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# Prenatal Maternal Stress Predicts Methylation of Genes Regulating the Hypothalamic–Pituitary–Adrenocortical System in Mothers and Newborns in the Democratic Republic of Congo

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Exposure to stress early in life permanently shapes activity of the hypothalamic–pituitary–adrenocortical (HPA) axis and the brain. Prenatally, glucocorticoids pass through the placenta to the fetus with postnatal impacts on brain development, birth weight (BW), and HPA axis functioning. Little is known about the biological mechanisms by which prenatal stress affects postnatal functioning. This study addresses this gap by examining the effect of chronic stress and traumatic war-related stress on epigenetic changes in four key genes regulating the HPA axis in neonatal cord blood, placenta, and maternal blood: *CRH*, *CRHBP*, *NR3C1*, and *FKBP5*. Participants were 24 mother–newborn dyads in the conflict-ridden region of the eastern Democratic Republic of Congo. BW data were collected at delivery and maternal interviews were conducted to assess culturally relevant chronic and war-related stressors. Chronic stress and war trauma had widespread effects on HPA axis gene methylation, with significant effects observed at transcription factor binding (TFB) sites in all target genes tested. Some changes in methylation were unique to chronic or war stress, whereas others were observed across both stressor types. Moreover, stress exposures impacted maternal and fetal tissues differently, supporting theoretical models that stress impacts vary according to life phase. Methylation in several *NR3C1* and *CRH* CpG sites, all located at TFB sites, was associated with BW. These findings suggest that prenatal stress exposure impacts development via epigenetic changes in HPA axis genes.

Developmental processes during the prenatal phase are highly susceptible to environmental exposures such as maternal stress. Both low-level chronic stress and severe stress or trauma are associated with postnatal behavioral outcomes (Glover, O'Connor, & O'Donnell, 2010), with more severe stressors also associated with neonatal outcomes including lower birth weight (BW; Xiong et al., 2008). Notably, studies assessing chronic stress or traumatic life events have largely been conducted in Western populations. Little is known about prenatal stress effects in the developing world, although widespread poverty, social injustice, and political unrest can produce highly stressful living conditions.

Stress exposures have well-documented impacts on the development of the neuroendocrine branch of the biological stress response system, known as the hypothalamic–pituitary–adrenocortical (HPA) axis. Under low-stress conditions, the HPA axis is involved in homeostatic or regulatory processes important for growth and repair. Under high-stress conditions, the system shifts metabolic resources to meet the threat (Karatsoreos & McEwen, 2011). Stress exposures impact the HPA axis differently depending on the phase of the life course. Although psychosocial stress can activate the HPA axis

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throughout life, extensive evidence from animal models and human studies show early experiences have particularly long-term, programming effects (Anacker, O'Donnell, & Meaney, 2014; Lupien, McEwen, Gunnar, & Heim, 2009).

During the prenatal period, maternal and fetal physiology is connected, providing the fetus direct input regarding environmental stressors. Discrete or repeated stress exposure during pregnancy elevates maternal glucocorticoids (cortisol in humans), the hormonal endproduct of the HPA axis (Harris & Seckl, 2011). Cortisol passes through the placenta to the fetus, impacting brain development, BW, and HPA axis functioning pre- and postnatally (Glover et al., 2010; Harris & Seckl, 2011; Phillips & Jones, 2006). The mechanisms by which prenatal stress has these effects are largely unknown. Research in animal models suggests a role for epigenetic processes such as DNA methylation, the transfer of a methyl group to the fifth position of cytosine residues in CpG dinucleotides (Anacker et al., 2014; Zhang, Labonté, Wen, Turecki, & Meaney, 2013). Methylation affects gene transcription by altering local chromatin structure, thereby affecting transcription factors' ability to bind to DNA (Jones, 2012).

Interest in prenatal stress effects on DNA methylation emerged following evidence that postnatal stress impacted hippocampal DNA methylation of the gene coding the glucocorticoid receptor (GR), which regulates the stress response (McGowan et al., 2011; Zhang et al., 2013). Effects were specific to the exon $1^7$ promoter region, which contains a transcription factor binding (TFB) site for nerve growth factor inducible A (NGFI-A). Prenatal research demonstrated a comparable effect in hypothalamic tissue; specifically, exposure to chronic stress during pregnancy predicted increased methylation in the exon $1^7$ promoter region (Mueller & Bale, 2008).

Few studies have examined the effects of prenatal stress (or related phenotypes) on DNA methylation in humans. In neonatal cord blood, prenatal maternal anxiety (Hompes et al., 2013), interpartner violence (Radtke et al., 2011), and depressive symptoms (Conradt, Lester, Appleton, Armstrong, & Marsit, 2013; Oberlander et al., 2008) have been associated with methylation within or close to the homologous NGFI-A binding site in the promoter region of *NR3C1*, the gene encoding GR. This suggests environmental exposures impact neonatal methylation of the GR promoter in peripheral tissue. Although the Radtke et al. (2011) study had limitations, including wide variability in child age and retrospective recall of domestic violence, results suggest that prenatal stress effects on *NR3C1* may persist years after birth. This is consistent with a rich body of evidence suggesting long-term programming effects of early life stress (Glover et al., 2010; Lupien et al., 2009).

To date, effects in humans of prenatal psychosocial stress on DNA methylation of genes regulating the HPA axis have primarily focused on *NR3C1*. However, HPA axis activation and its effects in target tissues involve a complex biological cascade. The present study advances understanding of epigenetic mechanisms by which psychosocial stressors may program the HPA axis by examining methylation of multiple genes regulating this system. Four key genes were included: *CRH*, which codes for corticotrophin-releasing hormone (CRH); *CRHBP*, coding for CRH binding protein (CRHBP); *NR3C1*, coding the GR; and *FKBP5*, coding for FK506 binding protein 5 (FKBP5). These four genes were targeted based on (a) the role of the protein products in the initiation of HPA axis activation (*CRH*, *CRHBP*) and downstream effects of cortisol release at target tissues (*NR3C1*, *FKBP5*); (b) previous associations of polymorphisms or methylation levels with stress-related disorders, suggesting a potential functional role (*CRHBP*, *NR3C1*, *FKBP5*); and (c) evidence that DNA methylation is related to prenatal stress exposures (*CRH*, *NR3C1*, *FKBP5*) or birth outcomes (*CRH*, *CRHBP*, *NR3C1*; Binder, 2009; Hogg, Blair, McFadden, von Dadelszen, & Robinson, 2013; Kertes et al., 2011; Klengel et al., 2013; Mulligan, D'Errico, Stees, & Hughes, 2012; Xu, Sun, Gao, Cai, & Shi, 2014). Additional background on the role of these genes in stress regulation and prenatal development is described in Appendix S1.

In addition to examining effects in humans of prenatal psychosocial stress in multiple HPA axis genes, the present study enhances understanding of epigenetic processes in development in several ways. Consistent with contemporary theoretical models of HPA axis development (Lupien et al., 2009), which suggest that impacts of stress exposure differ by developmental phase, this study examines stress effects in both maternal and newborn tissues. Moreover, since maternal and fetal physiologies are connected through the placenta during pregnancy, analysis of neonatal cord and maternal venous blood, as well as placental tissue, provides an opportunity to assess the differing effects of prenatal stress on multiple relevant tissues. As the placenta regulates fetal exposure to maternal cortisol and impacts cortisol production in both mother and child during pregnancy, placental methylation may

represent an especially important conduit between maternal stress exposure, fetal epigenetic processes, and developmental outcomes. In sum, this study assesses HPA axis gene methylation in three tissues to compare the effects of maternal stress on methylation across life stage and tissue type.

