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Childhood adversity and DNA methylation of genes involved in the hypothalamus–pituitary–adrenal axis and immune system: Whole-genome and candidate-gene associations

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Abstract

In recent years, translational research involving humans and animals has uncovered biological and physiological pathways that explain associations between early adverse circumstances and long-term mental and physical health outcomes. In this article, we summarize the human and animal literature demonstrating that epigenetic alterations in key biological systems, the hypothalamus–pituitary–adrenal axis and immune system, may underlie such disparities. We review evidence suggesting that changes in DNA methylation profiles of the genome may be responsible for the alterations in hypothalamus–pituitary–adrenal axis and immune system trajectories. Using some preliminary data, we demonstrate how explorations of genome-wide and candidate-gene DNA methylation profiles may inform hypotheses and guide future research efforts in these areas. We conclude our article by discussing the many important future directions, merging perspectives from developmental psychology, molecular genetics, neuroendocrinology, and immunology, that are essential for furthering our understanding of how early adverse circumstances may shape developmental trajectories, particularly in the areas of stress reactivity and physical or mental health.

The experience of early-life stress is associated with increased risk for a variety of long-term mental and physical health disorders. Adults exposed to early-life stress show higher levels of chronic health problems including depression, anxiety, auto-immune disorders, cardiovascular diseases, diabetes, hypertension, and premature mortality (Anda et al., 2009; Dube et al., 2009; Gluckman, Hanson, Cooper, & Thornburg, 2008; Goodwin & Stein, 2004; Matthews & Gallo, 2011; Rich-Edwards et al., 2010; Riley, Wright, Jun, Hibert, & Rich-Edwards, 2010; Shonkoff, Boyce, & McEwen, 2009). These long-term health disparities persist even when individuals' life quality improves after early developmental periods (Keinen-Boker, Vin-Raviv, Liphshitz, Linn, & Barchana, 2009; Kittleson et al., 2006). The notion that the origins of long-term human health and disease can begin in early childhood has enormous implications for public health and prevention. Therefore, there is growing interest in uncovering mechanisms that explain how early adverse experiences become biologically embedded in human physiology early in life, shaping long-term trajectories.

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Supplementary Materials and Methods

The supplementary material referred to in this article can be found online at http://journals.cambridge.org/dpp

Early Stress and the Hypothalamus–Pituitary–Adrenal (HPA) Axis

One biological system particularly affected by early adverse experiences is the HPA axis. The HPA axis is one of the primary stress response systems among humans and animals. In addition to its role in mounting a stress response, this neuroendocrinological system influences many biological functions related to physical growth, reproduction, metabolism, and maintaining a circadian rhythm. As part of this system, corticotropin releasing hormone is transported from the hypothalamus to the pituitary gland, stimulating the release of adrenocorticotropic hormone, which acts on the adrenal gland to release corticosteroids, including the glucocorticoid cortisol. The activity and regulation of this system is driven by the adrenal cortisol release, which, via a negative feedback loop, inhibits the HPA axis activity initiated in the hypothalamus and pituitary.

Mounting evidence indicates associations between early-life stress and HPA axis functioning in both animals and humans. Among humans, early trauma (Debellis et al., 1994; Gunnar & Donzella, 2002; Heim, Newport, Mletzko, Miller, & Nemeroff, 2008), child abuse and neglect (Cicchetti, Rogosch, Gunnar, & Toth, 2010; Cicchetti, Rogosch, & Oshri, 2011; van der Vegt, van der Ende, Kirschbaum, Verhulst, & Tiemeier, 2009), severe institutional deprivation (Wismer-Fries, Shirtcliff, & Pollack, 2008), removal from biological parents and placement into foster care (Dozier et al., 2006), and being reared in harsh early environments (Taylor, Lerner, Sage, Lehman, & Seeman, 2004) have been found to be associated with alterations in the HPA axis. These perturbations have been observed during the period of childhood in which the adversity occurs, and also in adolescence and adulthood, long after the cessation of the early adverse experience (Carpenter et al., 2007, 2009; Kittleson et al., 2006; Miller et al., 2009; Tyrka et al., 2008).

Such chronic alterations in HPA axis functioning are thought to have serious consequences for later health and development. Among humans and animals, alterations in the HPA axis have been implicated in the pathogenesis of cellular functioning, impaired neuronal growth and survival, modification of neurotropins and immune system activity, and acceleration of cellular aging (Ceccatelli, Tamm, Zhang, & Chen, 2007; Duman, 2009; Epel, 2009). Altered HPA axis functioning is thought to be a primary agent in increasing risk for numerous chronic physical and psychiatric diseases (Duman, 2009; Simon et al., 2006; Wolkowitz et al., 2001). The HPA axis is known to interact with the immune system in that dys-regulation in HPA axis activity results in a generalized increase in inflammatory activity (Gill, Saligan, Woods, & Page, 2009). In summary, perturbations in the HPA axis system can have a widespread effect on long-term health trajectories, especially when alterations are chronic and long lasting.

Early Stress and Biomarkers Associated With the Immune System

There is growing evidence that early adversity may also affect regulatory activities of the immune system that operate independently of the HPA axis. These alterations have been observed among humans at multiple developmental phases. Children who experience significant deprivation and neglect have been found to show elevated immune system activity, signified by increased antibody levels to herpes simplex virus type 1 (Shirtcliff, Coe, & Pollak, 2009). Variables including low socioeconomic status and harsh early environmental circumstances and maltreatment have also been linked with elevated levels of C-reactive protein, a biomarker of inflammatory processes in adults (Danese, Pariante, Caspi, Taylor, & Poulton, 2007; Phillips, Marsland, Flory, & Muldoon, 2009; Politt et al., 2007; Taylor, Lehman, Kiefe, & Seeman, 2006). Child maltreatment and/or trauma has predicted individual levels of fibrinogen (a plasma glycoprotein involved in inflammatory activity) and white blood cell counts (Danese et al., 2008), interleukin-6 (a cytokine that

regulates inflammatory activity), and level of nuclear factor kappa B (a protein linked with inflammatory and immune diseases) in response to an acute stressor (Caroll, Cohen, & Marsland, 2011; Pace et al., 2006). Lifetime stress, trauma, low socioeconomic conditions, and child abuse have been associated with alterations in peripheral markers of inflammation including interleukin-4, interleukin-2, and tumor necrosis factor α (Smith et al., 2011). There is evidence that these aberrations in immunoactivity are persistent, even when accounting for adult socioeconomic and health status (Caroll et al., 2011). Together, these studies indicate that environmental conditions during childhood ultimately program immune system phenotypes in adults that are associated with increased risk for chronic illnesses.

