Epigenetic Programming by Early-Life Stress: Evidence from Human Populations

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Background: A substantial body of experimental and epidemiological evidence has been accumulated suggesting that stressful events in early life including acute perinatal stress, maternal deprivation or separation, and variation in maternal care may lead to neuroendocrine perturbations thereby affecting reproductive performance, cognitive functions, and stress responses as well as the risk for infectious, cardio-metabolic and psychiatric diseases in later life. Results: Findings from recent studies based on both genome-wide and candidate gene approaches highlighted the importance of mechanisms that are involved in epigenetic regulation of gene expression, such as DNA methylation, histone modifications, and non-coding RNAs, in the long-term effects of exposure to stress in early life. Conclusions: This review is focused on the findings from human studies indicating the role of epigenetic mechanisms in the causal link between early-life stress and later-life health outcomes. Developmental Dynamics 244:254–265, 2015. © 2014 Wiley Periodicals, Inc.

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Key words: perinatal stress; childhood adversity; epigenetics; developmental programming of adult disease

Submitted 19 August 2014; First Decision 29 September 2014; Accepted 29 September 2014; Published online 9 October 2014

Introduction

A substantial body of experimental and epidemiological evidence has been accumulated in recent years, demonstrating that the health status of an adult organism may be strongly influenced by experiences in early life. The developmental origins of an adult health and disease (DOHaD) hypothesis states that adverse fetal and early childhood exposures can “program” risks for later chronic disorders such as obesity, metabolic syndrome, as well as cardiovascular, neurodegenerative and cognitive diseases in adult life (Hanson, 2013; Tarantal and Berglund, 2014). The DOHaD concept originally focused on long-term health outcomes of fetal malnutrition. Over the past years, however, it has been extended to other, non–dietary, factors that were demonstrated to have great potential to influence the organism’s health status. Among them, the early-life exposure to stress (i.e., challenges to physical and/or psychological homeostasis impacting the functional integrity and survival of the organism) is likely one of the most important risk factors for developmental programming of adverse outcomes in adulthood.

Findings from recent animal studies highlighted the importance of mechanisms that are involved in epigenetic regulation of gene expression on the long-term effects of exposure to stress in early life. Human data on this subject are similar, though more limited. Current reviews highlighting progress in this research field are mostly focused on animal models (Curley et al., 2011; Roth, 2012; Champagne, 2012, 2013; Sasaki et al., 2013). The present review, in contrast, is primarily focused on the findings from human studies indicating the role of epigenetic mechanisms in the causal link between early-life stress and later-life health outcomes.

Long-Term Outcomes of Exposure to Early-life Stress

Several lines of experimental evidence were obtained in various rodent models, such as models of acute perinatal stress (including perinatal maternal distress, complications during the birth process, asphyxia, and hypothermia), maternal deprivation or separation, and variation in maternal care, suggesting that exposure to stressful conditions in early life may lead to neuroendocrine perturbations thereby affecting reproductive performance, cognitive functions, and stress responses in later life. A large number of animal and epidemiological studies have revealed that prenatal stress as well as excess exogenous glucocorticoids or inhibition of 11b-hydroxysteroid dehydrogenase type 2 (the placental barrier to maternal glucocorticoids) may be linked to adverse health outcomes including low birth weight, neuroendocrine dysfunction, and increased risk of infectious, cardio-metabolic, and psychiatric diseases in later life (for reviews, see Kajantie, 2006; Beydoun and Saftlas, 2008; Cottrell and Seckl, 2009; Mastorci et al., 2009; Harris and Seckl, 2011; Nielsen et al., 2011).

At the behavioral level, the neuroendocrine effects induced by prenatal stress are shown to be accompanied by various anxiety- and depressive-like phenotypes, such as changed physical activity level, decreased exploration of novel and/or anxiogenic...
environments, and increased immobility during a forced swim test (Weinstock, 2008). It has been demonstrated that the long-term effects of early-life stress are mediated through alterations in the maternal and fetal hypothalamic-pituitary-adrenal (HPA) axes leading to the in-utero exposure to excess glucocorticoids (Reznikov et al., 1999; Reynolds et al., 2013a, 2013b). The role of the fetal exposure to stress-induced maternal glucocorticoids, which may pass the placental barrier and disrupt the fetal brain development, was evident, for example, from research in the male adult offspring born to either rat dams with intact corticosterone secretion or to prenatally stressed adrenalectomized dams (Barbazanges et al., 1996). The maternal–placental–fetal endocrine and immune responses have been proposed as a plausible candidate mechanism mediating the lasting effects of the exposure to stress during gestational development on various physiological systems of the developing organism (Entringer et al., 2010).

The early postnatal life is another sensitive period with high potential to induce programming effects. Impairment of mother-infant interactions (such as maternal separation or deprivation) during the early postnatal period was shown to disrupt the neuroendocrine regulations, such as upregulation of hippocampal glucocorticoid receptor (GR) and hypothalamic corticotropin-releasing factor (CRF), along with the increased corticosterone and adrenocorticotropic hormone levels (Lehmann and Feldon, 2000; Lippmann et al., 2007; Korosi and Baram, 2010; Nishi et al., 2013). Such early stress-induced neuroendocrine alterations may cause behavioral problems in adulthood, such as impaired memory, learning, and anxiety- and depressive-like behaviors (Curley et al., 2011). Lasting effects associated with natural variations in postnatal maternal care in rodents (namely, high or low levels of licking and grooming, LG) have also been indicated. Male rats that were reared by high LG dams demonstrated lower levels of stress responsivity and greater performance on cognitive tasks in adulthood, and they were more exploratory in a novel environment than the offspring of low-LG dams (Liu et al., 1997, 2000; Caldi et al., 2000).

