J Med Biochem 2013; 32 (4) DOI: 10.2478/jomb-2013-0047

UDK 577.1 : 61 **ISSN 1452-8258**

J Med Biochem 32: 313–324, 2013

Review article
Pregledni članak

PRENATAL PROGRAMMING OF PSYCHOPATHOLOGY: THE ROLE OF EPIGENETIC MECHANISMS

PRENATALNO PROGRAMIRANJE PSIHIJATRIJSKIH POREMEĆAJA: ULOGA EPIGENETSKIH MEHANIZAMA

Marija Kundakovic

Department of Psychology, Columbia University, New York, NY, USA

Summary

The Human Genome Project, completed ten years ago, widely opened the door for the field of Epigenetics as a new venue to study the causes of human disease and to search for predictive biomarkers and therapeutic targets for a wide range of disorders. The field of behavioral and psychiatric epigenetics is still very young, but increasing evidence suggests that epigenetic mechanisms contribute to the development of neuropsychiatric disorders. The prenatal period is particularly vulnerable to epigenetic disruption, and it seems likely that adverse in utero environments can induce epigenetic dysregulation and predispose an individual to mental disease later in life. Emerging evidence from animal studies has shown that maternal exposure to drugs, stress, and toxicants can alter epigenetic gene programming in the brain and contribute to neurodevelopmental and behavioral deficits in the offspring. The evidence from human studies is more limited but is in agreement with animal data. Several human studies have shown that prenatal risk factors, such as maternal food deprivation and stressful life events, are associated with persistent epigenetic changes in genes that are linked to neurodevelopmental disorders and psychopathology. Although these studies support the hypothesis that epigenetic mechanisms may be involved in prenatal programming of psychopathology, a collaborative effort of basic, clinical and epidemiological research is needed to advance this field. Nevertheless, this field holds great promise to facilitate our understanding of environmental contribution to human mental disease and to reveal new predictive biomarkers as well as preventive and therapeutic approaches for various neuropsychiatric disorders.

Keywords: epigenetic, DNA methylation, prenatal programming, psychiatric disorders, neurodevelopment

Kratak sadržaj

Projekat humanog genoma (HGP), završen pre deset godina, širom je otvorio vrata epigenetici kao novoj oblasti za proučavanje uzroka humanih bolesti i za pronalaženje novih dijagnostičkih biomarkera i terapeutskih pristupa za širok spektar poremećaja. Epigenetika ponašanja i psihijatrijska epigenetika su još uvek mlade oblasti ali je sve više dokaza koji ukazuju na to da epigenetski mehanizmi doprinose razvoju neuropsihijatrijskih poremećaja. Prenatalni period je posebno osetljiv i smatra se da negativni uslovi tokom intrauterinog razvoja mogu da izazovu epigenetsku disregulaciju i predisponiraju osobu za razvoj duševnih bolesti kasnije u životu. Istraživanja na životinjama pokazala su da izlaganje majke lekovima, stresu i otrovnim materijama može da promeni epigenetsko programiranje gena u mozgu i da doprinese poremećajima razvoja mozga i ponašanja kod potomaka. Istraživanja na ljudima su znatno ograničenija, ali su rezultati u skladu sa podacima dobijenim kod životinja. Nekoliko studija na ljudima ukazuje na to da su prenatalni faktori rizika, kao što su gladovanje majke ili izlaganje majke stresu, povezani sa trajnim epigenetskim promenama u genima koji su dovedeni u vezu sa neurorazvojnim poremećajima i psihopatološkim promenama. Iako ove studije podržavaju hipotezu da epigenetski mehanizmi mogu biti uključeni u prenatalno programiranje psihijatrijskih oboljenja, potreban je zajednički napor istraživača iz osnovnih, kliničkih i epidemioloških istraživanja da bi se ova oblast unapredila. Ovo polje istraživanja omogućiće nam da bolje razumemo doprinos životne sredine razvoju mentalnih bolesti kod ljudi i ima potencijal da otkrije nove biomarkere, kao i preventivne i terapeutske pristupe različitim neuropsihijatrijskim poremećajima.

Ključne reči: epigenetika, DNK metilacija, prenatalno programiranje, psihijatrijski poremećaji, neuralni razvoj

Address for correspondence:

Dr. Marija Kundakovic
Department of Psychology, Columbia University
1190 Amsterdam Avenue
406 Schermerhorn Hall, New York, NY 10027
Phone: (212) 854-2490, Fax: (212) 854-3609
e-mail: mk3242@columbia.edu

Introduction

The completion of the Human Genome Project in 2003 represents an important milestone in biomedical sciences; approximately 20,500 genes in human DNA have been identified and the sequences of the 3 billion base pairs that make up human DNA have been determined (1–3). While the information derived from this project is precious and has significantly changed the way life science is being performed, certainly not all promises have been met. The results were actually somewhat perplexing for the scientific community; we had to face the fact that our genome contains only »a few« more genes than the genomes of mice or fruit flies as well as that only up to 2% of our genome contains protein-coding sequences. In general, our understanding of human disease and diagnostic and therapeutic approaches did not significantly improve. It has become clear that genetic mutations and polymorphisms may account for only part of disease risk for the vast majority of the disorders. And, we understood that we have to learn how to »read« our genome and to unravel its complexity that extends way beyond the naked DNA sequence (4, 5). In fact, the Human Genome Project and its outcomes widely opened the door for the field of gene regulation, and in particular *Epigenetics*, as a new venue to study the causes of human disease and to search for predictive biomarkers and therapeutic targets for a variety of disorders.