In addition to affecting peripheral levels of inflammatory markers, adverse events early in life have been found to affect patterns of gene expression involved in immune system responses. Differential regulation in genes involved in inflammation have been found among adults with exposure to low socioeconomic conditions during childhood (Miller & Chen, 2007). Individuals exposed to low socioeconomic status early on in life show an upregulation of genes involved in inflammatory activity and a downregulation of genes involved in glucocorticoid signaling, which play a critical role in anti-inflammatory activity (Miller et al., 2009). Furthermore, adult victims of prior trauma have been found to show differential expression of genes involved in activation of the HPA axis (Segman et al., 2005) and immune system in numerous studies (see, e.g., Segman et al., 2005; Zieker et al., 2007). Based on the accumulation of findings to date, Chen, Miller, Kobor, and Cole (2011) have theorized that early-life stress may promote states of heightened “pro-inflammatory signaling” that may increase risk for disease.

Interdisciplinary research in recent years has moved the field of developmental science beyond uncovering biomarkers associated with dysfunction and pathogenesis to understanding the mechanistic properties that “program” long-term phenotypic trajectories. There are a variety of theoretical models and perspectives that explain links between early experience and long-term disease profiles (for a review, see Miller, Chen, & Parker, 2011). It is likely that more than one biological mechanism is responsible for the life-altering changes observed among individuals exposed to chronic stress. In this article, however, we focus on the emerging evidence suggesting that epigenetic changes, established early in life, drive connections between long-term outcomes, specifically implicated in long-term perturbations in HPA axis and immune system functioning.

Early Adversity and Epigenetic Changes

“Epigenetic alterations” refer to modifications in gene expression, which occur without the presence of specific changes to the nucleotide sequence of DNA. In addition to their role in cellular differentiation during embryogenesis, X chromosome activation, imprinting of genes, and a variety of adverse cellular events (e.g., cancer), epigenetic events appear to function as a biological mechanism for translating environmental signals into organismal molecular events. Importantly, there is emerging evidence that environmental signals give rise to de novo epigenetic changes, which alter phenotypic trajectories by altering the expression of genes.

Epigenetic alterations of DNA can occur in a variety of ways. First, the proteins that serve to pack DNA into chromatin can be chemically modified so that transcriptional machinery is prevented from accessing regulatory regions in DNA (Jenuwein & Allis, 2001; Strahl & Allis, 2000). Second, gene expression can be affected by noncoding RNA (including microRNA), which promotes RNA degradation, inhibits translation, restructures chromatin, and regulates gene expression overall (Bergmann & Lane, 2003). Third, the actual DNA molecule itself can be modified through the alteration of the methyl molecule patterns on

nucleotide bases. At the nucleotide level, methyl groups become bonded to a specific combination of nucleotide sites, known as cytosine nucleotide–phosphate–guanine nucleotide (CpG) sites, on the regulatory region of a gene and block the access of transcriptional chemicals to specific recognition elements of a gene (Comb & Goodman, 1990; Inamdar, Ehrlich, & Erlich, 1991). An additional enzymatic oxidation to the set of nucleic acid methylation modifications in a genome was recently discovered (5-hydroxy-methylcytosine), which seems particularly relevant during embryogenesis and has been found to be abundant in brain tissue (Jin, Win, Li, & Pfeifer, 2011; Kriaucionis & Heintz, 2009).

Although it may be critical to consider the complexity of the multiple epigenetic changes that are elicited from environmental signals, there is mounting evidence that early-life stress is specifically associated with alterations in the third mechanism, related to changes in the DNA "methylome." Early adverse events have been found to affect DNA methylation profiles of candidate genes and across the entire genome. Thus, our review of the literature focuses on DNA methylation changes in response to environmental signals that have implications for HPA axis functioning and immune system activity across development.

Early-life events and DNA methylation: Research with animal models

Several lines of evidence involving rodent models suggest that caregiving quality in the early stages of life alters DNA methylation patterns of genes involved in the HPA axis. For a variety of reasons, researchers have become especially interested in epigenetic alterations in the glucocorticoid receptor (*GR*) gene. First, this gene is critically involved in the negative feedback regulation of the HPA axis that occurs when peripheral cortisol binds to GRs. Second, early adverse events have been connected with long-term signaling differences in this gene in both humans and animals (Francis, Diorio, Liu, Meaney, 1999; Liu et al., 1997; Miller et al., 2009).

Among rodents, variability in parental caring during the first 10 days of life has been associated with changes in methylation patterns at several regulatory sites in the exon 1_7 of the *GR* gene located in the hippocampus (Weaver et al., 2004). The areas affected involve the nerve growth factor inducible-A (NGFI-A) consensus sequence, a regulatory region critical for the expression of this gene. Within this promoter region, a specific NGFI-A binding site has been observed to be unmethylated prior to birth. Immediately following birth, however, this nucleotide site becomes fully methylated. It is remarkable that at 6 days of postpartum life the same nucleotide returns to an unmethylated state, comparable to the methylation status observed prior to birth, but *only* for rodents that received high levels of licking, grooming, and arched back nursing. In contrast, rodents who received insufficient caregiving quality have methylation levels that remain high at 6 days postpartum. Most impressive is that methylation patterns established early in life by high- or low-quality care-giving conditions are maintained well into adulthood (Weaver et al., 2004).

Through cross-fostering studies, in which rodent pups born to insensitive mothers were reared by highly sensitive mothers and vice versa, Weaver and colleagues demonstrated that these epigenetic associations were related to early rearing conditions rather than patterns of inheritance (Weaver et al., 2004). Follow-up studies have revealed that differences in DNA methylation between pups who receive high- or low-quality caregiving were not limited to select binding sites, but ranged across a broad area (7 million base pairs) of the chromosome that contained the *GR* gene (McGowan et al., 2011). Therefore, caregiving quality appears to affect epigenetic structures of DNA both at site-specific areas of candidate genes and across the genome. Moreover, because they remain stable and are not genetically inherited, these epigenetic signatures provide a model for how early caregiving quality influences long-term HPA activity.