In all the models of prenatal and postnatal stress that are listed above, the biochemical and physiological changes have been shown to be accompanied by corresponding alterations in gene expression and associated epigenetic alterations.

Epigenetic Changes Mediating Lasting Effects of Early-Life Stress

The epigenetic regulation of gene expression has been convincingly demonstrated to play a pivotal role in mediating the programming effects of stressful conditions during early development, with implications for neurobiological functioning and behavior (Wadhwa et al., 2009; Champagne, 2013). Epigenetic modifications refer to mitochiondrial and/or mitochiondrial heritable changes in gene expression that occur without changes in underlying DNA sequence (Hochberg et al., 2011). Whereas DNA is known to be relatively static in a course of ontogeny, the epigenetic code is changed dramatically during the embryonic development to initiate differential patterns of gene expression among developing tissues. This code consists of chemical modifications to DNA and to histones (proteins that play a central role in tightly coiling and packing the DNA into nucleosomes).

In mammals, methylation of DNA, which consists of the addition of a methyl group at the 5-carbon position of cytosine by specific enzymes, DNA methyltransferases, resulting in 5-methylcytosine (5mC), is the most common mechanism of epigenetic regulation (Smith and Meissner, 2013; Jeltsch and Jurkowska, 2014). This process predominantly occurs within the CpG-rich regions, where cytosine nucleotides are directly followed by guanidine nucleotides (so-called “CpG islands”). CpG islands typically occur near or at transcription start sites. Generally, methylation of CpG islands in promoter regions of various genes is linked with their transcriptional silencing. Post-translational histone modifications, such as acetylation, methylation, phosphorylation, and ubiquitination of histone tails, are another important mechanism responsible for epigenetic regulation of transcription (Wang and Higgins, 2013). Among all these modifications, the histone acetylation seems to play a greater role in transcriptional regulation. Acetylation of lysine residues on the amino-terminal tails of the histones leads to neutralizing their positive charge and to decreasing their affinity for the DNA, thereby causing an increased accessibility of transcriptional regulatory proteins to chromatin templates. The enzymes catalyzing the processes of addition or removal of acetyl groups from lysine residues on the histone N-terminal tails, are histone acetyltransferases and deacetylases, respectively (Selvi and Kundu, 2009). These epigenetic processes are interrelated with one another: that is, methylation of DNA may influence histone modifications or vice versa, thereby collectively influencing the accessibility of the chromatin to RNA polymerases and transcription factors.

A novel, recently discovered level of epigenetic regulation is the modualation of gene expression by non-coding RNAs (ncRNAs) that regulate activity of the gene at both the transcriptional and post-transcriptional levels. NcRNAs were shown to play a substantial role in all major epigenetic pathways including DNA methylation, histone modification, formation of heterochromatin, and gene silencing (Adifo and Pasquinelli, 2012; Doglioni et al., 2014). A schematic representation of the main epigenetic mechanisms involved in regulation of gene expression is presented in Figure 1.

There are specific stages of development during which the epigenome (the totality of epigenetic marks across the entire genome) is the most labile and, therefore, most sensitive to various hormonal and environmental cues (Thompson and Einstein, 2010; Bateson et al., 2014). In mammals, genome-wide DNA
demethylation is known to occur during germ cell development, followed by remethylation before fertilization. Early embryogenesis is then characterized by a second wave of the genome-wide demethylation, and methylation patterns are re-established after the implantation. The phases of postfertilization demethylation and remethylation likely play a role in the removal of acquired epigenetic modifications (Lee et al., 2014). The epigenome. Therefore, seems to be particularly vulnerable to the adverse influences during the periods of gametogenesis and early embryogenesis (Vickaryous and Whitelaw, 2005). There is convincing evidence that environmental cues during early life can induce de novo epigenetic changes, which change phenotypic trajectories via altering the gene expression profile (Bick et al., 2012). Once established in early development, the epigenetic marks are stably maintained through somatic cell divisions (Shipony et al., 2014). In humans, the window of epigenetic developmental plasticity is shown to extend from preconception to early childhood (Hochberg et al., 2011). The epigenetic “tuning” through developmental plasticity can be advantageous because it attempts to match the organism’s responses to environmental conditions that are likely to prevail during the life-course (Bateson et al., 2014). Such match to predicted environment will obviously promote the individual’s health and social well-being. However, a mismatch between the predicted and actual conditions may lead to disease in later life (Burdge and Lillycrop, 2014).