Epigenetics is the study of changes in gene activity and phenotype that do not involve changes in the DNA sequence. Epigenetics involves several mechanisms that control chromatin structure and gene expression, including DNA methylation, histone modifications, chromatin remodeling factors, nucleosome positioning, and non-coding RNAs (6). Roughly, epigenetics can explain how genetically-identical cells of a single organism can have such diverse morphologies and functions as well as how genetically identical individuals (monozygotic twins) may present different phenotypes including the occurrence of certain diseases and disorders. One important characteristic makes epigenetics particularly attractive target for the study of complex, environmentally-contributed disorders : epigenetic marks are dynamic and responsive to the environment throughout life making the epigenome much more plastic than the genome (7). As such, epigenetic marks represent a plausible biological substrate through which the environment could influence our genes and phenotype over the life course contributing to risk for many diseases, including neuropsychiatric disorders.

The role of epigenetics has been extensively studied in cancer research, which resulted in the discovery of potential clinical biomarkers and in a successful implementation of epigenetic therapy in the treatment of certain types of cancers (8). On the other hand, the field of behavioral and psychiatric epigenetics is still very young but there is increasing evidence that epigenetic mechanisms contribute to the development of psychiatric disorders and the potential of epigenetic therapy has been proposed (9–11). However, it may be even more important to understand the factors and mechanisms that initiate psychopathology as this may result in preventive approaches and early interventions. Recent findings have shown that epigenetic changes may contribute to psychopathology throughout life, although early life alterations likely have the most significant impact (12, 13). This review is focused on the prenatal period that is highly vulnerable to epigenetic disruption and will summarize emerging evidence from both human and animal studies showing that epigenetic mechanisms may underlie prenatal programming of psychopathology.

Psychopathology and prenatal environment

Increasing epidemiological evidence shows that early life adverse environments can significantly increase the risk for developing mental disease in later life (14). One of the best examples is schizophrenia, a complex mental disorder that is now widely accepted to have a neurodevelopmental origin (15, 16). Schizophrenia is considered a complex polygenic genetic disorder. It has a clear genetic component; the disorder runs in families and the closer genetic relatedness to the affected individual the higher the risk that a person will develop schizophrenia (17). However, the concordance of monozygotic twins for this disorder is only about 50% and this shows that environmental factors play an important role as well, likely by influencing fetal brain development (18). The increased risk for schizophrenia has been associated with several *in utero* exposures, including severe food restriction, exposures to viral infections (influenza, measles, polio), stress (in male offspring), and hypoxia associated with gestational and birth complications (19, 20).

Importantly, these environmental risk factors do not seem to be specific for schizophrenia. Maternal exposure to famine during periconceptional period was associated with increased risk for schizophrenia (21), while the same exposure during the second and third trimesters of pregnancy was linked to affective disorders in offspring (22, 23). Furthermore, maternal stress during gestation is associated with increased risk for the development of many neuropsychiatric disorders besides schizophrenia, including depression, autism, and anxiety (14, 24). Similarly, attention deficit hyperactivity disorder (ADHD) has been linked to poor maternal diet and maternal stress during pregnancy, but also to exposure to recreational drugs and toxins such as nicotine, alcohol, polychlorinated biphenyls, and hexachlorobenzene (25).

It is not clear why a single environmental factor could induce different psychiatric outcomes nor how

different factors can lead to the same disorder. It seems likely that the timing of exposure and sex of the offspring both play significant roles. In addition, a complex interaction of genes and the environment (both prenatal and postnatal) is likely to determine the final outcome. However, it is important to understand biological mechanisms through which prenatal environmental exposures can affect brain development and predispose an individual for mental disease later in life. Epigenetic mechanisms have emerged as a plausible biological substrate through which the environment could change gene expression associated with normal brain development leading to lasting consequences for brain function and behavior (14, 25–27). The next section will provide a rationale for this hypothesis.