Research on prenatal stress has been predominantly conducted in Western populations. Responding to the call to globalize child development research with culturally sensitive, interdisciplinary, international research (SRCD Governing Council, 2005), this study examined effects of stress exposures on DNA methylation in mother–newborn dyads in the eastern Democratic Republic of Congo (DRC). This region is plagued by severe violence against women, including military and civilian-perpetrated rape and other war-related traumas, alongside widespread poverty, political strife, and social injustice contributing to chronic stress (Johnson et al., 2010). Rodney and Mulligan (2014) recently reported reduced global (genome-wide) methylation among Congolese women experiencing high rates of war stress. Prior research suggests different effects of traumatic stress on HPA axis functioning compared to more moderate stressors with potential transgenerational effects (Yehuda & Bierer, 2007). Thus, this study tested impacts of both chronic stress and war-related trauma on HPA axis gene methylation in mothers and their newborns, with the understanding that the two types of stress would likely be correlated, consistent with studies of trauma-exposed individuals (e.g., Cigrang et al., 2014; Fjeldheim et al., 2014).

This study tested the hypothesis that stress exposure during pregnancy would be associated with methylation in multiple HPA axis-related genes. Owing to the severe nature of war-related stressors for Congolese women, including displacement (as a refugee), kidnapping, rape, and family members killed, we hypothesized that effects would be stronger for war-related stressors compared to chronic stressors. With respect to similarities or differences across tissue types, two alternate hypotheses were posed. The first hypothesis—that prenatal stress effects would be similar across tissue sources—was based on evidence that stress impacts *NR3C1* methylation in both brain and peripheral tissue (Hompes et al., 2013; McGowan et al., 2011). The alternate hypothesis was that prenatal stress effects would be tissue specific. This was based on (a) different physiologic functions of blood versus placental tissue, (b) the life cycle model of stress positing that stress effects differ by phase of the life course (Lupien et al., 2009), (c) developmental origins of health and disease models positing that the developing fetus is uniquely sensitive to intrauterine environmental cues (Entringer, Buss, & Wadhwa, 2010), and (d) tissue and developmental specificity of mammalian DNA methylation patterns (Liang et al., 2011; Pai, Bell, Marioni, Pritchard, & Gilad, 2011).

As a global index of neonatal development and health, this study also examined BW. Based on a rich literature in humans linking prenatal stressors, alterations in HPA axis activity, and BW (Harris & Seckl, 2011; Phillips & Jones, 2006), and evidence that DNA methylation is associated with birth outcomes (Filiberto et al., 2011; Hogg et al., 2013), we hypothesized that DNA methylation of CpG sites in HPA axis genes associated with stress exposures would, in turn, predict BW.

## Method

### *Participants*

Participants were recruited from women delivering their babies at HEAL Africa hospital in Goma, DRC. Informed consent was obtained from Western Institutional Review Board, the University of Goma, and an ethical review committee at HEAL Africa hospital. During oral consent, participants were able to have family members consent with them, provided a detailed explanation of the project and possible uses of results, and encouraged to ask questions. Participation was voluntary and confidential.

The study included 24 mother–newborn dyads (newborns = 54% male); 83% were vaginal deliveries. Mean maternal age was 26.91 years ($SD$ = 5.63) and 29% of mothers were primiparous. All mothers were nonsmokers.

DNA samples were collected within several hours of delivery from maternal venous blood, placental tissue (from the largest fetal cotyledon), and umbilical cord blood. All three tissue types were obtained from each dyad, except one cord blood sample, yielding 71 tissue samples.

### *Measures*

BW was collected at delivery. Mothers were interviewed within 24 hr of birth regarding general health, reproductive history, and stressful life experiences. Because Western-generated questionnaires would not have adequately captured women's experiences in this cultural context, stress exposure data were obtained by culturally sensitive, semistructured oral history methods and ethnographic

interviews (Spradley, 1979) in the Congolese dialect of Swahili. An emphasis was placed on establishing rapport, and women had the option to bring their newborn or family member to the private interview room. Interview questions tapped chronic socioeconomic and socioemotional stress along with war-related traumatic experiences, with some questions overlapping constructs assessed in stress and trauma inventories (e.g., Hassles Scale, Kanner, Coyne, Schaefer, & Lazarus, 1981; Trauma History Questionnaire, Green, 1996) and others capturing stressors relevant to this population (e.g., traveling alone, kidnap, soldier rape). Interviews were transcribed and initially coded for the presence of 32 items reflecting chronic stress or war-related trauma. Two measures, termed chronic stress and war trauma, were computed as continuous variables reflecting the total number of endorsed items with all items weighted equally. Development of the stress scales, frequencies of specific stressors, and examination of stressors by demographic characteristics are provided in Appendix S1 and Table S1.

### *Methylation Measurements*

Genomic DNA was isolated on site using Qiagen QIAamp DNA Midi Kits (Qiagen, Valencia, CA) according to the manufacturer's instructions. Following bisulfite conversion, 500 ng DNA was processed on Illumina HumanMethylation450 BeadChips at the Hussman Institute for Human Genomics, Miami, FL. Output processed through Illumina's GenomeStudio V2011.1 Methylation Module v1.9.0 yielded average beta estimates indicating methylation level. Quality control protocols are provided in Appendix S1. The final data set had 13 *CRH*, 14 *CRHBP*, 35 *NR3C1*, and 31 *FKBP5* CpG sites.

### *Statistical Analyses*

Analyses were conducted using SPSS v22.0 (IBM Corp., Armonk, NY) and R v3.1.2 (R Foundation for Statistical Computing, Vienna, Austria). As the dependent variable (methylation) lay on a beta distribution of 0 = *fully unmethylated* to 1 = *fully methylated*, beta regression was used to test associations with chronic stress and war trauma. Infant sex was included as a covariate in analyses of cord blood and placenta. To address multiple testing, $q$ values were computed to estimate false discovery rate (FDR) with $q < .25$ demonstrating "medium confidence" of statistically significant findings (Lam et al., 2012). For nominally significant sites ($p < .05$), findings were considered meaningful if at least one of the following two additional criteria were met: previous association of the CpG with stress exposure or birth outcomes, or situated in known TFBs. Putative TFBs were identified using PhysBinder. UCSC genome browser was used to compile ENCODE TFB data. Hierarchical regressions tested whether methylation at stress-associated CpG sites predicted BW, controlling for infant sex because of known sex differences in BW (WHO Multicentre Growth Reference Study Group, 2006).

## Results

### *Descriptives*

There was wide variability in the number of chronic stress items endorsed ($M = 6.54$; $SD = 6.32$). Thirty-three percent of the sample endorsed 0–1 chronic stressors, with the other two thirds endorsing multiple (2–18) stressors. Chronic stress was indicated by high rates of socioemotional stress (unhappy marriage, crying during pregnancy, and no help with domestic chores) and socioeconomic stress (not owning home, trouble paying bills, and past food insufficiency). There was also variability in war trauma ($M = 1.58$, $SD = 2.21$, range = 0–8). Of women endorsing war traumas (55% of the total sample), 31% reported one event, with an equivalent number (23%) reporting 2, 3, or 4 or more events. The most common war traumas were rape, refugee status, and family member killed.