Early-life events and DNA methylation: Research with humans

Similar patterns have been observed among humans in recent years. In a seminal examination of postmortem hippocampal brain tissue of adult suicide victims, McGowan et al. (2009) demonstrated that individuals with histories of child abuse showed higher methylation levels at specific CpG sites in the exon1_F of the promoter region of the *GR* gene. Paralleling findings with animals, methylation of target CpG sites (i.e., those that contain the NGFI-A recognition sequence, critical for gene transcription) showed a corresponding decrease in transcriptional activity (i.e., NGFI-A binding and NGFI-A inducible gene transcription), suggesting associations between increased methylation patterns of specific CpG sites and decreased transcription in the *GR* gene (McGowan et al., 2009). These studies provide an important illustration of how a specific epigenetic mechanism, occurring as a result of early-life stress, can explain the long-term dysregulation in HPA axis activity and co-occurring susceptibility to chronic disease often observed among humans.

Epigenetic changes caused by early caregiving quality do not appear to be limited to brain tissues, but can be observed across several peripheral tissues. Methylation levels of CpG sites measured in the exon1_F region of the *GR* gene in leukocyte cells have been associated with early adverse experiences in healthy adults (Tyrka, Price, Marsit, Walters, & Carpenter, 2012), adults with borderline personality disorder (Perroud et al., 2011), and adults whose mothers experienced domestic violence during pregnancy (Radtke et al., 2011). Similar to patterns observed in human brain tissue, methylation differences in leukocyte CpG sites located around the NGFI-A binding regions seem to be particularly affected by early adverse experiences (Perroud et al, 2011; Tyrka et al., 2012). In an examination of differential methylation patterns of mixed mononuclear cells from human cord blood, Oberlander et al. (2008) demonstrated associations between prenatal maternal depression symptoms and site-specific methylation levels of the *GR* gene. Consistent with findings among adults, methylation levels were associated with infants' salivary cortisol levels at 3 months of age, further suggesting connections between DNA methylation profiles and ongoing HPA axis activity (Oberlander et al., 2008).

Because GRs in peripheral tissues may serve different functions from those of hippocampal cells, the findings suggesting connections between methylation levels in GR in peripheral tissue and HPA axis functioning have been somewhat unexpected. Nevertheless, in numerous studies, the methylation status of CpG sites in the *GR* gene has mediated the association between early adverse experiences and cortisol production (Oberlander et al., 2008; Tyrka et al., 2012). Results such as these have garnered increased interest in the use of noninvasive measurements of peripheral tissues in examining epigenetic changes related to environmental signals.

Despite the growing body of research connecting early adverse conditions with alterations in genes involved in the HPA axis, there has been considerably less focus on epigenetic changes to genes in the immune system. Existing studies that have examined epigenetic alterations of genes involved in the immune system have taken a whole-genome approach. An overrepresentation of genes involved in metabolism (in addition to various signaling pathways) have been found to be differentially methylated among individuals reared in disadvantaged socioeconomic conditions early in life or exposed to parental stress during infancy or toddlerhood (Borghol et al., 2012; Essex et al., 2011). Follow-up analyses from whole-genome DNA methylation profiles revealed that several highly differentially methylated candidate genes between children with and without early stress were implicated in health and the immune system (insulin-like growth factor 2 [*IGF2AS*], which produces an antisense transcript of the insulin-like growth factor 2 gene [*TGFB2*], which encodes a

member of the transforming growth beta factor family involved in cytokine production; Essex et al., 2011).

With regard to immune activity, children with a history of severe early deprivation show differential patterns of DNA methylation across clusters of genes implicated in immune system functioning, in addition to genes involved in various pathways important for brain development (Naumova et al., 2012). Recent evidence suggests that children exposed to violence or extreme neglect early in life show variations in the length of telomeres, a marker of cellular aging, in comparison to children reared by intact families (Drury et al., 2012; Shalev et al., 2012). This epigenetic change may have particular relevance to long-term disease states, given connections between telomere lengths and onset of cardiovascular disease and diabetes (Fitzpatrick et al., 2007).

Although preliminary, there is some evidence that changes in DNA methylation profiles in response to stressful life events alter inflammatory activity in peripheral systems. Relative to individuals without prior traumatic experiences, individuals who report experiencing prior traumas or increased lifetime stress show differential methylation of genes distinctly involved in the immune system at the whole-genome level (Uddin et al., 2010) and with respect to specific candidate genes (Smith et al., 2011). The functional significance of these stress-induced epigenetic changes is implied by an associated increase in antibody levels to cytomegalovirus and cytokines associated with inflammatory activity, serving as a physiological marker of immune functioning (Smith et al., 2011; Uddin et al., 2010).

Preliminary Data for Genome-Wide and Candidate-Gene Exploration

Scientific advances in recent years have furthered our understanding of how early adversity becomes systemically embedded in the genome and at the level of specific candidate genes with functional implications for HPA axis and immune system activity. In this section, we present preliminary data illustrating two approaches for exploring how early adverse events are associated with differential DNA methylation profiles. Our goal is to illustrate how examinations from both genome-wide and candidate-gene explorations may be fruitful for understanding the epigenetic mediation of associations between early-life stress and HPA axis and immune system functioning.

Our data come from a longitudinal cohort of adolescents who range in the degree to which they have experienced early and/or chronic stress throughout their lives. Individuals participating in the current study were recruited approximately 14 years ago and were between 7 and 14 years of age (see Luthar & Sexton, 2007 for a comprehensive description of the sample). At the time of epigenetic analyses, participants ranged in age from 20 to 28 years ($M = 23.7$, $SD = 2.8$). Five out of the 9 participants presented in these preliminary analyses were male. Four out of 9 total participants reported a significant history of DCF involvement entailing placement into foster care at some point during their childhood. Foster placements lasted at least 1 year for all participants in this study.

During the first two waves of the study, participants and their mothers completed the Parent–Adolescent Relationship Questionnaire (PARQ), a 60-item measure that assesses perceptions of maternal aggression/hostility, neglect/indifference, rejection, and warmth/affection (Rohner, 1991). Appropriate reliability (with Cronbach α coefficients for the subscales ranging from 0.86 to 0.95) and convergent, concurrent, and discriminant validity have been established for this measure (Rohner, 1991). Due to the presence of missing PARQ data at Wave 1, the current data illustration examined mothers and children's PARQ scores at Wave 2, which occurred 5 to 10 years prior to Wave 3. At Wave 3, blood samples were taken from participants in order to examine DNA methylation profiles. Subsequent to the donation, DNA was extracted from CD3 T-cells, which were isolated from the peripheral

blood mononuclear cells using Dynabeads Flow-Comp Human CD3 according to the manufacturer's protocol (Invitrogen). Thus, one advantage of the current study is that DNA methylation signatures were assessed from specific cells that are tightly involved in immune reactivity.