Epigenetic disruption as a plausible mechanism for the early initiation of psychopathology

Mammalian development starts with the zygote, a single totipotent cell, which divides and differentiate into diverse cell lineages and specific cell types that will eventually give rise to an organism. Each cell in a single organism contains identical genetic material,

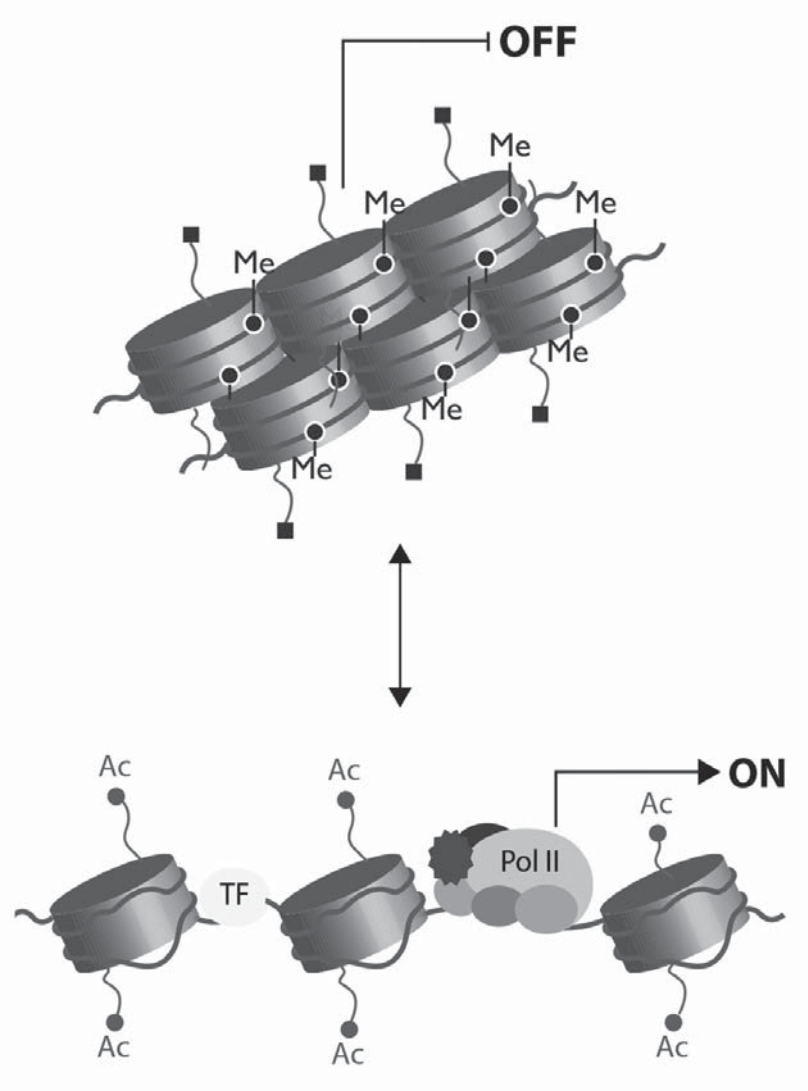

Figure 1 Epigenetic gene regulation. Chromatin can exist in a closed configuration (repressive chromatin; above) and more open configuration (transcriptionally permissive chromatin; below). Repressive chromatin is typically associated with high levels of DNA methylation (•-Me) and repressive histone marks (▪), such as dimethylation of histone H3 at lysine 9 (2meH3K9), which do not allow transcription factor access and therefore inhibit gene transcription (OFF). Transcriptionally permissive chromatin is typically associated with low levels of DNA methylation and active histone marks, such as 3meH3K4 and H3/H4 histone acetylation (Ac), and allows the binding of transcription factors (TF) and general transcription machinery (including polymerase II - Pol II) to gene regulatory regions that initiates transcription (ON).