Mean BW was 3.18 kg ($SD = 0.76$), with 25% of newborns meeting criteria for low BW (LBW; WHO Multicentre Growth Reference Study Group, 2006). There was no significant sex difference for LBW status, $\chi^2(1) = 1.40$, *ns*. Partial correlations indicated that, controlling for infant sex, BW was associated with both chronic stress ($r = -.65$, $p = .001$) and war trauma ($r = -.63$, $p = .001$).

### *Associations of Stressors and DNA Methylation*

Beta regressions tested whether chronic stress and war trauma predicted methylation levels in any gene from any of the tissues. In total, 18 CpG sites were significantly associated with chronic stress or war trauma in one or more tissues at $p < .05$ (Table 1). Eleven were associated with chronic stress and 14 with war trauma, including six sites predicted by both stressors (Table 1, underlined sites). A total of eight CpG sites survived

Table 1
*Association Estimates of Stress Exposures and Methylation of CpG Sites Using Beta Regression*

| CpG | Gene | Tissue | Gene region | Regression coefficient | *p* | $R^2$ | *q* | Abs β | Δβ |
|---|---|---|---|---|---|---|---|---|---|
| ***Chronic Stress*** | | | | | | | | | |
| cg03405789 | *CRH* | Cord | Body | 2.74 | .022 | .20 | .927 | .23 | .12 |
| **cg15971888**[a] | ***CRH*** | **Cord** | **Body** | **9.48** | **.024** | **.18** | **.927** | **.05** | **.05** |
| **cg17305181**[a] | ***CRH*** | **Venous** | **Body** | **−3.81** | **.024** | **.20** | **.576** | **.13** | **.09** |
| **cg08215831**[1] | ***CRH*** | **Venous** | **Promoter** | **−2.49** | **.008** | **.25** | **.576** | **.71** | **.18** |
| cg26196496 | *CRHBP* | Placenta | Promoter | −3.17 | .049 | .16 | .670 | .86 | .17 |
| **cg15910486**[a,2,3] | ***NR3C1*** | **Placenta** | **Promoter** | **−6.08** | **.010** | **.23** | **.636** | **.09** | **.04** |
| **cg24026230**[b,4] | ***NR3C1*** | **Cord** | **Body** | **11.12** | **.039** | **.16** | **.927** | **.04** | **.03** |
| **cg13648501**[b,5] | ***NR3C1*** | **Cord** | **Body** | **5.38** | **.044** | **.16** | **.927** | **.08** | **.16** |
| cg12466613 | *NR3C1* | Venous | Body | 7.61 | .032 | .18 | .576 | .94 | .06 |
| cg17085721 | *FKBP5* | Placenta | Body | −13.57 | .014 | .19 | .636 | .97 | .03 |
| cg03546163 | *FKBP5* | Placenta | Body | 6.21 | .022 | .17 | .665 | .92 | .10 |
| cg03546163 | *FKBP5* | Venous | Body | 1.84 | .022 | .19 | .576 | .57 | .24 |
| ***War trauma*** | | | | | | | | | |
| **cg03405789** | ***CRH*** | **Cord** | **Body** | **3.28** | **.002** | **.34** | **.093** | **.23** | **.12** |
| **cg15971888**[a] | ***CRH*** | **Cord** | **Body** | **11.37** | **.002** | **.31** | **.093** | **.05** | **.05** |
| **cg17305181**[a] | ***CRH*** | **Cord** | **Body** | **5.42** | **.031** | **.17** | **.728** | **.08** | **.07** |
| **cg08215831**[1] | ***CRH*** | **Venous** | **Promoter** | **−1.91** | **.043** | **.16** | **.450** | **.71** | **.18** |
| **cg16664570** | ***CRH*** | **Placenta** | **Promoter** | **2.01** | **.010** | **.23** | **.178** | **.42** | **.22** |
| **cg17448335**[a] | ***CRHBP*** | **Placenta** | **Body** | **3.33** | **.037** | **.13** | **.269** | **.11** | **.37** |
| **cg17448335**[a] | ***CRHBP*** | **Venous** | **Body** | **−13.77** | **.018** | **.22** | **.450** | **.04** | **.03** |
| **cg27122725**[b] | ***NR3C1*** | **Placenta** | **Body** | **−4.18** | **.024** | **.20** | **.241** | **.12** | **.13** |
| **cg20753294**[b] | ***NR3C1*** | **Venous** | **Body** | **−5.17** | **.037** | **.17** | **.450** | **.09** | **.08** |
| **cg18019515**[b] | ***NR3C1*** | **Placenta** | **Promoter** | **−34.55** | **.015** | **.22** | **.205** | **.02** | **.02** |
| **cg15910486**[a,2,3] | ***NR3C1*** | **Placenta** | **Promoter** | **−6.10** | **.007** | **.24** | **.178** | **.09** | **.04** |
| **cg12466613** | ***NR3C1*** | **Venous** | **Body** | **10.32** | **.003** | **.35** | **.202** | **.94** | **.06** |
| **cg03546163** | ***FKBP5*** | **Placenta** | **Body** | **7.56** | **.004** | **.30** | **.178** | **.92** | **.10** |
| **cg00052684**[b] | ***FKBP5*** | **Venous** | **Body** | **−1.70** | **.034** | **.17** | **.450** | **.46** | **.29** |
| **cg25114611**[a,b,5] | ***FKBP5*** | **Venous** | **Promoter** | **−2.08** | **.025** | **.19** | **.450** | **.30** | **.08** |

*Note.* Bolded entries are CpG sites identified by prior studies, located at transcription factor binding site(s), or significant after FDR correction. Underline indicates CpG sites associated with both chronic stress and war trauma. $R^2$ values indicate percent variance explained (after controlling for infant sex in cord blood and placenta). Due to potential error in array data at the extremes of the beta distribution, CpG sites with absolute (mean) methylation < 5% or > 95% should be interpreted with caution. Gene region information was obtained from Illumina and confirmed with Ensembl 77 annotation (hg38).
[a]CpG site within transcription factor consensus binding site. [b]CpG site within transcription factor binding region (ENCODE data).
[1]McGill et al. (2006). [2]Radtke et al. (2011). [3]Hompes et al. (2013). [4]Hogg et al. (2013). [5]Weder et al. (2014).

FDR correction with moderate confidence ($q < .25$); all of these were associated with war trauma. Among the significant associations at $p < .05$, only CpG sites that survive FDR correction, show prior evidence of association, or are situated at a TFB region (Table 1, bolded sites) are described below. Locations of CpG sites situated at or near TFBs are shown in Figure 1. TFB region information is available in Table S2.

Based on these criteria, chronic stress predicted methylation in *CRH* and *NR3C1* and explained 16%–25% of the variance, as indicated by $R^2$ values (Table 1). War trauma predicted methylation in all four genes (variance explained 13%–35%). For placenta and maternal blood, effects were seen in all four genes, whereas in cord blood significant associations with stressors were observed in *CRH* and *NR3C1* only. Methylation correlations among each pair of tissues were computed; none of the CpG sites in Table 1 had significant cross-tissue correlations.