Analyses of methylation patterns were performed using the Infinium HumanMethylation27 BeadChip assay (Illumina). This assay contains 27,578 CpG sites, which represent more than 13,500 promoters of well-annotated genes and about 110 microRNA loci. The number of CpG sites per gene ranges from one site in 2,541 genes, two sites in 11,711 genes, to more than three sites in 195 genes. Bisulfite treatment of genomic DNA, whole-genome amplification, labeling, hybridization, and scanning were performed at the Yale Center for Genome Analysis (http://medicine.yale.edu/keck/ycga/index.aspx). The Illumina methylation data were analyzed using GenomeStudio software (Illumina). The methylation status of each CpG site was measured as the ratio of signal from methylated probe to the sum of both methylated and unmethylated signals and is reported as a β value. The number of detected Illumina probes (detection $p < .01$) was high for all DNA samples and varied between 99.39% and 99.99%. The comparison of methylation profiles of the technical replicates have shown excellent reproducibility of methylation level measurements ($r^2 = .9946$). To ensure that detection of methylation was sensitive, our analyses only included sites that were highly distinguishable from those of negative controls at $p < .001$. This excluded 296 CpG sites from the whole-genome analyses. We also excluded CpG sites that were located on either sex chromosome to prevent interindividual and intergroup variability by gender. This left a total of 26,011 CpG sites to be included in primary analyses.

Early adversity and whole-genome DNA methylation profiles

To illustrate the manner in which early adverse experiences are associated with genome-wide epigenetic signatures, we compared DNA methylation patterns between participants with and without histories of foster care. Through these comparisons, we illustrate how genome-wide approaches can be useful for (a) detecting which of the 27,000 CpG sites were differentially methylated across "early risk" groups, (b) differentiating between CpG sites that show increased levels of methylation versus those that show decreased levels of methylation across risk groups, and (c) discerning the functional biological and metabolic pathways associated with differentially methylated genes across the genome, with particular focus on those implicated in immune system activity.

Comparisons between individuals with and without previous placement into foster care were based on the average β value of each CpG site across the genome. Given the large number of potential comparisons, only targets with significant intergroup differences in methylation level (Illumina DiffScore > 20, corresponding to $p < .01$) were considered as differentially methylated CpG sites. Results of genome-wide analyses revealed that individuals with and without prior placement into foster care showed differential methylation of 180 CpG sites localized in 173 genes. Of these 173 genes, children with histories of prior foster care showed significantly higher levels of methylation in 72 genes, and significantly lower levels of methylation in 101 genes, relative to children without such histories (see online-only supplementary Table S.1 for DAVID results).

The functional significance of this subset of differentially methylated genes was ascertained through the use of bioinformatics software, which identifies common biological processes and pathways, molecular functions, and cellular components that are overrepresented among the subset of differentially methylated genes. For the functional annotation and characterization of the differentially methylated genes, we used DAVID bioinformatics software (http://david.abcc.ncifcrf.gov; Huang, Sherman & Lempicki, 2009). Taking into account the 173 differentially methylated genes, DAVID software generates enrichment

scores for subsets of functional relationships among the genes of interest. We used the default "medium stringency" setting of the DAVID analysis, which compares the enrichment of the functional clusters derived from the 173 genes using a Fisher exact test. For the purposes of this study, we considered clusters that had an enrichment score of >1.3 (which corresponded to $p < .05$) as a reliably identified cluster.

Among 173 genes, differentially methylated across individuals with and without previous placement into foster care, a number of genes were identified as involved in the control of two major metabolic pathways of the immune system functioning (see online-only supplementary Table S.2 for DAVID results). The first metabolic pathway involved ubiquitin mediated proteolysis (enrichment score = 5.01), known to play an important role in modulation of the immune and inflammatory responses in addition to other basic cellular processes involving the cell cycle, cell development and differentiation, and control of signal transduction pathways. The second identified metabolic pathway involved antigen processing and presentation (enrichment score = 5.81), both processes that instigate antibody activity.

We also examined the functional properties of the subset of genes identified as differentially methylated between individuals with and without histories of foster care placement. Results of analyses yielded several significant functional clusters involving numerous cellular processes with enrichment factors higher than 1.3. These clusters included genes involved in negative regulation of transcription (enrichment score = 2.13), apoptosis and cell death (enrichment score = 2.08), posttranslation protein modifications (enrichment score = 1.9), regulation of translation (enrichment score = 1.8), and muscle tissue development (enrichment score = 1.8; see online-only supplementary Table S.2 for DAVID results). The functional properties of the 72 genes with elevated methylation levels in children with histories of foster care relative to control children were primarily related to the control of transcriptional regulation and apoptosis. The remaining 101 genes that showed a decrease in their methylation levels of children with histories of foster care relative to individuals without prior histories of placement into foster care were primarily involved in control of posttranslational protein modifications and protein catabolic processes.

Early adversity and candidate-gene DNA methylation profiles

In addition to uncovering the functional systems and metabolic pathways through genome-wide explorations of methylation, there is an additional advantage to understanding how early adverse experiences may be connected with methylation levels of specific regions of candidate genes. Examinations of several candidate genes have shed light on how methylation levels, associated with early adverse experiences, may be interrupting important biological processes linked with stress regulation and social behavior. Methylation levels of the estrogen receptor-alpha (*ER-alpha*) and *GR* genes have illustrated the specific mechanism by which neurohormonal systems (oxytocinergic and HPA, respectively) become perturbed under conditions of early adversity and lead to phenotypic differences in stress regulation and social behavior. For example, methylation levels of specific regulatory sites assessed on the *GR* gene (i.e., the NGFI-A consensus sequence) and *ER-alpha* gene (i.e., the Stat5 consensus sequence) are linked with altered cortisol production (in the case of the GR gene) and oxytocin production (in the case of the *ER-alpha* gene). These hormonal alterations further predict fear response and/or maternal behavior exhibited to offspring later in life (Champagne et al., 2006; Weaver et al., 2004). As highlighted by these studies, the candidate-gene approach can offer a more nuanced, mechanistic understanding of biological events that mediate connections between early stress and HPA axis or oxytocinergic activity, which functionally affects phenotypic behavior.