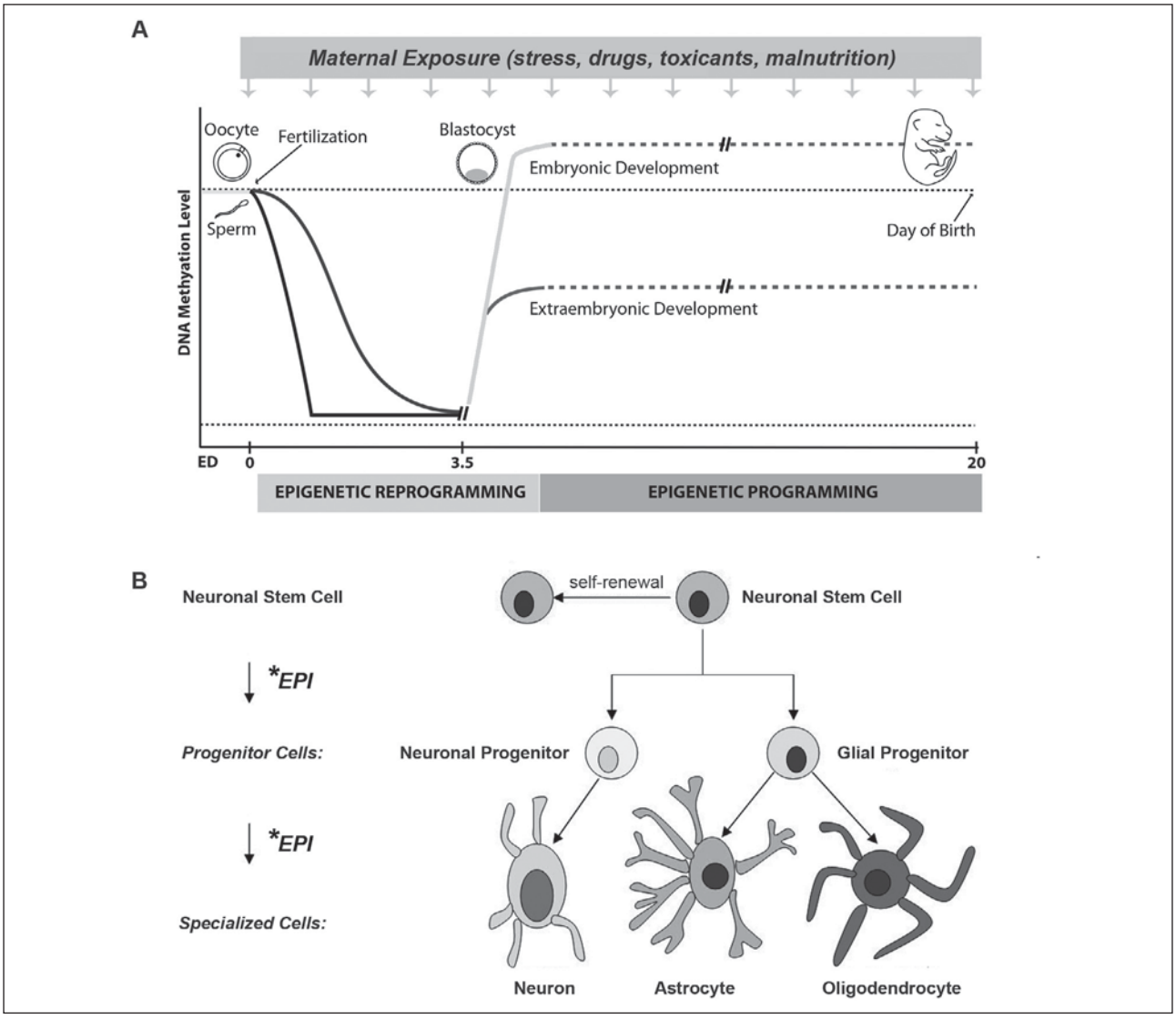

Figure 2 Maternal environmental exposures can disrupt epigenetic (re)programming in the developing embryo/fetus. Epigenetic marks are extremely dynamic during prenatal development when an extensive reprogramming and programming of epigenetic modifications takes place. A. During post-fertilization »epigenetic reprogramming« (zygote to blastocyte stage), DNA methylation is almost completely erased (in both paternal and maternal genome) and then re-established leading to differential DNA methylation and gene expression patterns in the first two cell lineages that precede embryonic and extra-embryonic development. In the later stages of development, epigenetic marks are less dynamic (dashed lines) but still participate in gene programming relevant for later stages of cellular differentiation (»epigenetic programming«). B. For instance, during differentiation of brain cells, epigenetic marks (*EPI; DNA methylation and histone modifications) are involved in gene programming that differentiate neuronal stem cells into neuronal and glial progenitors and further into more specialized neuronal and glial cells (astrocytes and oligodendrocytes). Therefore, maternal exposure to environmental factors that affect the epigenome (stress, drugs, toxicants, malnutrition) can disrupt gene expression programming in the embryo/fetus resulting in developmental deficits, including abnormal brain development [adapted from (64)].

however cells are differentiated based on a set of genes they are programmed to express. Gene expression programming largely occurs at the level of transcriptional regulation (synthesis of mRNA from DNA) and involves epigenetic gene regulation as a critical level of control (28).

In each cell, DNA is packaged in a very specialized structure called chromatin, which consists of DNA wrapped around the octamers of histone proteins (*Figure 1*) (29). If local chromatin configuration is more open, this allows the binding of transcription factors and general transcription machinery to the gene promoters leading to gene activation; on the contrary, closed chromatin configuration is not permissive for transcription. Epigenetic modifications, such as DNA methylation and histone modifications, control the state of chromatin in the vicinity of gene regulatory regions and thereby directly regulate gene

expression. DNA methylation occurs at position 5 of cytosine residues and predominantly in the context of CpG dinucleotides; it is catalyzed by a family of enzymes called DNA methyltransferases (DNMTs), and is typically involved in gene silencing (30). DNA methylation can either : 1) directly interfere with the binding of transription factors; or 2) can recruit the biding of repressors proteins and histone modifiers to a gene regulatory region resulting in repressive chromatin state. Many different post-translational modifications of histones have been described so far, including acetylation, methylation, phosporylation, ubiquitination, sumoylation, and ADP ribosylation. Specific combinations of histone modifications can affect local chromatin configuration and mark genes for either enhanced activity or transcriptional repression (31). In addition, DNA methylation and histone marks often work in concert to regulate gene expression (32).

