-0.34** | **0.41˙** | **0.41˙** | 0.06 | **0.31** | -0.17 | 0.04 | 0.12 | 0.07 |
| BPD associated symptoms | 46 | -0.17 | 0.26 | **0.53**** | 0.16 | 0.03 | -0.20 | 0.22 | 0.06 | -0.05 |
| strength and difficulties | 45 | -0.15 | **0.50*** | **0.34** | -0.03 | 0.02 | -0.07 | 0.07 | -0.09 | -0.02 |
| perceived health related life quality | 43 | 0.14 | **-0.36** | **-0.42** | **-0.31** | -0.29 | **0.31** | -0.17 | -0.12 | -0.09 |

Only CpG sites correlating with at least one psychometric measurement at medium effect size are shown. To adjust for multiple testing adjusted p-values were computed according to Benjamin-Hochberg (Benjamini *et al.*, 1995) applying a false discovery rate of 0.05. Bold depicted correlation coefficients display at least medium effect sizes. (r/rho > 0.3).
˙:adj p≤0.1; *: adj p≤0.05; **: adj p≤0.01; adj p: adjusted p value

## V Child abuse and *POMC* methylation

**Table S-V-1: ANOVAs analyzing the effect of tissue on DNA methylation in CpGs associated with the *AVP*, *POMC*, *NR3C1* and *CRH* gene**

| Gene | CpG | *F* | $\eta^2$ | *p* | *adj. p* |
|---|---|---|---|---|---|
| *AVP* | cg02187522 | 0.96 | 0.01 | >.10 | >.10 |
| *AVP* | cg04632887 | 14.25 | 0.11 | <.001 | <.001 |
| *AVP* | cg05136169 | 99.64 | 0.46 | <.001 | <.001 |
| *AVP* | cg11491381 | 24.27 | 0.18 | <.001 | <.001 |
| *AVP* | cg15189567 | 4.47 | 0.04 | <.05 | <.10 |
| *AVP* | cg16536918 | 0.34 | 0 | >.10 | >.10 |
| *AVP* | cg23169111 | 3.79 | 0.03 | <.10 | <.10 |
| *AVP* | cg25551168 | 2.84 | 0.02 | <.10 | >.10 |
| *AVP* | cg25673357 | 0.38 | 0 | >.10 | >.10 |
| *CRH* | cg00269606 | 0.05 | 0 | >.10 | >.10 |
| *CRH* | cg03405789 | 20.17 | 0.15 | <.001 | <.001 |
| *CRH* | cg08215831 | 7.25 | 0.06 | <.01 | <.05 |
| *CRH* | cg15971888 | 10.42 | 0.09 | <.01 | <.01 |
| *CRH* | cg16664570 | 15.16 | 0.11 | <.001 | <.001 |
| *CRH* | cg17305181 | 127.22 | 0.53 | <.001 | <.001 |
| *CRH* | cg18640030 | 0 | 0 | >.10 | >.10 |
| *CRH* | cg19035496 | 0.83 | 0.01 | >.10 | >.10 |
| *CRH* | cg20329958 | 0.12 | 0 | >.10 | >.10 |
| *CRH* | cg21240762 | 5.34 | 0.05 | <.05 | <.05 |
| *CRH* | cg21878188 | 5.53 | 0.05 | <.05 | <.05 |
| *CRH* | cg23409074 | 5.7 | 0.05 | <.05 | <.05 |
| *NR3C1* | cg00629244 | 2.59 | 0.02 | >.10 | >.10 |
| *NR3C1* | cg03857453 | 27.31 | 0.2 | <.001 | <.001 |
| *NR3C1* | cg04111177 | 0.85 | 0.01 | >.10 | >.10 |
| *NR3C1* | cg06521673 | 7.67 | 0.06 | <.01 | <.05 |
| *NR3C1* | cg06952416 | 11.9 | 0.1 | <.001 | <.01 |
| *NR3C1* | cg06968181 | 0.99 | 0.01 | >.10 | >.10 |
| *NR3C1* | cg07528216 | 18.28 | 0.14 | <.001 | <.001 |
| *NR3C1* | cg07589972 | 5.97 | 0.05 | <.05 | <.05 |
| *NR3C1* | cg07733851 | 34.31 | 0.23 | <.001 | <.001 |
| *NR3C1* | cg11152298 | 1.24 | 0.01 | >.10 | >.10 |
| *NR3C1* | cg12466613 | 7.48 | 0.06 | <.01 | <.05 |
| *NR3C1* | cg13648501 | 41.96 | 0.27 | <.001 | <.001 |
| *NR3C1* | cg14558428 | 0.04 | 0 | >.10 | >.10 |
| *NR3C1* | cg15645634 | 0.01 | 0 | >.10 | >.10 |
| *NR3C1* | cg15910486 | 6.66 | 0.06 | <.05 | <.05 |
| *NR3C1* | cg16335926 | 7.76 | 0.06 | <.01 | <.05 |
| *NR3C1* | cg16586394 | 6.01 | 0.05 | <.05 | <.05 |