Changes in epigenetic pathways are considered to be a key mechanism linking early-life nutritional disturbance (both under- and overnutrition) to many age-associated chronic pathologies such as obesity, type 2 diabetes, cardiovascular disease, osteoporosis, neurodegenerative and cognitive disorders (Lillycrop and Burdge, 2012; Lupu et al., 2012; Reynolds et al., 2013a,b; Vaiserman, 2012, 2014), as well as to cancer (Kaur et al., 2013; Lillycrop and Burdge, 2014). The key role of epigenetic mechanisms in mediating the long-term effects of exposure to non-nutritional stresses in early life was also revealed in a number of rodent studies, where substantial variations in DNA methylation and histone modifications were obtained in offspring exposed to prenatal stress, maternal deprivation and/or separation, variation in maternal care, and juvenile social enrichment and/or isolation (reviewed, e.g., in Curley et al., 2011; Gudsnuk and Champagne, 2012; Champagne, 2012, 2013). Importantly, the earlier the organism is subjected to stressful events in utero development, the more pronounced lasting effects are generally observed, further suggesting the causal role of epigenetic processes in pathways to later health outcomes. For example, in the Mueller and Bale (2008) study, prenatal exposure to different stressors has been found to contribute to the enhanced stress sensitivity during adulthood in mice, manifested in altered expression of GR and CRF, along with increased HPA axis responsivity. These changes were accompanied by corresponding alterations in the levels of methylation and expression of the GR and CRF genes. Remarkably, the early gestational stage was shown to be especially sensitive to stress exposure, suggesting strong evidence for developmental epigenetic programming. Epigenetic changes were also found with respect to exposures in postnatal life, such as neonatal handling, which has been shown to induce a persistent elevation in the level of transcription of the NR3CI gene encoding GR (O'Donnell et al., 1994). A high level of maternal care has been demonstrated to cause epigenetic changes similar to those induced by handling, such as a decreased level of DNA methylation within the promoter region of the NR3CI gene in the hippocampal tissue of male offspring reared by high-LG dams (Weaver et al., 2004). The lasting epigenetic influence of early-life adversity on the methylation levels of the brain-derived neurotrophic factor (BDNF) gene, playing a key role in the neural and behavioral plasticity, along with its expression in the adult prefrontal cortex, has also been demonstrated (Roth et al., 2009).

Human data confirming the involvement of epigenetic mechanisms in long-lasting effects of early-life stress are more scarce relative to animal data because of the limited access to relevant biological materials, but they clearly indicate that such mechanisms may operate in man as well. In the subsections below, the data obtained in human populations are summarized.

Prenatal Stress and Programming of Human Health and Disease Risk

In a number of human studies, prenatal exposure to maternal stressful conditions, including acute and chronic stressors, anxiety or depression (which are all known to be accompanied by elevated cortisol levels), was associated with an increased risk of multiple neurobiological and behavioral problems in adult life (for reviews, see, e.g., Monk et al., 2012; Del Giudice, 2014). So, there is compelling evidence linking prenatal exposure to maternal distress to increased risk of autism, anxiety, and schizophrenia in the offspring’s adult life (Beversdorff et al., 2005; Khashan et al., 2008; Fine et al., 2014). In research by Entringer and coworkers, it has been found that those individuals who were prenatally exposed to maternal psychosocial stress, demonstrated a higher percentage body fat, body-mass index, lipid profile, primary insulin resistance (Entringer et al., 2008a), impaired immune function (Entringer et al., 2008b) and cognitive performance (Entringer et al., 2009), as well as obesity and dysregulated glycemic control (Entringer, 2013) in adult life.

In a series of recent studies, the potential role of epigenetic mechanisms in mediating the lasting effects of maternal prenatal distresses on subsequent bio-behavioral outcomes was highlighted. It has been suggested that developmental deregulation of epigenetic pathways results in genome-wide changes in gene expression in various tissues including the brain, thereby affecting the connectivity and functioning of neural circuitry and conferring risk for both psychiatric and physical disorders in later life (Monk et al., 2012). In Oberlander et al.'s (2008) research, exposure to the maternal depressed/anxious mood during the 3rd trimester of gestational development resulted in an increased methylation of a CpG-rich region in the promoter and exon1F of the GR gene (NR3CI) in the cord blood of newborns, and these effects on methylation of the NR3CI gene have been shown to be persistent beyond infancy. Surprisingly, the epigenetic effects obtained have been demonstrated in offspring but not in maternal blood samples. Levels of methylation of NR3CI gene in fetal cord blood were correlated with levels of response to stress in infants at 3 months of age (as measured by salivary cortisol levels), suggesting a functional consequence of this epigenetic variation on HPA stress reactivity.

In the study by Devlin et al. (2010), the exposure to maternal depressed mood during the second trimester of gestational development was shown to be associated with decreased levels of methylation in promoter of SLC6A4 gene, encoding the serotonin transporter, in maternal peripheral leukocytes and in umbilical cord leukocytes collected from their infants at birth, while no such effects on BDNF gene have been revealed (Devlin et al.,
DEVELOPMENTAL DYNAMICS of parents or poor quality parenting, parental psychiatric disorder, adversity during early childhood linked to maltreatment, loss exposures, early postnatal stressful experiences may have potential throughout the life span (Weaver et al., 2004). During the postnatal period, and that demethylation of DNA may occur in neurons and fully differentiated neurons and may thereby support neuronal functions and plasticity in adult brain (Riccio, 2010). The importance of epigenetic mechanisms in these processes is supported by observations that de novo DNA methylation may occur in neurons during the postnatal period, and that demethylation of DNA may occur throughout the life span (Weaver et al., 2004).