The epigenome is highly susceptible to environmental exposures during early prenatal development when an extensive reprogramming and programming of epigenetic modifications takes place in order to establish cell- and tissue-specific gene expression (*Figure 2*). Epigenetic reprogramming involves genome-wide erasure and remodeling of DNA methylation and histone modifications, and occurs at two stages in mammalian development: 1) at pre-implantation stage (from the zygote through the blastocyst stage), and 2) during gametogenesis (beginning at mid-gestation) (33). Post-fertilization epigenetic reprogramming is associated with almost complete erasure of DNA methylation marks within the zygote followed by *de novo* methylation of the genome, and this seems to be accompanied by different patterns of histone modifications (*Figure 2A*). These events are likely needed for the establishment of the zygote's totipotency, initiation of embryonic gene expression, and early lineage development in the embryo (26). The first two lineages, the embryonic lineage (the inner cell mass) and extraembryonic lineage (trophectoderm), are the first to show different epigenetic marks and it seems likely that these early lineages setup the DNA methylation status of their somatic and placental derivatives. Therefore, interference with epigenetic reprogramming during early embryogenesis has a significant potential to influence early gene programming in developing embryo (28, 34).

Besides their key role in gene regulation during early development, increasing evidence shows that, in concert with transcription factors, histone modifications and DNA methylation still actively participate in controlling gene expression in later lineage commitment (*Figure 2*). Of relevance for neurodevelopment, it has been shown that there is an epigenetic »programming« of stem cells into neuronal precursors followed by further differentiation into specific neurons or glia cells (*Figure 2B*) (35–37). For instance, during neurogenesis, neural precursor cells express factors that promote transcription of neuronal genes, while the activity of some glia-specific genes is inhibited by formation of repressive chromatin conformation induced by DNA methylation (37). *In vivo* evidence for this was provided by the experiments with conditional knockout mice carrying a deletion of DNA methyltransferase 1 gene specifically in neural progenitor cells of the developing brain (38). Brains of these mice show decreased numbers of neurons and precocious astroglial differentiation, implying that neuro- to gliogenic switch involves DNA hypomethylation. Therefore, epigenetic mechanisms represent plausible molecular substrate through which environmental exposures can change the patterns of gene expression associated with normal neurodevelopment.

In summary, it seems likely that the whole developmental period is vulnerable to epigenetic disruption, and that any agent with the ability to affect the epigenome can cause adverse developmental effects (*Figure 2*). Importantly, once established, DNA methylation patterns can be passed from one cell generation to another and persist into adulthood, thus providing the mechanism through which the early life environment can exert long-lasting effects on gene expression and phenotype (39). In the next section, I will review experimental evidence implying that prenatal environmental exposures can affect the epigenome leading to long-lasting consequences for later behavior and brain function.

Evidence from animal studies

Emerging evidence from animal studies has shown that maternal exposure to drugs, stress, and toxicants can alter epigenetic gene programming in the brain and contribute to neurodevelopmental and behavioral deficits in the offspring. However, I will start this section by introducing the landmark studies with the Agouti mouse model, which practically established the field of environmental epigenetics.

Agouti mouse model

Agouti viable yellow (A^{vy}) mice represent the first animal model that provided the evidence that maternal environmental exposure during pregnancy can induce phenotypic changes in the offspring that are mediated by epigenetic mechanisms (40). An advantage of this animal model is its ability to provide a direct link between environmentally-induced changes in DNA methylation and easily-observed changes in phenotype. A^{vy} mice (the A^{vy}/a genotype) are genetically-identical but they contain a meta-stable, DNA methylation sensitive, A^{vy} allele in the Agouti gene locus that determines coat color. The expression of the *Agouti* gene varies depending on the DNA methylation status of the intracisternal A particle (IAP)

retrotransposon inserted upstream of the *Agouti* gene. The establishment of epigenetic marks at A^{vy} occurs during early embryogenesis and is a probabilistic event, resulting in a wide distribution of the coat color of A^{vy} mice ranging from pure yellow (hypomethylation of the A^{vy} IAP) to pseudoagouti brown (hypermethylation of the A^{vy} IAP). Importantly, developmental exposure to maternal diet rich in methyl group donors (such as folate) or supplemented with phytoestrogen genistein was able to increase A^{vy} IAP methylation in the offspring and shift the coat color distribution in these animals toward pseudoagouti brown (41, 42). Although this study provided a strong evidence that maternal dietary supplementation can induce lasting changes in A^{vy} DNA methylation in offspring with consequences for gene expression and phenotype, no comparable *Agouti* gene containing a retroviral insert is present in the human genome (43), and there was a need to show similar changes in endogenous genes of relevance to neurodevelopment and brain function. The following studies explored the effects of other environmental exposures in the context of the brain epigenome and behavior.