As a means of replicating the extant human and animal studies that have adopted a candidate-gene approach, we first examined associations between reports of parenting quality and methylation levels of a specific CpG site, located 250 bp upstream from the transcription start site, of the *GR* gene. Results from using nonparametric correlational analyses (which was employed due to the small sample size) indicated a strong, significant negative association between mothers' reports of their affection and warmth provided to their child, and their child's methylation patterns of the *GR* gene (Spearman $\rho = -0.81$, $p < .05$; surviving corrections for multiple comparisons). Because caregiving quality was assessed 5 to 10 years prior to the assessment of methylation levels, this provides preliminary evidence that prior caregiving experience may shape (or at least be associated with) future epigenetic signatures in the long term.

Our second examination focused on a candidate gene, macrophage migration inhibitory factor (*MIF*), that is functionally involved in *GR* expression and immune system activity. Considered a "glucocorticoid antagonist," *MIF* overrides the glucocorticoid-mediated suppression of inflammation and cytokine release (Calandra et al., 1995). The close associations with the *GR* gene and involvement in pro-inflammatory signaling make the *MIF* gene a candidate gene of interest in the current examination. The specific CpG site examined in the current study was located 273 bp from the transcription start site on the *MIF* gene. Results from using nonparametric correlational analyses indicated a strong, significant negative association between mothers' reports of their affection and warmth provided to their child, and their child's methylation patterns for the *MIF* gene CpG site (Spearman $\rho = -0.80$, $p < .05$). Furthermore, children who report higher levels of rejection show higher levels of methylation on the same CpG site (Spearman $\rho = 0.76$, $p < .05$). Both findings survived corrections for multiple comparisons.

Discussion

The results of these pilot analyses are consistent with previous research studies that illustrate how epigenetic signatures, both across the whole genome level and at the level of candidate genes, may mediate associations between early adverse events and long-term alterations in human stress response and immune systems. Using a genome-wide approach, we illustrated how the experience of entering foster care in childhood was associated with methylation profiles of an array of genes functionally implicated in immune system activity. By exploring methylation levels of select candidate genes, we show how histories of maternal warmth or rejection were associated with methylation levels of specific loci on genes involved in the HPA axis and immune system activity. Despite these promising preliminary results, we acknowledge that the sample size limits our ability to draw strong conclusions from these results. Furthermore, regarding the genome-wide approach, we identified several significant clusters with enrichments scores greater than 1.3, but note that false detection ratios and tests controlling for multiple comparisons ranged across clusters (see online-only supplementary Table S.2). Therefore, we emphasize the need to replicate these results with a larger sample. Notwithstanding these limitations, these results serve as a model for how both genome-wide and candidate-gene approaches offer differing perspectives on how early adverse events epigenetically program phenotypic trajectories related to stress response and immune system activity.

As demonstrated from this article and data presented, both the genome-wide and candidate-gene approaches have important, yet distinct implications for understanding early adversity's effect on stress response and immune system activity. As can be seen by our demonstration, the genome-wide approach offers a more exploratory approach to understanding which genes are differentially methylated across groups of children who differed in terms of their histories of early adversity. This genome-wide method allows for a

top down appreciation for how broadly measured epigenetic patterns mapped on to alterations in physiological and metabolic pathways associated with immunoactivity. Furthermore, the broad-based scan of differential DNA methylation profiles across the genome inherently generates general hypotheses and is useful for generating more systematic, hypothesis-driven analyses that can be carried out at the candidate-gene level.

In contrast, the candidate-gene approach allows for a more nuanced, bottom up understanding of how early adversity may be associated with specific methylome structure of loci on candidate genes. This more a priori approach, narrowing the focus on the role of specific genes whose functional properties are known, examines whether alterations of systems known to be affected by early-life stress are mediated by epigenetic events. Although lacking in ability to explain the entire genomic picture underlying phenotypic variability, this method can provide incremental information for how individual elements of larger biological systems become perturbed in the face of early-life stress. Furthermore, understanding methylation patterns at the level of specific candidate genes may be beneficial when applying and assessing the effectiveness of clinical treatments, as has been successfully performed in prior animal research (Weaver et al., 2004).

In addition to the relative advantages to each approach, it is important to consider the respective limitations of the whole-genome and candidate-gene approaches in uncovering epigenetic signatures associated with early adversity. Advantages and disadvantages of genome-wide approaches to understanding genetic risk for disease have been widely discussed, and these same issues apply to the questions of interest in the current study. Technological advancements in the past decade have allowed for the simultaneous exploration of functionally annotated genes across the genome in relation to functional outcomes. Depicted in the pilot demonstration above, the major advantage of the genome-wide approach is the possibility of discovering unknown (epi)genetic pathways that have functional relevance. This approach, however, is not without drawbacks. Frequently discussed is the lower-powered nature of the genome-wide analyses due to the inherently high number of comparisons being tested through this approach (Amos, Driscoll, & Hoffman, 2010; McCarthy et al., 2008; Schulze & McMahon, 2002). Questions of sufficient power have been raised even in genome-wide investigations involving samples that contain thousands of participants (Yoo et al., 2010). Furthermore, the number of statistical comparisons carried out in genome-wide approaches confers a high risk for false positives, which underscores the need to carefully consider issues of sample size (Hirschorn & Daly, 2005).

The strengths of the candidate-gene approach, in terms of increased statistical power and grounding in a priori theory and knowledge, may combat some of the weaknesses of the genome-wide approach. The candidate-gene approach, however, inevitably falls short in fully uncovering the (epi)genetic mechanisms underlying complex phenotypic variability (Zhu & Zhao, 2007). As we demonstrated in this article, many studies of epigenetic profiles of candidate genes focus on genetic markers whose biological functions are typically known and functionally implicated in development. Although furthering our understanding of certain genetic pathways that underlie complex traits, the study of human development has moved well beyond hypotheses linking a small number of candidate genes with complex psychological outcomes (Kendler, 2005). Therefore, it is important to consider the positive candidate-gene findings as a small part of the complex picture of epigenetic mediators related to human development.

This literature review and empirical illustration suggest that both candidate-gene and genome-wide explorations are important for understanding the intricate associations between early adversity and long-term health, bearing in mind known limitations. As Vrieze

et al. discuss in this issue, scientific progress will advance most rapidly by taking advantage of both approaches, using systematic, error-reducing methods, especially as approaches become more cost effective and approachable to developmental scientists. Regardless of the approach, it will be critical for future research to consider epigenetic research not as deterministic, but as a biological signature that may increase the risk for developing chronic mental or physical health problems later in life. Emerging preliminary data indicate that high levels of maternal warmth can protect individuals from the epigenetic alterations related to patterns of immune system and inflammatory activity typical under conditions of early adversity (Chen et al., 2011). Although widely explored in the behavioral and psychological literature, future investigations on the protective factors that shape or reverse "risky" epigenetic signatures will be needed.