It is therefore not surprising that in addition to prenatal stress exposures, early postnatal stressful experiences may have potential for long-term programming. There is compelling evidence that adversity during early childhood linked to maltreatment, loss of parents or poor quality parenting, parental psychiatric disorders, exposure to sexual, physical, emotional/psychological abuse, etc., may cause a range of physical and mental problems in adulthood including hypertension, obesity, cardiovascular disease, substance abuse, smoking, psychosis, depression, and attempted suicide (reviewed, e.g., in Varese et al., 2012; Brent and Silverstein, 2013), as well as cancer (Kelly-Irving et al., 2013). Immune dysregulation is considered to be a key pathway linking the childhood adversity to elevated rates of morbidity and mortality from a number of chronic diseases later in life (Fagundes et al., 2013). Adult individuals with a history of childhood adversity also demonstrated reduced volumes of hippocampus and prefrontal cortex, elevated HPA axis activation in response to stress, and enhanced inflammation in comparison with the non-maltreated individuals (Danese and McEwen, 2012). According to the concept of allostatic load, exposure to stressful conditions during childhood may lead to depletion or failure of normal physiologic processes. Adverse experiences throughout childhood induce substantial biological changes (biological embedding), modifying the maturation of allostatic systems (Sasaki et al., 2013). The frequent or chronic activation of allostatic systems in early life may cause progressive wear and tear, or allostatic overload, and thereby result in adverse health outcomes in adulthood (Katz et al., 2012; Misra et al., 2013; Ellis and Del Giudice, 2014).

Several lines of animal evidence support a role for epigenetic processes in lasting effects of childhood adversity in insulin resistance and immune dysregulation, as well as in emotional and cognitive impairments in adulthood (Meany et al., 2007; Hackman et al., 2010). These experimental findings have been extended to human beings by identifying an association between early-life adversity and epigenetic marks in adult life, primarily, changed expression of the hippocampal GR gene (Bick et al., 2012; Zhang et al., 2013; Lester et al., 2013; Vialou et al., 2013).

An association between the stressful conditions in early life and long-term alterations in epigenetic regulation at the whole-genome level has been shown repeatedly. Several such findings were obtained from studies using low socioeconomic status (SES) as an indicator for stressful events in early life. This condition, which is generally accompanied by a higher stress load through a poor quality of dietary intake and higher physical work load, was shown to strongly predict a variety of psycho-emotional disorders, such as depression and schizophrenia (Hackman et al., 2010), as well as respiratory, metabolic, and cardiovascular disorders (Tamayo et al., 2010; Azad et al., 2012; Spencer et al., 2013) in adult life. In the study by the Borghol et al. (2012), disadvantaged socio-economic position in early life was found to be associated with adult blood DNA methylation profiles. Functionally, most genes shown to be differentially methylated in association with low SES early in life were genes involved in various metabolic and cell signaling pathways. Miller et al. (2009) have also found by performing genome-wide transcriptional profiling that in those healthy adults who had low-SES backgrounds, the genes bearing response elements for the CREB/ATF family of transcription factors that conveys adrenergic signals to leukocytes were significantly up-regulated, while the genes with response elements for the glucocorticoid receptor, which regulates the secretion of cortisol and transduces its antiinflammatory actions in the immune system, were significantly down-regulated. Those persons who had low SES in early life also demonstrated elevated cortisol levels in daily life, along with enhanced expression of transcripts bearing response elements for NF-kappaB, and...
enhanced stimulated production of the proinflammatory cytokine interleukin 6. The authors conclude that “low early-life SES programs a defensive phenotype characterized by resistance to glucocorticoid signaling, which in turn facilitates exaggerated adrenocortical and inflammatory responses. Although these response patterns could serve adaptive functions during acute threats to well-being, over the long term they might exact an allostatic toll on the body that ultimately contributes to the chronic diseases of aging.” Findings from the study by Chen et al. (2011) demonstrate that the detrimental effects of low SES in early life on patterns of the immune system and inflammatory activity in adulthood may be at least partly buffered by high-level maternal warmth. These changes were accompanied by alterations in genome-wide transcriptional profiling. Specifically, individuals with low early-life SES whose mothers expressed high warmth toward them, demonstrated less Toll-like receptor-stimulated production of interleukin 6, and reduced activity of both pro-inflammatory transcription factor (NF-κB) and immune activating transcription factor (AP-1) relative to those persons who had low SES early in life but experienced low maternal warmth. These data suggest that the deleterious effects of low socioeconomic environments early in life could be at least partly reduced through supportive family climate.

Some data have been also obtained in studies of the lasting effects of adverse experiences such as physical and sexual abuse or neglect in early life, which are well-known risk factors for mental and behavioral disorders (Sasaki et al., 2013), as well as for affective disorders and suicide (Mann and Currier, 2010) in adulthood. In a genome-wide study of promoter methylation in individuals with history of severe abuse during childhood, Labonté et al. (2012a) have identified 362 differentially methylated promoters in hippocampal neurons from postmortem brain tissues of individuals with a history of severe childhood abuse compared to control subjects. Among these genes those involved in cellular/neuronal plasticity have been revealed to be most differentially methylated. More recently, Suderman et al. (2014) have demonstrated that 997 gene promoters in whole blood DNA of adult subjects were differentially methylated in association with childhood abuse. Most of these genes are known to be involved in key cell signaling pathways linked to development and regulation of transcription. Provençal et al. (2014), by analyzing the genome-wide promoter DNA methylation profiles in T cells from adult men, have revealed that 448 distinct gene promoters were differentially methylated in persons with a history of parental physical aggression from 6 to 15 years of age compared to a control group. Functionally, most of these genes were previously demonstrated to play a substantial role in aggression and were enriched in biological pathways that are affected by behavior.