In cord blood, three *CRH* sites of potential relevance were identified (Table 1). Methylation at cg15971888, located at multiple TFBs (Figure 1), and cg03405789 was associated with both stressors, with war trauma effects reaching significance at moderate confidence ($q < .25$). Entered together in regression analyses, the combined stressors accounted for 31% of the variance in cg15971888 methylation and 34% of the variance in cg03405789

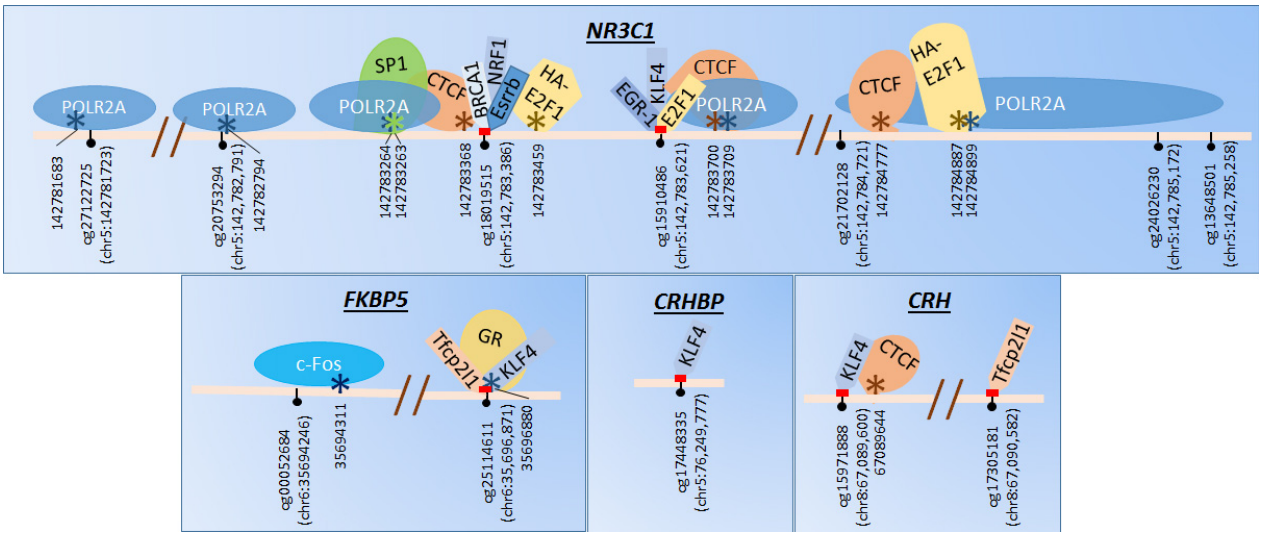


*Figure 1.* Schematic representation of CpG sites within or near transcription factor binding site(s). Black dots along the chromosome indicate CpG sites significantly associated with prenatal stress. An asterisk in an oval represents the nearest peak point position to the CpG site from the ENCODE database. A horizontal red bar indicates the putative binding motif for the transcription factor at the CpG site from the PhysBinder database.

methylation. A third *CRH* CpG, cg17305181 (associated with war trauma), as well as *NR3C1* cg24026230 and cg13648501 (associated with chronic stress) did not survive FDR correction but were situated within TFBs or previously identified in the literature (TFBs and citations shown in Table 1). For all sites in cord blood, higher methylation was associated with higher reports of maternal stress.

In placenta, reduced methylation at *NR3C1* cg15910486, cg18019515, and cg27122725 was significantly associated with war trauma after FDR correction (Table 1). For cg15910486, also nominally associated with chronic stress, both stressors collectively explained 27% of the variance at this site. All CpG sites were in genomic regions with one or more TFBs (Figure 1) and thus may be functionally relevant for gene transcription. War trauma–methylation associations were also observed at *CRH* cg16664570 and *FKBP5* cg03546163 (surviving FDR correction) and at *CRHBP* cg17448335 (uncorrected significance, TFB site; Figure 1).

In maternal blood, *NR3C1* cg1246613 methylation was significantly associated with war trauma after FDR correction and was nominally associated with chronic stress. Stress–methylation associations observed at *CRH* cg17305181 and cg08215831 (chronic and war), *CRHBP* cg17448335, *NR3C1* cg20753294, and *FKBP5* cg00052684 and cg25114611 (war) did not survive FDR correction, but these sites remained of interest because of locations at TFBs and prior associations with stress phenotypes (Table 1).

The vast majority of significant associations were tissue specific. The one exception was *FKBP5* cg03546163, for which higher methylation was associated with chronic stress and war trauma in placenta and with chronic stress in maternal blood. Methylation at *CRH* cg17305181 and *CRHBP* cg17448335 were also significant in two tissues but in opposite directions. In mothers, cg17448335 methylation was extremely low (3%) and should be interpreted with caution.

Methylation at six CpG sites was associated with both stressor types in the same tissue. Post hoc analyses (see Appendix S1) revealed chronic stress and war trauma were unique predictors of methylation at *CRH* cg03405789 and cg15971888, *NR3C1* cg12466613, and *FKBP5* cg03546163, whereas associations were due to shared variance among the two stressor types at *CRH* cg08215831 and *NR3C1* cg15910486.

### *Methylation as a Predictor of BW*

Hierarchical linear regressions tested whether variation in methylation predicted BW. Analyses were conducted with CpG sites reaching at least nominal significance in the first set of analyses (Table 1). Significant results reflect regressions with normally distributed residuals and no influential outliers.

Controlling for sex, methylation at four sites across two genes predicted BW (Table 2). They were: *NR3C1* cg15910486, associated with chronic stress and war trauma in placenta and located at seven TFBs including the consensus NGFI-A binding site; *NR3C1* cg18019515, associated with war trauma in placenta and located at six known TFBs; *NR3C1* cg24026230, associated with chronic stress (not FDR corrected) in cord blood and situated at a POLR2A binding site; and *CRH* cg17305181, associated with chronic stress (non-FDR corrected) in maternal blood and with war trauma in cord blood and situated at a Tfcp2l1 binding site. Scatter plots are shown in Figure 2. Entered simultaneously in a single linear regression, methylation at these four sites accounted for 55% of the variance in BW.

## Discussion

The purpose of this study was to examine the effects of prenatal stress exposure on DNA methylation in genes regulating the HPA axis and associations of methylation with BW. Chronic stress and war trauma were assessed in mother–newborn dyads in the conflict-ridden region of eastern DRC. Given the complexity of the HPA axis, analyses examined multiple genes regulating this system: *CRH*, *CRHBP*, *NR3C1*, and *FKBP5*. Stress exposures were expected to predict methylation levels in maternal blood, cord blood, and placental tissue, with effects stronger for war trauma. Results showed widespread effects of prenatal stress in predicting methylation in all genes tested, with effects in cord blood restricted to *CRH* and *NR3C1* and effects in placenta and maternal blood observed across the four genes. Some impacts on methylation were unique to chronic stress or war trauma, whereas others were observed across both stressor types. Only war trauma associations survived multiple test correction at moderate confidence using FDR-adjusted $q$ values. This supports the hypothesis that war-related stressors have stronger effects, presumably due to their extreme, uncontrollable, and unpredictable nature. Nevertheless, several CpG sites nominally significant with chronic stress were situated at TFBs or previously associated with stress exposures or birth outcomes, and thus are likely to be biologically meaningful.