Research in the epigenetic consequences of early-life stress is in its infancy; certain conclusions, however, can be made from the extant body of research. First, it is clear that signals from the social environment can be detected on an epigenetic level, across the genome and at the level of candidate genes. Second, research to date demonstrates that a number of early-life stressors, including child abuse and maltreatment, stressful separations from caregivers, and being reared in an impoverished environment can lead to epigenetic alterations that have functional implications for health and development. Third, it is becoming apparent that the epigenetic structure of genes underlying critical stress response and immune systems are especially vulnerable to environmental signaling. With the implementation of more prospective, longitudinal studies, incorporating both genome-wide and candidate-gene approaches, the field will move forward in its understanding of the stability (or reversibility) and functional consequences of epigenetic signatures specifically involving HPA axis and immune system activity, with the overall goal of promoting positive mental and physical health among at risk individuals.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

Amos W, Driscoll E, Hoffman J. Candidate genes versus genome-wide associations: Which are better for detecting genetic susceptibility to infectious disease? Proceedings of Biological Science. 2011; 278:1183–1188.

Anda RF, Brown DW, Dube SR, Bremner JD, Felitti VJ, Giles WH. Adverse childhood experiences and chronic obstructive pulmonary disease in adults. American Journal of Prevention Medicine. 2009; 34:396–403.

Bergmann A, Lane ME. Hidden targets of microRNAs for growth control. Trends in Biochemical Sciences. 2003; 28:461–463. [PubMed: 13678953]

Borghol N, Suderman M, McArdle W, Racine A, Hallett M, Pembrey M, et al. Associations with early-life socio-economic position in adult DNA methylation. International Journal of Epidemiology. 2012; 41:62–74. [PubMed: 22422449]

Calandra T, Bernhagen J, Metz CN, Spiegel L, Bacher M, Donnely T, et al. *MIF* as a glucocorticoid-induced modulator of cytokine production. Nature. 1995; 377:68–71. [PubMed: 7659164]

Carpenter LL, Carvalho JP, Tyrka AR, Wier LM, Mello AF, Mello MF, et al. Decreased adrenocorticotropic hormone and cortisol responses to stress in healthy adults reporting significant childhood maltreatment. Biological Psychiatry. 2007; 62:1080–1087. [PubMed: 17662255]

Carpenter LL, Tyrka AR, Ross NS, Khoury L, Anderson GM, Price LH. Effect of childhood emotional abuse and age on cortisol responsivity in adulthood. Biological Psychiatry. 2009; 66:69–75. [PubMed: 19375070]

Carroll JE, Cohen S, Marsland AL. Early childhood socioeconomic status is associated with circulating interleukin-6 among mid-life adults. Brain, Behavior, and Immunology. 2011; 7:1468–1474.

Ceccatelli S, Tamm C, Zhang Q, Chen M. Mechanisms and modulation of neural cell damage induced by oxidative stress. Physiology & Behavior. 2007; 92:87–92. [PubMed: 17628619]

Champagne FA, Weaver IC, Diorio J, Dymov S, Szyf M, Meaney MJ. Maternal care associated with methylation of the estrogen receptor-alpha1b promoter and estrogen receptor-alpha expression in the medial preoptic area of female offspring. Endocrinology. 2006; 147:2909–2915. [PubMed: 16513834]

Chen EE, Miller GE, Kobor MS, Cole SW. Maternal warmth buffers the effects of low early-life socioeconomic status on pro-inflammatory signaling in adulthood. Molecular Psychiatry. 2011; 16:729–737. [PubMed: 20479762]

Cicchetti D, Rogosch FA, Gunnar MR, Toth SL. The differential impacts of early physical and sexual abuse and internalizing problems on daytime cortisol rhythm in school-aged children. Child Development. 2010; 81:252–269. [PubMed: 20331666]

Cicchetti D, Rogosch FA, Oshri A. Interactive effects of corticotropin releasing hormone receptor 1, serotonin transporter linked polymorphic region, and child maltreatment on diurnal cortisol regulation and internalizing symptomatology. Development and Psychopathology. 2011; 23:1125–1138. [PubMed: 22018085]

Comb M, Goodman HM. CpG methylation inhibits proenkephalin gene expression and binding of the transcription factor AP-2. Nucleic Acids Research. 1990; 18:3975–3982. [PubMed: 1695733]

Danese A, Moffitt T, Pariante C, Ambler A, Poulton R, Caspi A. Elevated inflammation levels in depressed adults with a history of childhood maltreatment. Archives of General Psychiatry. 2008; 65:409–416. [PubMed: 18391129]

Danese A, Pariante C, Caspi A, Taylor A, Poulton R. Childhood maltreatment predicts adult inflammation in a life-course study. Proceedings of the National Academy of Sciences. 2007; 104:1319–1324.

De Bellis MD, Chrousos GP, Dorn LD, Burke L, Helmers K, Kling MA, et al. Hypothalamic–pituitary–adrenal axis dysregulation in sexually abused girls. Journal of Clinical Endocrinology and Metabolism. 1994; 78:249–255. [PubMed: 8106608]

Dozier M, Manni M, Gordon M, Peloso E, Gunnar MR, Stovall-McClough K, et al. Foster children's diurnal production of cortisol: An exploratory study. Child Maltreatment. 2006; 11:189–197. [PubMed: 16595852]

Drury S, Theall K, Gleason MM, Smyke AT, De Vivo I, Wong JY, et al. Telomere length and early severe social deprivation: Linking early adversity and cellular aging. Molecular Psychiatry. 2012; 17:719–727. [PubMed: 21577215]

Dube SR, Fairweather D, Pearson WS, Felitti VJ, Anda RF, Croft JB. Cumulative childhood stress and autoimmune diseases in adults. Psychosomatic Medicine. 2009; 71:243–250. [PubMed: 19188532]

Duman RS. Neuronal damage and protection in the pathophysiology and treatment of psychiatric illness: Stress and depression. Dialogues in Clinical Neuroscience. 2009; 11:239–255. [PubMed: 19877493]

Epel ES. Psychological and metabolic stress: A recipe for accelerated cellular aging? Hormones. 2009; 8:7–22. [PubMed: 19269917]

Essex MJ, Shirtcliff EA, Burk LR, Ruttle PL, Klein MH, Slattery MJ, et al. Influence of early life stress on later hypothalamic–pituitary–adrenal axis functioning and its covariation with mental health symptoms: A study of the allostatic process from childhood into adolescence. Development and Psychopathology. 2011; 23:1039–1058. [PubMed: 22018080]