In a number of studies, it has been shown that those children who experience parental neglect as a result of early-life institutionalization, subsequently tend to demonstrate profound intellectual impairment (Beckett et al., 2006). In the study by Naumova et al. (2012), differential patterns of whole-genome DNA methylation in blood samples were clearly evident among institutionalized children and children raised by their biological parents. Most of the differentially methylated genes either contributed to immune function or to cell signaling pathways, including those implicated in neural communication and the development and functioning of the brain. In the Bick et al. (2012) study, 173 genes have been demonstrated to be differentially methylated among persons with and without previous placement into foster care. Most of these genes were identified as implicated in the control of the ubiquitin-mediated proteolysis pathway, playing a substantial role in the inflammatory/immune responses, as well as in several basic cellular processes, and in the pathways of antigen processing and presentation. In addition, 72 genes known to be linked to the control of transcriptional regulation and apoptosis demonstrated enhanced levels of methylation in children with the history of foster care, whereas 101 genes implicated in control of posttranslational protein modifications and protein catabolic processes demonstrated decreased levels of methylation compared to control individuals.

In addition to genome-wide association studies, the role of epigenetic regulation in linking early stressful experiences to later health outcomes is also evident from several candidate gene studies. While genome-wide analysis allows for generating hypotheses regarding underlying molecular pathways, the candidate gene approach restricts inference to the physiologically relevant gene under consideration. In determining the differences in epigenetic marks in the critical loci in the brain implicated in the pathophysiology of suicide, McGowan et al. (2008) have examined the postmortem hippocampal brain tissues in the suicide subjects with the history of childhood neglect/abuse, which is associated with decreased hippocampal volume and cognitive impairments. In this study, the ribosomal RNA (rRNA) gene promoter has been shown to be significantly hypermethylated throughout the promoter and 5′ regulatory region in the brain of suicide subjects, consistent with the decreased level of rRNA expression in the hippocampus. In their subsequent study, McGowan et al. (2009) examined epigenetic differences in a neuron-specific GR promoter (NR3C1) between postmortem hippocampal tissues obtained from suicide victims with or without the history of child abuse. This gene was selected for analysis because decreased levels of GRs within the hippocampus are expected to cause enhanced HPA response to stress and thereby can account for the increased risk of poorer emotional regulation and psychopathology in individuals abused in childhood. In this study, the expression of total GR was significantly reduced in suicide victims with a history of childhood abuse relative to non-abused suicide victims or controls; there were, however, no differences between non-abused suicide victims and controls. There was also a significant effect on the expression of transcripts containing the exon 1F NR3C1 promoter. The levels of expression of GR 1F were significantly lower in samples obtained from suicide victims with a history of childhood abuse relative to the suicide victims without child abuse or controls. Similarly, in the study by Labonté et al. (2012b), the childhood abuse–associated decrease in GR expression was observed to be linked to elevated levels of DNA methylation in the promoter of the GR (1F) variant in the hippocampus of suicide completers compared to both suicide completers with no history of abuse and to control individuals.

In several studies, childhood trauma was shown to be a potent risk factor for developing depression in adulthood, particularly in response to additional stress (Heim et al., 2008; Heim and Binder, 2012). For example, in the study by Heim et al. (2000), women with a history of childhood abuse and a current major depression diagnosis demonstrated a six-fold greater adrenocorticotropic hormone response to stress than their age-matched controls, suggesting that there can be a permanent alteration in the set-point for HPA activity in the face of stress among those subjects who were exposed to stress in early life. By summarizing data from a series of studies, Heim et al. (2008) concluded that early-life
trauma is linked to immune activation, sensitization of the neuroendocrine stress response, glucocorticoid resistance, increased central CRF activity, and reduced hippocampal volume in adulthood. According to the authors, these neuroendocrine changes secondary to early-life stress likely reflect risk to develop depression in response to stress. More recent findings highlight the role of epigenetic factors in linkage between early-life trauma and adult depression (Heim and Binder, 2012; Hornung and Heim, 2014). For instance, Klengel et al. (2013) have demonstrated that an increased risk of developing adult stress-related psychiatric disorders may be associated with allele-specific, childhood trauma–dependent DNA demethylation in functional glucocorticoid response elements of FKBP5, an HPA-axis regulating gene. Such demethylation of FKBP5 gene was shown to be related to an increased stress-dependent gene transcription followed by a long-term dysregulation of the stress hormone system and a global effect on immune functions and brain areas linked to stress regulation.