Table 2
*Association Estimates of Significant CpG Sites and Birth Weight Using Linear Regression*

| CpG | Gene | Tissue | Regression coefficient | $p$ | $R^2$ |
|---|---|---|---|---|---|
| cg24026230[a,1] | *NR3C1* | Cord | −.46 | .022 | .26 |
| cg15910486[b,2,3] | *NR3C1* | Placenta | .44 | .029 | .22 |
| cg18019515[a] | *NR3C1* | Placenta | .45 | .028 | .23 |
| cg17305181[b] | *CRH* | Venous | .53 | .010 | .29 |

*Note.* All analyses were conducted controlling for infant sex. [a]CpG site within transcription factor binding region (ENCODE data). [b]CpG site within transcription factor consensus binding site.
[1]Hogg et al. (2013). [2]Radtke et al. (2011). [3]Hompes et al. (2013).

This study also tested competing hypotheses regarding common versus unique effects of stressors across multiple tissues. Given that multiple sites in a gene may have functional consequences for transcription and protein product, results demonstrating prenatal stress associations with methylation in 2–3 tissue types for all genes tested suggest broad, common impacts of prenatal stress across tissues. At the same time, significant associations were generally unique to each tissue type, consistent with evidence that mammalian DNA methylation is tissue and developmentally specific (Liang et al., 2011; Pai et al., 2011) and different physiologic functions of blood versus placental tissue. Specificity is also consistent with the life cycle model of stress that posits differential effects of stress exposure on the HPA axis according to life phase (Lupien et al., 2009). Long-term programming effects of prenatal stress may result when systems regulating the HPA axis are still developing, whereas biological stress effects in adulthood may reflect a manifestation of incubated effects of early adversity or maintenance of chronic stress. It is possible that gene-level impacts of stress exposures affect DNA methylation across tissues and throughout life, whereas variation in methylation at specific sites, especially at TFBs, may partially underlie differential effects of stress on HPA axis activity at different phases of the life course. Future research should examine the relations of global (gene-level) and specific (CpG-level) methylation with HPA axis activity throughout development.

Since BW predicts numerous developmental outcomes including physical and mental health, language, and cognitive ability (Barre, Morgan, Doyle, & Anderson, 2011; Boulet, Schieve, & Boyle, 2011), this study also tested the hypothesis that HPA axis gene methylation has downstream consequences using BW as a global index of neonatal development and health. Results showed methylation levels at four CpG sites, all situated at TFBs, collectively explained over half of the variance in BW. These included two *NR3C1* sites in placenta, both associated with war trauma and situated at binding

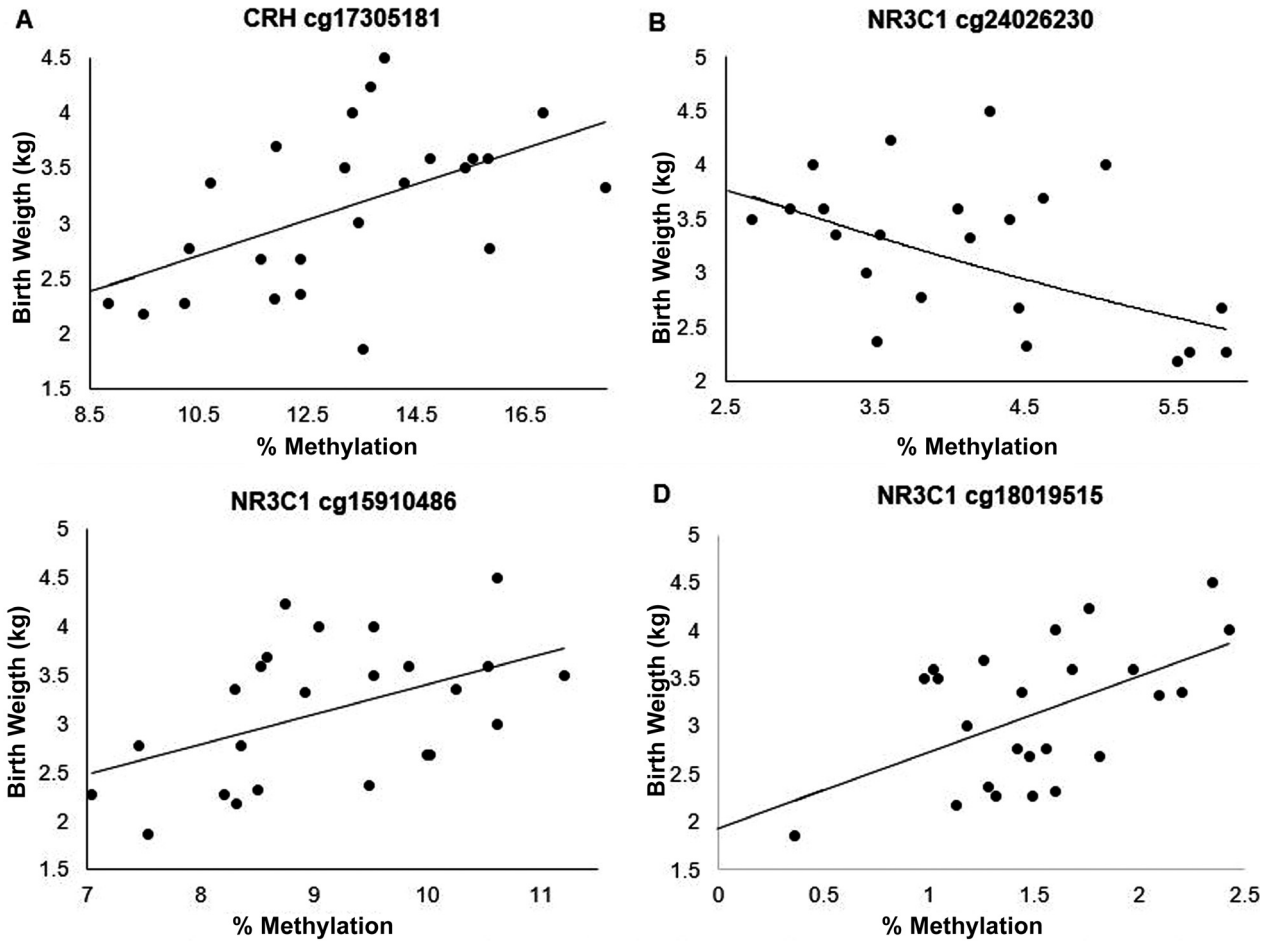


*Figure 2.* Scatter plots of the CpG sites significantly associated with birth weight. (A) *CRH* cg17305181 in venous blood, (B) *NR3C1* cg24026230 in cord blood, (C) *NR3C1* cg15910486 in placenta, (D) *NR3C1* cg18019515 in placenta.

regions for 6–7 transcription factors. The third CpG was in *NR3C1* in cord blood and the fourth was in *CRH* in maternal blood, with one known TFB at each site. The fact that the significant CpG sites were not isolated to one tissue source and were all situated at TFBs in *CRH* and *NR3C1* speaks to the potential role of methylation in these genes as a biological pathway of prenatal stress effects on BW.