Fitzpatrick A, Kronmal R, Gardner J, Pstay B, Jenny N, Tracy R, et al. Leukocyte telomere length and cardiovascular disease in the cardiovascular health study. American Journal of Epidemiology. 2007; 165:14–21. [PubMed: 17043079]

Francis D, Diorio J, Liu D, Meaney MJ. Nongenomic transmission across generations of maternal behavior and stress responses in the rat. Science. 1999; 286:1155–1158. [PubMed: 10550053]

Gill JM, Saligan L, Woods S, Page G. PTSD is associated with an excess of inflammatory immune activities. Perspectives in Psychiatric Care. 2009; 45:262–277. [PubMed: 19780999]

Gluckman PD, Hanson MA, Cooper C, Thornburg KL. Effect of inutero and early-life conditions on adult health and disease. New England Journal of Medicine. 2008; 359:61–73. [PubMed: 18596274]

Goodwin RD, Stein MB. Association between childhood trauma and physical disorders among adults in the United States. Psychological Medicine. 2004; 34:509–520. [PubMed: 15259836]

Gunnar M, Donzella B. Social regulation of the cortisol levels in early human development. Psychoneuroendocrinology. 2002; 27:199–220. [PubMed: 11750779]

Heim C, Newport D, Mletzko T, Miller AH, Nemeroff CB. The link between childhood trauma and depression: Insights from HPA axis studies in humans. Psychoneuroendocrinology. 2008; 33:693–710. [PubMed: 18602762]

Hirschhorn J, Daly M. Genome-wide association studies for common diseases and complex traits. Nature Reviews Genetics. 2005; 6:95–108.

Huang DW, Sherman B, Lempicki R. Bioinformatics enrichment tools: Paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Research. 2009; 37:1–13. [PubMed: 19033363]

Inamdar NM, Ehrlich KC, Ehrlich M. CpG methylation inhibits binding of several sequence-specific DNA-binding proteins from pea, wheat, soybean and cauliflower. Plant Molecular Biology. 1991; 17:111–123. [PubMed: 1831056]

Jenuwein T, Allis CD. Translating the histone code. Science. 2001; 293:1074–1080. [PubMed: 11498575]

Jin SG, Wu X, Li AX, Pfeifer GP. Genomic mapping of 5-hydroxymethylcytosine in the human brain. Nucleic Acids Research. 2011; 39:5015–5024. [PubMed: 21378125]

Keinan-Boker L, Vin-Raviv N, Lipshitz I, Linn S, Barchana M. Cancer incidence in Israeli Jewish survivors of World War II. Journal of National Cancer Institute. 2009; 101:1489–1500.

Kendler K. “A gene for …”: The nature of gene action in psychiatric disorders. American Journal of Psychiatry. 2005; 162:1243–1252. [PubMed: 15994704]

Kittleson MM, Meoni LA, Wang NY, Chu AY, Ford DE, Klag MJ. Association of childhood socioeconomic status with subsequent coronary heart disease in physicians. Archives of Internal Medicine. 2006; 166:2356–2361. [PubMed: 17130389]

Kriaucionis S, Heintz N. The nuclear DNA base 5-hydroxymethylcytosine is present in Purkinje neurons and the brain. Science. 2009; 324:929–930. [PubMed: 19372393]

Liu D, Diorio J, Tannenbaum B, Caldji C, Francis D, Freedman A, et al. Maternal care, hippocampal glucocorticoid receptors, and hypothalamic–pituitary–adrenal responses to stress. Science. 1997; 277:1659–1662. [PubMed: 9287218]

Luthar S, Sexton C. Maternal drug abuse versus maternal depression: Vulnerability and resilience among school-age and adolescent offspring. Development and Psychopathology. 2007; 19:205–225. [PubMed: 17241491]

Matthews KA, Gallo LC. Psychological perspectives on pathways linking socioeconomic status and physical health. Annual Review of Psychology. 2011; 62:501–530.

McCarthy MI, Abecasis GR, Cardon LR, Goldstein DB, Little J, Ionnidis JP, et al. Genome-wide association studies for complex traits: Consensus, uncertainty and challenges. Nature Review Genetics. 2008; 9:356–369.

McGowan PO, Sasaki A, D’Alessio AC, Dymov S, Labonte B, Szyf M, et al. Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. Nature Neuroscience. 2009; 12:342–348.

McGowan PO, Suderman M, Sasaki A, Huang TC, Hallett M, Meaney MJ, et al. Broad epigenetic signature of maternal care in the brain of adult rats. PLoS One. 2011; 6:e14739. [PubMed: 21386994]

Miller G, Chen E. Unfavorable socioeconomic conditions in early life presage expression of proinflammatory phenotype in adolescence. Psychosomatic Medicine. 2007; 69:402–409. [PubMed: 17556642]

Miller GE, Chen E, Fok AK, Walker H, Lim A, Nicholls EF, et al. Low early-life social class leaves a biological residue manifested by decreased glucocorticoid and increased proinflammatory signaling. Proceedings of the National Academy of Science. 2009; 106:14716–14721.

Miller G, Chen E, Parker KJ. Psychological stress in childhood and susceptibility to the chronic diseases of aging: Moving toward a model of behavioral and biological mechanisms. Psychological Bulletin. 2011; 137:959–997. [PubMed: 21787044]

Naumova O, Lee M, Koposov R, Szyf M, Dozier M, Grigorenko EL. Differential patterns of whole-genome DNA methylation in institutionalized children and children raised by their biological parents. Development and Psychopathology. 2012; 24:143–155. [PubMed: 22123582]

Oberlander TF, Weinberg J, Papsdorf M, Grunau R, Misri S, Devlin AM. Prenatal exposure to maternal depression, neonatal methylation of human glucocorticoid receptor gene (*NR3C1*) and infant cortisol stress responses. Epigenetics. 2008; 3:97–106. [PubMed: 18536531]

Pace TW, Mletzko TC, Alagbe O, Musselman DL, Nemeroff CB, Miller AH, et al. Increased stress-induced inflammatory responses in male patients with major depression and increased early life stress. American Journal of Psychiatry. 2006; 163:1630–1633. [PubMed: 16946190]

Perroud N, Paoloni-Giacobino A, Olie P, Salzmann A, Nicastro R, Guillaume S, et al. Increased methylation of glucocorticoid receptor gene (*NR3C1*) in adults with a history of childhood maltreatment: A link with the severity and type of trauma. Translational Psychiatry. 2011; 1:e59. [PubMed: 22832351]

Phillips JE, Marsland AL, Flory JD, Muldoon MF. Parental education is related to C-reactive protein among female middle-aged community volunteers. Brain, Behavior, and Immunology. 2009; 23:677–683.