Because access to the neural tissues is obviously limited in human subjects, many candidate gene investigations evaluating the role of specific epigenetic pathways in developmental programming of adult physical and mental problems by early-life stressful conditions were based on peripheral tissue samples. As indicated by some studies, the early childhood adversity-induced epigenetic alterations are not limited to brain tissues, but may occur in peripheral tissues as well. A significant negative association between the mothers’ reports on the parenting quality provided to their children and the offspring’s methylation levels of the GR gene and other candidate gene (namely, a macrophage migration inhibitory factor gene that is functionally involved in GR expression and immune responses) in blood samples 5 to 10 years after assessing the caregiving quality was observed in the study by Bick et al. (2012). The findings from the study by Tynka et al. (2012) also support that childhood adversity can cause the epigenetic modifications in the GR gene in human blood samples. In this research, the elevated NR3C1 methylation levels have been detected in leukocyte DNA from healthy adults exposed to maltreatment or inadequate nurturing during childhood. In the Perroud et al. (2011) study, childhood maltreatment and its severity have been found to be linked to enhanced levels of methylation of the exon 1p NR3C1 promoter in peripheral blood of persons with major depressive disorder and borderline personality disorder. No such changes were, however, observed in bulimic women who were exposed to abuse in childhood (Steiger et al., 2013). The differential methylation patterns were also detected in DNA extracted from buccal epithelial cells in adolescents whose parents were subjected to severe stresses during their early childhood (Essex et al., 2013). Maternal exposure to stress in infancy and paternal exposure to stress in the preschool years were found to be stronger predictors of differential methylation.

In several candidate gene studies, the low early-life SES was used as a strong indicator of adverse perinatal conditions. To examine whether early-life SES is associated with persistent changes in expression of genes implicated in inflammation response, Miller and Chen (2007) quantified the levels of mRNA transcripts encoding GR and toll-like receptor 4 (TLR4) in the peripheral blood leukocytes. In this research, it has been found that those participants whose families owned homes during their early childhood years demonstrated higher levels of GR and lower levels of TLR4 mRNAs throughout the adolescence, suggesting better regulation of the inflammatory response. The data of this research suggest that low early-life SES can induce a proinflammatory phenotype throughout the adolescence.

Overall, findings from these investigations have provided important insights into the causal molecular mechanisms involved in early stress-induced impairment of biological pathways related to stress reactivity and social behavior in adulthood. In particular, it is assumed that childhood adversity-induced changes in the levels of methylation of the GR gene may disturb neuro-endocrine systems, resulting in altered cortisol production (Weaver et al., 2004; Davidson and McEwen, 2012), and leading, in turn, to the development of various pathological conditions in adult life.

Long-Lasting Consequences of Acute Intrapartum Events

The intrapartum period, which refers to the time from the onset of labor until delivery of baby and placenta, is relatively short in comparison with the entire perinatal period. However, there is consistent evidence to support the significance of this time frame in reprogramming the human epigenome. A variety of environmental factors surrounding the antenatal and early postpartum period have been described as potential triggers of modulation of the epigenome (Moshe, 2009). Acute intrapartum events such as maternal pyrexia during labor, instrumental vaginal delivery, cesarean section, induction of labor using artificial oxytocin or prostaglandins and general anesthesia were also shown to cause long-term health outcomes including type 1 diabetes, asthma, eczema, allergies, gastroenteritis, obesity, multiple sclerosis, leukemia, and testicular cancer (Nelson, 1989; Hyde et al., 2012; Dahlen et al., 2013).

Among all intrapartum events, the cesarean section is the most studied in the context of developmental epigenetic programming. It is known that the cesarean mode of delivery can cause more severe stress in newborn infants compared with that of those born by vaginal delivery, who adapt to the new conditions better, and may lead to significant short- and long-term health outcomes, including different aspects of metabolic syndrome (Bohanick et al., 2014) as well as increased risk for asthma (Huang et al., 2014) later in life. By studying whether the mode of delivery may affect epigenetic patterning, Schlinzig et al. (2009) examined the components of the immune response as a candidate system that might likely be sensitive to epigenetic disturbances at birth. It has been revealed that neonates born by cesarean section exhibited significantly higher DNA methylation levels in leukocytes compared with that of those born by vaginal delivery. At 3–5 days post-birth, the patterns of methylation remained unchanged in infants born vaginally but were significantly decreased in those born by cesarean section. These findings, however, were not confirmed in a study by Virani et al. (2012), where type of delivery has not been linked to global methylation levels in DNA isolated from umbilical venous cord blood.

In a recent study by Franz et al. (2014), no difference in global methylation levels between newborn infants in the vaginal delivery group compared to the elective cesarean section group was observed, similarly to the findings of Virani et al. (2012). The levels of methylation of specific genes, such as genes encoding neutrophil elastase (ELA2) and the interferon regulatory factor-1 (IRF1), however, were found to be significantly higher in newborn infants delivered by elective cesarean section than in those
who were vaginally delivered. In another recent study, Almgren et al. (2014) found that the mode of delivery may influence the epigenetic state of neonatal hematopoietic stem cells. Specifically, it has been shown by the genome-wide methylation analysis that 343 loci were differentially methylated in neonatal CD34+ cells in relation to mode of delivery. These differentially methylated loci were functionally enriched for processes such as the immunoglobulin biosynthetic process, regulation of the response to food and regulation of glycolysis and ketone metabolism. Given the functional relevance of these pathways, the authors concluded that modulation of these processes can have important implications for health status in later life. Their main assumption is that “altered methylation may create poised, replication-heritable epigenetic marks, not immediately influencing gene transcription until a second hit arrives, causing disease.”