Chronic stress and war trauma predicted reduced methylation at a CpG site in the NGFI-A binding region in placenta, adding to a growing literature documenting effects of stressors on methylation at the NGFI-A binding site in the gene encoding GR (e.g., Zhang et al., 2013). Prenatal stress explained 27% of the variation in methylation at cg15910486, which in turn predicted 22% of the variation in BW. An association with lower BW highlights the functional role of NGFI-A, which controls brain glucocorticoid levels and acts as a transcription factor in brain and adrenals (Anacker et al., 2014). These results are the first to document prenatal stress effects at this site in human placenta. The direction of effects is consistent with pregnancy-related anxiety and methylation in cord blood (Hompes et al., 2013), but reversed compared to other stress studies using nonplacental tissues (e.g., Radtke et al., 2011). Notably, in other *placental* studies, reduced methylation in the *NR3C1* promoter region correlated with decreased newborn attention, quality of movement, and being born small for gestational age (Bromer, Marsit, Armstrong, Padbury, & Lester, 2013; Filiberto et al., 2011). The present results suggest that reduced DNA methylation, specifically at the NGFI-A site in placenta, may serve as an epigenetic pathway for stress effects on lower BW.

This study also identified several novel associations of *CRH*, *CRHBP*, *NR3C1*, and *FKBP5* methylation with prenatal stress exposure. In cord blood, chronic stress and war trauma predicted methyla-

tion at two *CRH* CpG sites, one of which (cg15971888) is located in CTCF and KLF4 binding regions. CTCF regulates chromatin structure and defines active DNA boundaries to promote or repress gene transcription. KLF4 is present at telomerase, which preserves DNA at chromosome ends, and interacts with cyclic adenosinemonophosphate (cAMP) responsive element binding protein, a transcriptional activator. A third CpG, cg17305181, associated with war trauma at uncorrected significance, is located at a Tfcp2l1 binding site, which suppresses DNA transcription. Stress exposures were associated with increased methylation at all *CRH* sites in cord blood, which may hinder TFB at these locations and subsequently limit their ability to exert regulatory functions in the body. To the best of our knowledge, this is the first report documenting stress effects (either pre- or postnatal) on *CRH* methylation in humans.

Two additional *NR3C1* sites in cord blood significantly associated with chronic stress (before FDR correction) are noteworthy. cg24026230 is situated within the TFB region for POLR2A, the largest subunit of RNA polymerase II, and predicted reduced BW. Increased placental methylation at this site has previously been associated with early onset preeclampsia (Hogg et al., 2013). Methylation at cg13648501, positively associated with chronic stress, has previously been associated with child maltreatment (Weder et al., 2014; direction of effect not reported). This study is the first to report an association of methylation at these two *NR3C1* CpG sites with prenatal stress. As these sites are located within the gene body of the longest *NR3C1* transcripts, where methylation may be more likely to predict enhanced rather than repressed gene expression (Jones, 2012; Lou et al., 2014), it is tempting to speculate that these findings imply enhanced GR levels in cord blood. However, they also sit in the putative regulatory region of shorter *NR3C1* transcripts, in which case increased methylation would be expected to associate with decreased expression. This complexity epitomizes the challenges inherent in speculating on the effects of methylation changes for mRNA or protein levels and highlights the need for functional studies targeting these genomic regions.

The largest number of CpG sites surviving FDR correction was observed in placenta. The most noteworthy findings were in *NR3C1*. In addition to the CpG in the NGFI-A binding site, we report for the first time an association of prenatal stress with methylation at cg18019515 after FDR correction. Although caution is warranted in interpreting cg18019515 given the very low absolute methylation level, this CpG is in a genomic region binding six transcription factors and was one of the four sites significantly predicting BW. Also in placenta, *CRHBP* cg17448335 methylation was predicted by war trauma at uncorrected significance levels. This CpG is situated at a KLF4 binding site. Additionally, *CRH* cg16664570 showed increased methylation at FDR-corrected significance in association with war trauma.

In maternal blood, effects were observed in all genes tested. *NR3C1* cg1246613 methylation was associated with prenatal stress at FDR corrected significance; however, no known TFBs are present at this site and therefore the functional relevance is unknown. Four other nominally significant effects were notable. Methylation of cg25114611 in the *FKBP5* promoter region, recently linked in saliva to child maltreatment (Weder et al., 2014), was significantly predicted by war trauma. This CpG is in binding sites for Tfcp2l1 and KLF4, transcription factors with opposing regulatory functions, and is in close proximity to the peak of a GR binding site. This suggests a complex interplay of transcription factors while also implying functional importance for the site in stress-related activity, consistent with evidence of lower blood FKBP5 levels in PTSD patients (Yehuda et al., 2009). In *CRH*, cg17305181 methylation may reflect another potentially functionally relevant site as it is situated at a Tfcp2l1 binding site and showed a negative relation with BW. In addition, a difference in methylation at cg08215831 has previously been reported in rodents (McGill et al., 2006), although the tissue source (hypothalamus) differed from the present study. Finally, war trauma was negatively associated with methylation at *CRHBP* cg17448335, situated at a KLF4 binding site.

These findings should be interpreted in light of some limitations. First, the study was conducted with 24 newborn–mother dyads. However, small samples sizes ($N$ = 19–36) are not uncommon in methylation analyses (Naumova et al., 2012; Radtke et al., 2011; van Dongen et al., 2014; Yuen, Jiang, Peñaherrera, McFadden, & Robinson, 2011). Moreover, research on the developmental neurobiology of stress indicates different physiological consequences for extreme or chronic stressors compared to milder or time-limited stressors (Lupien et al., 2009). The high degree of unpredictability and uncontrollability of stressors faced by participants, especially severe war-related traumas, likely increased our ability to detect biological effects (Dickerson & Kemeny, 2004). In addition, the use of

culturally sensitive ethnographic interviews focused on establishing rapport and trust (Spradley, 1979), as opposed to questionnaire methods unfamiliar in this cultural context, likely increased accuracy in ascertaining valid stressors among Congolese women, which offsets the limitations of a smaller sample size.

Second, the overlap observed for chronic stress and war trauma associations limits the ability to fully disentangle effects of both types of stress. This issue is not unique to this study as trauma-exposed individuals often report high chronic stress due to broader impacts on social relationships and emotional functioning (Cigrang et al., 2014; Fjeldheim et al., 2014). Notably, this study is among the first to examine epigenetic patterns in a developing country and one of the few studies that has attempted to collect comprehensive biological and psychological data outside of the Western world, including the assessment of multiple tissue types.

We did not specifically recruit mothers from another geographic region as a nonstressed comparison group. However, a wide range of stressors was reported, with some women reporting few stressors, which indicates the sample was not restricted to highly stressed women.

Finally, as in other recent epigenetic studies (e.g., van Dongen et al., 2014; Weder et al., 2014), methylation was assessed via microarray, which is known to contain some degree of error (Michels et al., 2013). Although this method facilitates examining many genes and tissues simultaneously, and high correlations with whole genome bisulfite sequencing have been reported (Yuen et al., 2011), future research is warranted to validate the findings reported here with sequencing methods.