Pollitt RA, Kaufman JS, Rose KM, Diez-Roux AV, Zeng D, Heiss G. Early-life and adult socioeconomic status and inflammatory risk markers in adulthood. European Journal of Epidemiology. 2007; 22:55–66. [PubMed: 17225957]

Radtke KM, Ruf M, Gunter HM, Dohrmann K, Schauer M, Meyer A, et al. Transgenerational impact of intimate partner violence on methylation in the promoter of the glucocorticoid receptor. Translational Psychiatry. 2011; 1:1–6.

Rich-Edwards JW, Spiegelman D, Hibert E, Jun H, Todd T, Kawachi I, et al. Abuse in childhood and adolescence as a predictor of type 2 diabetes in adult women. American Journal of Preventive Medicine. 2010; 39:529–536. [PubMed: 21084073]

Riley EH, Wright RJ, Jun HJ, Hibert EN, Rich-Edwards JW. Hypertension in adult survivors of child abuse: Observations from the Nurses' Health Study II. Journal of Epidemiology and Community Health. 2010; 64:413–418. [PubMed: 20445210]

Rohner, RP. Handbook for the study of parental acceptance and rejection. Storrs, CT: University of Connecticut, Center for the Study of Parental Acceptance and Rejection; 1991.

Schulze TG, McMahon FJ. Genetic association mapping at the crossroads: Which test and why? Overview and practical guidelines. American Journal Medical Genetics. 2002; 114:1–11.

Segman RH, Shefi NN, Goltser-Dubner TT, Friedman NN, Kaminski NN, Shalev AY. Peripheral blood mononuclear cell gene expression profiles identify emergent post-traumatic stress disorder among trauma survivors. Molecular Psychiatry. 2005; 10:500–513. [PubMed: 15685253]

Shalev I, Moffit T, Sugden K, Williams B, Houts R, Danese A, et al. Exposure to violence during childhood is associated with telomere erosion from 5 to 10 years of age: A longitudinal study. Molecular Psychiatry. 2012 Advance online publication. 10.1038/mp.2012.32

Shirtcliff EA, Coe CL, Pollak SD. Early childhood stress is associated with elevated antibody levels to herpes simplex virus type 1. Proceedings of the National Academy of Sciences. 2009; 106:2963–2967.

Shonkoff JP, Boyce W, McEwen BS. Neuroscience, molecular biology, and the childhood roots of health disparities building a new framework for health promotion and disease prevention. Journal of the American Medical Association. 2009; 301:2252–2259. [PubMed: 19491187]

Simon NM, Smoller JW, McNamara KL, Maser RS, Zalta AK, Pollack MH, et al. Telomere shortening and mood disorders: Preliminary support for a chronic stress model of accelerated aging. Biological Psychiatry. 2006; 60:432–435. [PubMed: 16581033]

Smith AK, Conneely KN, Kilaru V, Mercer KB, Weiss TE, Bradley-Davino B, et al. Differential immune system DNA methylation and cytokine regulation in post-traumatic stress disorder. American Journal of Medical Genetics. 2011; 156B:700–708. [PubMed: 21714072]

Strahl BD, Allis CD. The language of covalent histone modifications. Nature. 2000; 403:41–45. [PubMed: 10638745]

Taylor SE, Lehman BJ, Kiefe CI, Seeman TE. Relationship of early life stress and psychological functioning to adult C-reactive protein in the coronary artery risk development in young adults study. Biological Psychiatry. 2006; 60:819–824. [PubMed: 16712805]

Taylor SE, Lerner JS, Sage RM, Lehman BJ, Seeman TE. Early environment, emotions, responses to stress, and health. Journal of Personality. 2004; 72:1365–1394. [PubMed: 15509286]

Tyrka AR, Price LH, Marsit C, Walters OC, Carpenter LL. Childhood adversity and epigenetic modulation of the leukocyte glucocorticoid receptor: Preliminary findings in healthy adults. PLoS One. 2012; 7:e30148. [PubMed: 22295073]

Tyrka AR, Wier L, Price LH, Ross N, Anderson GM, Wilkinson CW, et al. Childhood parental loss and adult hypothalamic–pituitary–adrenal function. Biological Psychiatry. 2008; 63:1147–1154. [PubMed: 18339361]

Uddin M, Aiello AE, Wildman DE, Koenen KC, Pawelec G, de los Santos R, et al. Epigenetic and immune function profiles associated with posttraumatic stress disorder. Proceedings of the National Academy of Sciences. 2010; 107:9470–9475.

van der Vegt EM, van der Ende J, Kirschbaum C, Verhulst FC, Tiemeier H. Early neglect and abuse predict diurnal cortisol patterns in adults: A study of international adoptees. Psychoneuroendocrinology. 2009; 34:660–669. [PubMed: 19128884]

Weaver IC, Cervoni N, Champagne FA, D'Alessio AC, Sharma S, Seckl JR, et al. Epigenetic programming by maternal behavior. Nature Neuroscience. 2004; 7:847–854.

Wismer-Fries AB, Shirtcliff EA, Pollak SD. Neuroendocrine dysregulation following early social deprivation in children. Developmental Psychobiology. 2008; 50:588–599. [PubMed: 18683181]

Wolkowitz OM, Mellon SH, Epel ES, Lin J, Dhabhar FS, Su Y, et al. Leukocyte telomere length in major depression: Correlations with chronicity, inflammation and oxidative stress—Preliminary findings. PLoS One. 2011; 6:e17837. [PubMed: 21448457]

Yoo YJ, Bull SB, Paterson AD, Waggott D, Sun L. The Diabetes Control and Complications Trial/ Epidemiology of Diabetes Interventions and Complications Research Group. Were genome-wide linkage studies a waste of time? Exploiting candidate regions within genome-wide association studies. Genetic Epidemiology. 2010; 34:107–118. [PubMed: 19626703]

Zhu M, Zhao S. Candidate gene identification approach: Progress and challenges. International Journal of Biological Science. 2007; 3:420–427.

Zieker JJ, Zieker DD, Jatzko AA, Dietzsch JJ, Nieselt KK, Schmitt AA, et al. Differential gene expression in peripheral blood of patients suffering from post-traumatic stress disorder. Molecular Psychiatry. 2007; 12:116–119. [PubMed: 17252001]