Based on these and many other contemporary research findings, Dahlen et al. (2013) proposed the Epigenetic Impact of Childbirth (EPIIC) hypothesis, according to which the adverse events during the intrapartum period, such as use of antibiotics, synthetic oxytocin, and cesarean section, may modulate the processes of epigenetic regulation and thereby influence the health status throughout the life course. The authors suggest that altered (both elevated and reduced) levels of adrenaline, cortisol, and oxytocin throughout the labor and delivery process can cause aberrant epigenomic reprogramming, which in turn leads to altered patterns of gene expression in later life. It seems likely that, due to such mechanism of “adaptive epigenetic tuning,” the fetal genome may be epigenetically remodeled throughout the intrapartum period to prepare a newborn for postnatal life; however, the higher risk for chronic disease in later life may be “an adaptive cost” of such tuning.

**Quasi-Experimental Evidence**

A causal link between early-life stress and subsequent health outcomes is evident from a range of quasi-experimental designs (also known as “natural experiments”). A natural experiment refers to “naturally occurring circumstances in which subsets of the population have different levels of exposure to a supposed causal factor, in a situation resembling an actual experiment where human subjects would be randomly allocated to groups” (Porta, 2008). This is a common research tool in fields where artificial experiments are impossible, such as epidemiology, demography, and population genetics. Exogenously imposed famine is the most commonly used type of quasi-experimental design (Mill and Heijmans, 2013). The nutritional insults and stress obviously tend to co-occur in famine-exposed populations. Stress is known to influence nutritional status at various levels, such as selection of food components, caloric intake, and utilization of metabolic fuels, while dietary factors may, in turn, affect stress reactivity through both central and peripheral pathways of stress response, evaluation of potentially stressful situations, and regulation of feedback loops. Therefore, the effects of either stress or nutrition may be modified by or conditioned upon the state of the other (Entreriguer and Wadhwa, 2013). Therefore, famine conditions have multiple features that are beneficial for their use as a natural experiment to study the lasting effects of early-life stresses.

Until now, the study from a birth cohort exposed to the Dutch famine in 1944–1945 is likely to provide the most convincing evidence of a relationship between exposure to starvation conditions in early life and adult physical and mental health outcomes, although similar associations for another famine episodes in the 20th century, such as the Ukraine famine of 1932–1933 and the Chinese Famine of 1959–1961, have been also reported (for reviews, see Lumey et al., 2011; Roseboom et al., 2011; Vaiserman, 2011). The long-lasting effects on adult health of other stressful historic events or natural disasters such as the Holocaust (Yehuda and Bierer, 2008; Bercovich et al., 2014), World War II in Europe (Kesternich et al., 2014), the Arab-Israeli war of 1967 (Malaspina et al., 2008), the 1918 influenza epidemic (Mazumder et al., 2010; Lin and Liu, 2014), the Quebec Ice Storm of 1998 (King et al., 2012; Walder et al., 2014), tropical storms in Louisiana over a period of 1980 to 1995 (Kinney et al., 2008), and Puerto Rico’s hurricanes of 1928 and 1932 (Sotomayor, 2013) were also observed. A variety of metabolic and cardiovascular problems, including dyslipidemia, hypertension, obesity, type 2 diabetes, and cardiovascular morbidity were shown to be highly prevalent in birth cohorts affected by these historic events in early life; the higher risks of mental and behavioral health problems such as schizophrenia, affective disorders, and other psychiatric illness were also evident in these populations (de Rooij et al., 2012; Huang et al., 2013; Susser and St Clair, 2013).

Although no relation between the prenatal exposure to the Dutch famine and overall global DNA methylation in adulthood has been found (Lumey et al., 2012), the gene-specific epigenetic differences associated with prenatal exposure to famine were evident in several studies. In one such investigations, Heijmans et al. (2008) identified patterns of DNA methylation in whole blood samples of siblings who were either exposed or not exposed to the Dutch famine conditions (both starvation and stress) during their intrauterine development. This analysis, conducted in adult persons aged 60+, has demonstrated that exposure to famine in prenatal life was associated with decreased methylation levels of the insulin-like growth factor 2 (IGF2) gene, which play a substantial role in human growth and development, in exposed compared to non-exposed siblings. In a subsequent study within the same cohort, some other genes, such as IL10, LEP, ABCA1, GNAS, MEG3, and INSIGF, were shown to have differential levels of DNA methylation in exposed and not exposed siblings (Tobi et al., 2009), suggesting ubiquitous epigenetic effects of the exposure to famine in early life. The differences in DNA methylation of HPA axis-associated genes, such as NR3C1 and CRH genes, were, however, non-significant in this study.

The effects of prenatal exposure to famine were found to depend strongly on the period of exposure, with differences being more evident when the exposure to the famine occurred during the periconceptional period rather than during the late gestational period. These data indicate that the early gestational period is the most vulnerable stage in human development (Heijmans et al., 2009; Roseboom et al., 2011). Since the epigenome is known to be most sensitive to different environmental cues during this developmental stage, these findings are strongly suggestive of a role of epigenetic processes in driving the long-lasting effects of early-life exposure to famine or other disasters on adult health and disease.