The results of this study demonstrated widespread effects of prenatal maternal stress on methylation in several genes regulating the HPA axis. Effects at CpG sites located in multiple TFBs suggested potential functional relevance for gene transcription. At the same time, maternal stress had unique effects on methylation in maternal and fetal tissues, consistent with theoretical models positing stress exposure effects vary by phase of the life course and consistent with the tenets of the developmental origins of health hypothesis that maternal stress modifies offspring biology. Differences were also seen in cord blood compared to placental tissue, likely related to the differential role of these tissues in prenatal development. Methylation in several *NR3C1* and *CRH* CpG sites, all located at TFBs, predicted BW. In sum, these findings are consistent with the hypothesis that prenatal exposure to maternal stress impacts development in offspring via epigenetic changes in genes regulating the HPA axis.

## References


Anacker, C., O'Donnell, K. J., & Meaney, M. J. (2014). Early life adversity and the epigenetic programming of hypothalamic-pituitary-adrenal function. *Dialogues in Clinical Neuroscience*, *16*, 321–333.

Barre, N., Morgan, A., Doyle, L. W., & Anderson, P. J. (2011). Language abilities in children who were very preterm and/or very low birth weight: A meta-analysis. *Journal of Pediatrics*, *158*, 766.e1–774.e1. doi:10.1016/j.jpeds.2010.10.032

Binder, E. B. (2009). The role of FKBP5, a co-chaperone of the glucocorticoid receptor in the pathogenesis and therapy of affective and anxiety disorders. *Psychoneuroendocrinology*, *34*(Suppl. 1), S186–S195. doi:10.1016/j.psyneuen.2009.05.021

Boulet, S. L., Schieve, L. A., & Boyle, C. A. (2011). Birth weight and health and developmental outcomes in US children, 1997–2005. *Maternal and Child Health Journal*, *15*, 836–844. doi: 10.1007/s10995-009-0538-2

Bromer, C., Marsit, C. J., Armstrong, D. A., Padbury, J. F., & Lester, B. (2013). Genetic and epigenetic variation of the glucocorticoid receptor (NR3C1) in placenta and infant neurobehavior. *Developmental Psychobiology*, *55*, 673–683. doi: 10.1002/dev.21061

Cigrang, J. A., Wayne Talcott, G., Tatum, J., Baker, M., Cassidy, D., Sonnek, S., . . . Smith Slep, A. M. (2014). Impact of combat deployment on psychological and relationship health: A longitudinal study. *Journal of Traumatic Stress*, *27*, 58–65. doi: 10.1002/jts.21890

Conradt, E., Lester, B. M., Appleton, A. A., Armstrong, D. A., & Marsit, C. J. (2013). The roles of DNA methylation of NR3C1 and 11β-HSD2 and exposure to maternal mood disorder *in utero* on newborn neurobehavior. *Epigenetics*, *8*, 1321–1329. doi: 10.4161/epi.26634

Dickerson, S. S., & Kemeny, M. E. (2004). Acute stressors and cortisol responses: A theoretical integration and synthesis of laboratory research. *Psychological Bulletin*, *130*, 355–391. doi: 10.1037/0033-2909.130.3.355

Entringer, S., Buss, C., & Wadhwa, P. D. (2010). Prenatal stress and developmental programming of human health and disease risk: Concepts and integration of empirical findings. *Current Opinion in Endocrinology, Diabetes, and Obesity*, *17*, 507–516. doi: 10.1097/MED.0b013e3283405921

Filiberto, A. C., Maccani, M. A., Koestler, D., Wilhelm-Benartzi, C., Avissar-Whiting, M., Banister, C. E., . . . Marsit, C. J. (2011). Birthweight is associated with DNA promoter methylation of the glucocorticoid receptor in human placenta. *Epigenetics*, *6*, 566–572. doi: 10.4161/epi.6.5.15236

Fjeldheim, C. B., Nöthling, J., Pretorius, K., Basson, M., Ganasen, K., Heneke, R., . . . Seedat, S. (2014). Trauma exposure, posttraumatic stress disorder and the effect

of explanatory variables in paramedic trainees. *BMC Emergency Medicine*, *14*, 11–17. doi: 10.1186/1471-227X-14-11

Glover, V., O'Connor, T., & O'Donnell, K. (2010). Prenatal stress and the programming of the HPA axis. *Neuroscience & Biobehavioral Reviews*, *35*, 17–22. doi:10.1016/j.neubiorev.2009.11.008

Green, B. (1996). Trauma History Questionnaire. In B. H. Stam & E. M. Varra (Eds.), *Measurement of stress, trauma, and adaptation* (pp. 366–368). Lutherville, MD: Sidran Press.

Harris, A., & Seckl, J. (2011). Glucocorticoids, prenatal stress and the programming of disease. *Hormones and Behavior*, *59*, 279–289. doi: 10.1016/j.yhbeh.2010.06.007

Hogg, K., Blair, J. D., McFadden, D. E., von Dadelszen, P., & Robinson, W. P. (2013). Early onset pre-eclampsia is associated with altered DNA methylation of cortisol-signalling and steroidogenic genes in the placenta. *PLoS ONE*, *8*, e62969. doi: 10.1371/journal.pone.0062969

Hompes, T., Izzi, B., Gellens, E., Morreels, M., Fieuws, S., Pexsters, A., . . . Freson, K. (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. *Journal of Psychiatric Research*, *27*, 58–65. doi: 10.1016/j.jpsychires.2013.03.009

Johnson, K., Scott, J., Rughita, B., Kisielewski, M., Asher, J., Ong, R., & Lawry, L. (2010). Association of sexual violence and human rights violations with physical and mental health in territories of the Eastern Democratic Republic of the Congo. *Journal of the American Medical Association*, *304*, 553–562. doi: 10.1001/jama.2010.1086

Jones, P. A. (2012). Functions of DNA methylation: Islands, start sites, gene bodies and beyond. *Nature Reviews Genetics*, *13*, 484–492. doi: 10.1038/nrg3230

Kanner, A. D., Coyne, J. C., Schaefer, C., & Lazarus, R. S. (1981). Comparison of two modes of stress measurement: Daily hassles and uplifts versus major life events. *Journal of Behavioral Medicine*, *4*, 1–39. doi: 10.1007/BF00844845

Karatsoreos, I. N., & McEwen, B. S. (2011). Psychobiological allostasis: Resistance, resilience and vulnerability. *Trends in Cognitive Sciences*, *15*, 576–584. doi: 10.1016/j.tics.2011.10.005

Kertes, D. A., Kalsi, G., Prescott, C. A., Kuo, P. H., Patterson, D. G., & Walsh, D.,. . . Riley, B. P. (2011). Neurotransmitter and neuromodulator genes associated with a history of depressive symptoms in individuals with alcohol dependence. *Alcoholism: Clinical and Experimental Research*, *35*, 496–505. doi: 10.1111/j.1530-0277.2010.01366.x

Klengel, T., Mehta, D., Anacker, C., Rex-Haffner, M., Pruessner, J. C., Pariante, C. M., . . . Bradley, B. (2013). Allele-specific FKBP5 DNA demethylation mediates gene-childhood trauma interactions. *Nature Neuroscience*, *16*, 33–41. doi: 10.3410/f.717970140.793468865

Lam, L. L., Emberly, E., Fraser, H. B., Neumann, S. M., Chen, E., Miller, G. E., & Kobor, M. S. (2012). Factors underlying variable DNA methylation in a human community cohort. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(Suppl. 2), 17253–17260. doi: 10.1073/pnas.1121249109