Stress

As previously mentioned, maternal stress during gestation is associated with increased risk for the development of many neuropsychiatric disorders in offspring, including schizophrenia, autism, depression, and anxiety (14, 19, 24). One of the mechanisms through which prenatal stress can influence disease risk is through changing the activity of the hypothalamic-pituitary-adrenal (HPA) axis, a key modulator of the neuroendocrine system controlling the release of various stress hormones including glucocorticoids. Early research in rodents showed that the genes encoding the regulators of the HPA axis, such as corticotropin-releasing factor (*Crf*) and glucocorticoid receptor (*Nrc1*), are regulated by DNA methylation and programmed early in life (39, 44). A recent study in mice explored whether epigenetic mechanisms may be involved in programming of neurobehavioral changes induced by prenatal stress (24). Importantly, this study showed that the effects of prenatal stress largely depend on the timing of stress exposure as well as on the sex of exposed animals; indeed, male (but not female) offspring exposed to chronic, variable stress early in gestation (the first gestational week) displayed depressive-like behavior in the adulthood accompanied by increased HPA response to stress. These changes were associated with changes in the expression of the genes important for HPA axis responsiveness, *Crf* and *Nrc1*, in the amygdala and the hippocampus, respectively. Changes in *Crf* and *Nrc1* DNA methylation inversely correlated with altered gene expression, providing evidence that epigenetic mechanisms may underlie behavioral and gene expression changes induced by prenatal stress exposure.

Interestingly, the same study established that early prenatal stress induces sex-specific changes in the expression of *Dnmt1*, the enzyme that controls DNA methylation levels, in the placenta. These results imply that stress may target epigenetic regulation in the placenta with consequences for fetal brain development and programming of neurodevelopmental outcomes. In accordance with this, a study in rats has shown that prenatal stress induces the expression of DNA methylating enzyme Dnmt3a in the placenta that is associated with increased DNA methylation and reduced expression of the gene that encodes 11b-hydroxysteroid dehydrogenase type 2 (11B-HSD) (45). Placental 11B-HSD is involved in the inactivation of the maternal glucocorticoids thus preventing excess hormonal exposure and increased HPA activation of the fetus. Therefore, stress-induced down-regulation of 11B-HSD via epigenetic mechanisms may diminish the protective effect of the placenta on the fetal HPA axis. In addition, a recent study has established a direct connection between an epigenetic regulator in the placenta and the regulation of neonatal brain gene expression (46). Together, these results further support the role of epigenetic mechanisms in programming the effects of prenatal stress exposure on neurodevelopmental outcomes.

Drugs

Maternal exposure to both therapeutical and recreational drugs has been associated with neurobehavioral outcomes in offspring and the possible mediating role of epigenetic mechanisms has been proposed (47). A large body of animal research shows that many drugs of abuse can affect the adult brain epigenome (48), however the link between maternal drug exposure and epigenetic changes in the brain of offspring is just emerging. The study of Novikova et al. (49) has shown that maternal cocaine exposure during the second and third weeks of gestation led to changes in the expression of DNA methyltransferases and global DNA methylation changes in the hippocampal neurons of male neonatal and prepubertal offspring. DNA methylation levels of several genes implicated in cell differentiation, survival and synaptic plasticity were shown to be hypermethylated or hypomethylated leading to decreased or increased gene expression, respectively. However, it is important to note that these molecular changes were not associated with differences in hippocampal tissue histology, requiring further studies to provide the link between maternal cocaine exposure, epigenetic changes and the disruption of neurodevelopment and behavior in offspring.

Another study explored the effects of maternal exposure to alcohol on the offspring epigenome and

brain structure. It is well-established that high levels of alcohol consumption during pregnancy can result in fetal alcohol syndrome (FAS) and other disorders that are associated with growth restriction, structural brain abnormalities as well as educational and behavioral problems later in life (50). The study used A^{vy} allele as an epigenetic biosensor, and showed that maternal exposure to ethanol from gestational days 0.5–8.5 increased the probability of hypermethylation and silencing at this locus and more mice with an agouti-colored coat (50). Importantly, the congenic siblings of these mice displayed postnatal growth restriction and craniofacial dysmorphology reminiscent of FAS in humans implying that the expression of genes other than A^{vy} was affected by prenatal ethanol exposure. This study provides an evidence that the epigenome is vulnerable to ethanol during early embryogenesis, a time when there is genome-wide epigenetic reprogramming, and suggests that epigenetic mechanisms may contribute to ethanol-induced effects on neurodevelopment and later brain function.