**Conclusions and Perspectives for Future Research**

There is increasing evidence that exposure to stressful events either during the periconceptional, gestational, and early childhood
Periods can be associated with long-lasting health problems. The molecular basis for such long-term effects remains unclear, but epigenetic regulation is thought to be involved. The epigenetic tuning early in life may likely prepare genes for responses to future second triggers (Scott et al., 2009). Thus, the performance of tissue and organ functions can be determined long before they are challenged. In evolutionary terms, such fine-tuning of gene expression through epigenetic mechanisms may enable the organism to adapt to changing environmental conditions in order to maintain the reproductive capacity throughout the reproductive period (Barouki et al., 2012). The interference with these developmentally-adaptive processes can, however, enhance the risk of pathology in the later life (Bateson et al., 2014; Burdge and Lillycrop, 2014). Thereby, epigenetic research promises to improve the understanding of the pathways by which organisms show widely diverse responses to stressful events in early life. In a variety of experimental models of stress-associated pathologies, specific epigenetic modifications maintaining the persistent patterns of gene expression and thereby mediating the aberrant neuropeptide interactions associated with adult-onset disease have been revealed. Several specific genes, such as GR in hippocampus, and CRF and arginine vasopressin in the neurons of the paraventricular nucleus, were identified as key candidate genes triggering early stress-associated disorders (Vialou et al., 2013). A scheme of hypothetical mechanisms linking exposure to early-life stress to later-life health outcomes is presented in Figure 2.

Although considerable advances have been made during the past decade in the field of environmental epigenetics, there are several research challenges that should be addressed in the coming years. An important issue is the general stability of epigenetic modifications induced by stress exposures during early development. Recently, experimental confirmations have been obtained in a range of studies that developmentally programmed epigenetic changes may persist throughout the life course, including old age (for reviews see, e.g., Lillycrop and Burdge, 2012; Lupu et al., 2012; Vaiserman, 2012, 2014). Such findings, however, are still relatively scarce, and this issue requires further investigation.

Since specific epigenetic patterns are likely peculiar to not only to specific cell types but also to particular neuronal pathways within the same tissue, further research will probably shift from candidate genes to candidate gene pathways that may be epigenetically labile in response to early-life stressful events (McGowan, 2012). Clarification of genetic pathways that are epigenetically labile under specific sets of stressful conditions will highlight the mechanisms by which the epigenome may mediate the development and progression of a complex disease in later life. Furthermore, since substantial epigenetic differences are likely to occur both within and across various tissue types, another important issue in human research is applicability of peripheral tissue samples for determining epigenetic profiles. Though exposure to stressful conditions in early life obviously results in widespread epigenetic alterations, these effects can vary significantly in magnitude and sign in neuronal tissue in comparison with non-neuronal tissues such as peripheral blood cells, thereby limiting opportunities to study the physiological, psychological, and behavioral effects of stress in early life based exclusively on peripheral blood or buccal samples. To solve this problem, appropriate animal models providing the ability to simultaneously measure the epigenetic profiles in both brain and peripheral tissues may have the potential for discovering fundamentally new knowledge on the epigenetic pathways controlling stress-associated phenomena. This clearly raises the question of the specificity of these pathways across mammal species and, thus, about similarities and differences between these pathways in animals and human beings. In addition, the prenatal-postnatal interplay may play an important role in the pathways by which exposure to stress in early life causes later health outcomes. As noted by Monk et al. (2012), “moderating and mediating influence of postnatal experiences following prenatal adversity must be carefully considered, as prenatal adversity is highly predictive of postnatal adversity, and postnatal experiences, particularly those involving variation in mother-infant interactions, can have significant epigenetic, neurobiological, and behavioral consequences.” The potential role of exposure to stressful events throughout the adulthood in altering epigenetic programming also needs to be considered since data from several studies suggest that the epigenome remains labile in adult life and thereby later-life impacts may interact with developmental programming influences (Talens et al., 2012).

Finally, it should be stressed that incorporating new knowledge on epigenetic programming by early-life stress into the modern paradigm of causation of chronic pathology will shift the focus of efforts targeted towards preventing the later-life disease from later stages of human life to the very early stages from conception to weaning. The reduction or elimination of stressful experiences in early life has a substantial potential to prevent the
adult-onset disease. Furthermore, since epigenetic marks, in contrast with genetic information that remains relatively stable throughout the life course, are potentially reversible (Johnson et al., 2012), the deeper understanding of mechanisms underlying the persisting effects of early-life stresses can lead to the development of effective diagnostic techniques and therapeutic procedures targeted to the prevention or removal of the adverse effects of stressful events in early life. In particular, the therapeutic use of drugs targeted at chromatin modifying enzymes such as histone deacetylase inhibitors, known to exhibit both antidepressant-like [Han et al., 2014] and life-extending [Vaizerman and Pasyukova, 2012] properties, may be potentially effective. The application of such preventive and therapeutic approaches during early-life sensitive periods is likely to be particularly promising. If one could modify the epigenetic patterns disrupted by exposure to stress through specific epigenome-targeted therapeutic interventions, then it would be possible to correct the impaired patterns of gene expression to prevent the stress-induced chronic pathologies and to improve human health and longevity.

Acknowledgments
The author thanks Oksana Zabuga for assistance in preparing the manuscript.

References