Liang, P., Song, F., Ghosh, S., Morien, E., Qin, M., Mahmood, S., . . . Held, W. A. (2011). Genome-wide survey reveals dynamic widespread tissue-specific changes in DNA methylation during development. *BMC Genomics*, *12*, 231–247. doi: 10.1186/1471-2164-12-231

Lou, S., Lee, H. M., Qin, H., Li, J. W., Gao, Z., Liu, X., . . . Yip, K. Y. (2014). Whole-genome bisulfite sequencing of multiple individuals reveals complementary roles of promoter and gene body methylation in transcriptional regulation. *Genome Biology*, *15*, 408–429. doi: 10.1186/s13059-014-0408-0

Lupien, S. J., McEwen, B. S., Gunnar, M. R., & Heim, C. (2009). Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nature Reviews Neuroscience*, *10*, 434–445. doi: 10.1038/nrn2639

McGill, B. E., Bundle, S. F., Yaylaoglu, M. B., Carson, J. P., Thaller, C., & Zoghbi, H. Y. (2006). Enhanced anxiety and stress-induced corticosterone release are associated with increased Crh expression in a mouse model of Rett syndrome. *Proceedings of the National Academy of Sciences of the United States of America*, *103*, 18267–18272. doi: 10.1073/pnas.0608702103

McGowan, P. O., Suderman, M., Sasaki, A., Huang, T. C., Hallett, M., Meaney, M. J., & Szyf, M. (2011). Broad epigenetic signature of maternal care in the brain of adult rats. *PLoS ONE*, *6*, e14739. doi: 10.1371/journal.pone.0014739

Michels, K. B., Binder, A. M., Dedeurwaerder, S., Epstein, C. B., Greally, J. M., Gut, I., . . . Irizarry, R. A. (2013). Recommendations for the design and analysis of epigenome-wide association studies. *Nature Methods*, *10*, 949–955. doi: 10.1038/nmeth.2632

Mueller, B. R., & Bale, T. L. (2008). Sex-specific programming of offspring emotionality after stress early in pregnancy. *Journal of Neuroscience*, *28*, 9055–9065. doi: 10.3410/f.1120766.577012

Mulligan, C. J., D'Errico, N. C., Stees, J., & Hughes, D. A. (2012). Methylation changes at NR3C1 in newborns associated with maternal prenatal stress exposure and newborn birth weight. *Epigenetics*, *7*, 853–857. doi: 10.4161/epi.21180

Naumova, O. Y., Lee, M., Koposov, R., Szyf, M., Dozier, M., & Grigorenko, E. L. (2012). Differential patterns of whole-genome DNA methylation in institutionalized children and children raised by their biological parents. *Development and Psychopathology*, *24*, 143–155. doi: 10.1017/S0954579411000605

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*, 97–106. doi: 10.4161/epi.3.2.6034

Pai, A. A., Bell, J. T., Marioni, J. C., Pritchard, J. K., & Gilad, Y. (2011). A genome-wide study of DNA methy-

lation patterns and gene expression levels in multiple human and chimpanzee tissues. *PLoS Genetics*, *7*, e1001316. doi: 10.1371/journal.pgen.1001316

Phillips, D. I., & Jones, A. (2006). Fetal programming of autonomic and HPA function: Do people who were small babies have enhanced stress responses? *Journal of Physiology*, *572*, 45–50. doi: 10.1113/jphysiol.2005.104695

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

Rodney, N. C., & Mulligan, C. J. (2014). A biocultural study of the effects of maternal stress on mother and newborn health in the Democratic Republic of Congo. *American Journal of Physical Anthropology*, *155*, 200–209. doi: 10.1002/ajpa.22568

Spradley, J. P. (1979). *The ethnographic interview*. New York, NY: Holt, Rinehart & Winston.

SRCD Governing Council. (2005). *The Society for Research on Child Development strategic plan*. Retrieved from http://www.srcd.org/sites/default/files/strategic-plan.pdf

van Dongen, J., Ehli, E. A., Slieker, R. C., Bartels, M., Weber, Z. M., Davies, G. E., . . . Boomsma, D. I. (2014). Epigenetic variation in monozygotic twins: A genome-wide analysis of DNA methylation in buccal cells. *Genes*, *5*, 347–365. doi: 10.3390/genes5020347

Weder, N., Zhang, H., Jensen, K., Yang, B. Z., Simen, A., Jackowski, A., . . . Perepletchikova, F. (2014). Child abuse, depression, and methylation in genes involved with stress, neural plasticity, and brain circuitry. *Journal of the American Academy of Child & Adolescent Psychiatry*, *53*, 417–424. doi: 10.1016/j.jaac.2013.12.025

WHO Multicentre Growth Reference Study Group. (2006). WHO Child Growth Standards based on length/height, weight and age. *Acta Paediatrica*, *450*(Suppl. 450), 76–85. doi: 10.1111/j.1651-2227.2006.tb02378.x

Xiong, X., Harville, E. W., Mattison, D. R., Elkind-Hirsch, K., Pridjian, G., & Buekens, P. (2008). Exposure to Hurricane Katrina, post-traumatic stress disorder and birth outcomes. *American Journal of the Medical Sciences*, *336*, 111–115. doi: 10.1097/MAJ.0b013e31820936b2

Xu, L., Sun, Y., Gao, L., Cai, Y.-Y., & Shi, S.-X. (2014). Prenatal restraint stress is associated with demethylation of corticotrophin releasing hormone (CRH) promoter and enhances CRH transcriptional responses to stress in adolescent rats. *Neurochemical Research*, *39*, 1192–1198. doi: 10.1007/s11064-014-1296-0

Yehuda, R., & Bierer, L. M. (2007). Transgenerational transmission of cortisol and PTSD risk. *Progress in Brain Research*, *167*, 121–135. doi: 10.1016/S0079-6123(07)67009-5

Yehuda, R., Cai, G., Golier, J. A., Sarapas, C., Galea, S., Ising, M., . . . Buxbaum, J. D. (2009). Gene expression patterns associated with posttraumatic stress disorder following exposure to the World Trade Center attacks. *Biological Psychiatry*, *66*, 708–711. doi: 10.1037/e717702011-009

Yuen, R. K., Jiang, R., Peñaherrera, M. S., McFadden, D. E., & Robinson, W. P. (2011). Genome-wide mapping of imprinted differentially methylated regions by DNA methylation profiling of human placentas from triploidies. *Epigenetics & Chromatin*, *4*, 1–16. doi: 10.1186/1756-8935-4-10

Zhang, T. Y., Labonté, B., Wen, X. L., Turecki, G., & Meaney, M. J. (2013). Epigenetic mechanisms for the early environmental regulation of hippocampal glucocorticoid receptor gene expression in rodents and humans. *Neuropsychopharmacology*, *38*, 111–123. doi: 10.1038/npp.2012.149

## Supporting Information

Additional supporting information may be found in the online version of this article at the publisher's website:

**Figure S1.** CpG Sites Within the Binding Motif and Sequence for Transcription Factor Binding Sites From the PhysBinder Database

**Table S1.** Items Comprising Chronic Stress and War Trauma Measures

**Table S2.** CpG Sites Within Transcription Factor Binding Regions

**Appendix S1.** Additional Background on HPA Axis Genes in Relation to Stress and Birth Outcomes