One study explored the role of histone modifications in the neurodevelopmental effects of Δ-9-tetrahydrocannabinol (THC), the main active component of marijuana or cannabis (51). Increasing evidence suggests that maternal cannabis use during pregnancy can have lasting negative consequences on offspring, including increased risk for developing drug addiction and neuropsychiatric disorders (52, 53), although the underlying mechanism is poorly understood. DiNieri et al. (51) hypothesized that prenatal cannabis exposure may induce enduring down-regulation of genes implicated in dopamine reward pathway, such as the gene encoding dopamine D2 receptor (*DRD2*). Importantly, reduced D2 receptor is a consistent feature observed in the adult drug abusers (54). Using aborted human fetuses from drug abusers, DiNieri et al. (51) were able to show that maternal cannabis use during pregnancy decreases *DRD2* gene expression in a key brain reward region, the nucleus accumbens (NAc), of the human fetus at approximately 20 weeks of gestation. With a rat model, they further examined whether disruption of NAc *Drd2* gene regulation persist into adulthood and whether epigenetic mechanisms are involved in this effect. Importantly, gestational maternal exposure to THC resulted in reduced *Drd2* gene expression in offspring NAc both at postnatal day (PND) 2 (corresponds to the human brain development at 20 weeks gestation) and in the adulthood (PND 62). Decreased *Drd2* gene expression in the adulthood was associated with increased repressive histone mark 2meH3K9, decreased active histone mark 3meH3K4, and increased binding of the RNA polymerase II at the *Drd2* gene locus close to the transcription start site. Furthermore, adult offspring prenatally exposed to THC also showed reduced D2 receptor binding sites in the NAc and increased sensitivity to opiate reward. Overall, this study provides strong evidence that *in utero* cannabis exposure can alter developmental programming of mesolimbic D2DR expression trough epigenetic mechanisms resulting in a lasting reduction of D2 receptors that contribute to addiction vulnerability. It is interesting to note that human genetic studies have also linked genetic polymorphisms in the *D2DR* gene to addiction phenotypes, including alcohol, opiates, and psychostimulant abuse (55). Therefore, both genetic and environmentally-induced epigenetic factors, alone or in combination, can possibly contribute to reduced *D2DR* gene expression that may promote vulnerability to addiction in drug abusers.

Toxicological exposures

Various maternal toxicological exposures during pregnancy have been associated with the increased risk for neurodevelopmental and behavioral changes in offspring (56–58) and epigenetic mechanisms have been proposed as possible mediators of these effects (59, 60). Bisphenol A (BPA) is an estrogenic endocrine disruptor widely used in the production of plastics and found in many consumer products (61). BPA has received a lot of public attention in recent years due to emerging evidence from both animal and epidemiological studies showing its neurodevelopmental toxicity (58, 62–64). Prenatal BPA exposure affects brain development, sexual differentiation, social and anxiety-like behavior, and learning/memory (64). Although underlying mechanisms are not well understood, increasing evidence shows that epigenetic mechanisms may play a critical role. The first convincing study suggesting that BPA can induce epigenetic changes came from Dolinoy et al. (65), using the above-mentioned Agouti viable yellow (A^{vy}) mouse model. Maternal exposure to BPA during pregnancy shifted the coat color distribution of offspring toward yellow by decreasing methylation at specific CpG sites in A^{vy} allele. Importantly, this BPA effect can be counteracted by maternal exposure to the diet rich in methyl group donors, including folic acid (65). However, the effect of BPA on the brain epigenome has just started to be explored. A study by Yaoi et al. (66) showed that maternal exposure to low-dose BPA can induce DNA methylation changes in multiple genes of the fetal brain, however this study did not examine the persistence of DNA methylation changes into later life. Recently, we have provided the first experimental evidence that low-dose prenatal BPA exposure induces long-lasting epigenetic disruption in the brain, which involves changes in the mRNA expression of DNA methyltransferases as well as DNA methylation changes at the regulatory region of the estrogen receptor gene 1 (*Esr1*) (67). *Esr1* is known to be crucial for sexual differentiation of the brain and behavior, including social and anxiety-like behavior (68, 69). Importantly, we were able to show that BPA-induced epigenetic changes are associated

with changes in *Esr1* gene expression in the juvenile brain as well as with enduring alteration in social and anxiety-like behavior. We have also shown that the BPA-induced molecular and behavioral outcomes are dose-dependent and sex-specific. As such, our data provide a strong evidence that epigenetic mechanisms may be particularly important as mediators of BPA effects on sexually dimorphic phenotypes. However, this study opens a possibility that epigenetic mechanisms may underlie enduring effects of prenatal BPA exposure on the brain, not only in the context of social and anxiety-like behavior, but also regarding cognitive function.

Evidence from human studies

Several studies in humans provide a possible link between prenatal environmental exposures, epigenetic changes, and psychopathology although the evidence is considerably more limited than that provided by animal studies. The first empirical support for the hypothesis that early-life environmental conditions can cause epigenetic changes in humans that persist throughout life was provided by the famous Dutch Famine Study.

Dutch Famine Study

Dutch Famine Study is a seminal epidemiological study that provided associations between the prenatal environment and offspring's long-term mental health outcomes (21, 70). The study is based on a »natural experiment« involving a severe famine that occurred during the Nazi blockade of occupied Western Holland toward the end of World War II, from October 1944 to May 1945. Over the time, the famine grew steadily worse and achieved its peak 2–3 months prior to liberation (March-April 1945), when the daily food rations were reduced to 500–1,000 kcal or even less, and the population was nutritionally depleted. Using excellent records of the exposed population spanning several decades following the famine, the series of studies have linked maternal periconceptional exposure at the famine's peak with increased risk of three neurodevelopmental outcomes in offspring: 1) neural tube defects; 2) schizoid diagnoses at age 18; and 3) hospitalization for schizophrenia in adulthood (about two-fold increase) (26). Two possible causal mechanisms of these effects have been suggested: 1) deficiency in many micro- and macronutrients due to the famine; and 2) maternal stress due to the famine, war, and many other hardships associated with this tragic event. Regarding increased risk for schizophrenia, the role of nutritional deprivation is likely to be more prominent (71), and although a deficiency of many nutrients may contribute to the adverse effects of famine on neurodevelopment, folic acid has been proposed as one of the main candidates (71). The role of folic acid in the brain development is very well-established, and independent epidemiological studies have shown that maternal folate supplementation during periconceptional period decreases the risk of neurodevelopmental disorders in children, including neural tube defects, severe language delays, autism, and cognitive impairments; the outcomes that could be antecedents of schizophrenia (26, 72, 73). Folate is important for the production of methyl donors and DNA methylation, and therefore this led to the hypothesis that nutritional (and in particular folate) deficiency could affect neurodevelopment and later risk for schizophrenia, at least in part, via epigenetic mechanisms.