| | | | | | |
|---|---|---|---|---|---|
| *NR3C1* | cg17342132 | 7.87 | 0.07 | <.01 | <.05 |
| *NR3C1* | cg17617527 | 0.91 | 0.01 | >.10 | >.10 |
| *NR3C1* | cg17860381 | 0.66 | 0 | >.10 | >.10 |
| *NR3C1* | cg18019515 | 3.26 | 0.03 | <.10 | >.10 |
| *NR3C1* | cg18068240 | 0.72 | 0.01 | >.10 | >.10 |
| *NR3C1* | cg18146873 | 0.18 | 0 | >.10 | >.10 |
| *NR3C1* | cg18484679 | 26.71 | 0.19 | <.001 | <.001 |
| *NR3C1* | cg18849621 | 11.05 | 0.09 | <.01 | <.01 |
| *NR3C1* | cg20753294 | 6.43 | 0.05 | <.05 | <.05 |
| *NR3C1* | cg21702128 | 1.74 | 0.02 | >.10 | >.10 |
| *NR3C1* | cg23273257 | 26.03 | 0.19 | <.001 | <.001 |
| *NR3C1* | cg24026230 | 0.49 | 0 | >.10 | >.10 |
| *NR3C1* | cg25535999 | 8.34 | 0.07 | <.01 | <.05 |
| *NR3C1* | cg26464411 | 0.25 | 0 | >.10 | >.10 |
| *NR3C1* | cg27122725 | 19.01 | 0.14 | <.001 | <.001 |
| *NR3C1* | cg27345592 | 53.05 | 0.32 | <.001 | <.001 |
| *POMC* | cg00293936 | 7.83 | 0.06 | <.01 | <.01 |
| *POMC* | cg00674304 | 48.82 | 0.3 | <.001 | <.001 |
| *POMC* | cg01926269 | 230.22 | 0.67 | <.001 | <.001 |
| *POMC* | cg02716646 | 0.52 | 0 | >.10 | >.10 |
| *POMC* | cg02757179 | 0.03 | 0 | >.10 | >.10 |
| *POMC* | cg06846259 | 3.7 | 0.03 | <.10 | <.10 |
| *POMC* | cg10045137 | 22.19 | 0.17 | <.001 | <.001 |
| *POMC* | cg11894631 | 19.21 | 0.14 | <.001 | <.001 |
| *POMC* | cg14170547 | 68.62 | 0.37 | <.001 | <.001 |
| *POMC* | cg14357535 | 22.22 | 0.16 | <.001 | <.001 |
| *POMC* | cg17736230 | 16.5 | 0.13 | <.001 | <.001 |
| *POMC* | cg22900229 | 228.96 | 0.67 | <.001 | <.001 |
| *POMC* | cg23598419 | 55.59 | 0.33 | <.001 | <.001 |
| *POMC* | cg23809645 | 4.91 | 0.04 | <.05 | <.05 |
| *POMC* | cg24425171 | 484.89 | 0.81 | <.001 | <.001 |
| *POMC* | cg24718866 | 1.47 | 0.01 | >.10 | >.10 |

*Note*. *F*: F statistic for abuse; *Adj. p*: adjusted p-value; $\eta^2$ = eta square effect size; *AVP = arginine-vasopressin gene; CRH = corticotropin-releasing hormone gene; NR3C1 = glucocorticoid receptor gene, POMC = proopiomelanocortin gene.*