Within the Dutch Famine cohort, recent studies tested this hypothesis by examining DNA methylation in the blood of the individuals that were prenatally exposed to famine compared to their unexposed, same-sex siblings at approximately 60 years of age (74, 75). Interestingly, six decades later, periconceptional exposure to famine was associated with decreased DNA methylation of the insulin-like growth factor 2 (*IGF2*) locus known to be epigenetically-regulated (74). *IGF2* is an imprinted gene that plays an important role in growth and development, and continues to play a role in cognitive processes over the life course (76). Subsequent studies have shown that DNA methylation changes in the additional genes implicated in growth and metabolic pathways were associated with the prenatal exposure to famine (75), suggesting widespread epigenetic dysregulation induced by this early environmental exposure. Together, these studies suggest that periconceptional maternal exposure to famine can have a lasting effect on offspring DNA methylation and that relevant genes can be affected with long-lasting consequences for brain structure and function. Importantly, many psychiatric disorders, including schizophrenia (77), show epigenetic changes in specific brain regions, further supporting the hypothesis that prenatal environmental exposure can contribute to the development of psychopathology by inducing long-lasting changes in the epigenome.

Prenatal stress and depression

The Dutch Famine Study also showed lasting DNA methylation changes in several genes of the offspring that was exposed to famine later in gestation (75). As previously stated, exposure to famine in the second and third trimester of the pregnancy was associated with increased risk for affective disorders. Interestingly, this study did not show changes in the genes that are associated with the HPA axis: corticotropin-releasing factor (*CRF*) and glucocorticoid receptor (*NRC1*) (75). However, other studies of prenatal maternal stress and depression have provided evidence of epigenetic dysregulation within HPA-axis associated genes (78). For instance, analysis of cord

blood samples from infants born to mothers suffering from depression during the third semester of pregnancy showed increased levels of DNA methylation of the *NRC1* gene (79). Importantly, levels of *NRC1* methylation in fetal cord blood were found to predict infant HPA reactivity (cortisol response to stress) at 3 months of age, implying that there may be a functional consequence of this epigenetic variation. An additional study explored whether maternal exposure to stressful events during pregnancy could affect offspring *NRC1* DNA methylation beyond infancy (80). This study has shown that maternal exposure to intimate partner violence during pregnancy was associated with elevated *NRC1* DNA methylation levels in the whole blood samples of their offspring at 10–19 years after birth. In both of these studies (79, 80), epigenetic changes were present in offspring blood samples but not in maternal blood samples suggesting that stress-induced epigenetic dysregulation of the *NRC1* gene occurs during developmental epigenetic programming. Together with the animal studies, these findings further support the hypothesis that maternal environmental exposures during pregnancy can alter *in utero* epigenetic gene programming contributing to neurodevelopmental and behavioral deficits in the offspring.

Future directions and challenges

The evidence presented in this article strongly suggests that: 1) *in utero* exposure to adverse environments, including stress, drugs, toxicants, and malnutrition, can predispose an individual for behavioral abnormalities and mental disease later in life; and 2) that epigenetic mechanisms may, at least in part, underlie these effects. While the epigenetic hypothesis of the developmental origin of adult disease is becoming widely accepted in scientific community (14, 26, 40, 60), which is reflected in the increasing number of articles and conferences covering this topic, it is important to note that the actual experimental evidence to support this hypothesis is still considerably scarce, particularly regarding neurobehavioral outcomes. Therefore, more studies involving various environmental exposures in both animals and humans will be needed to further our understanding of this field.

Future animal studies should incorporate well-controlled study designs that involve: 1) doses of prenatal environmental exposures that mimic those humans are exposed to; 2) epigenetic analyses at multiple time points throughout life; 3) analyses of multiple epigenetic modifications in relevant, specific brain regions;, and 4) epigenetic analyses of multiple genes including the incorporation of the genome-wide or epigenomic approach. Coupled with neurobehavioral analyses of the prenatally-exposed animals, these types of studies can give us more information on how environmentally-induced epigenetic dysregulation during early development can produce long-lasting effects on brain gene expression and neurobehavioral phenotypes.

Of particular interest is also to study the epigenetic effects of environmental exposures in both sexes as increasing evidence suggests that epigenetic programming during development and responses to environmental cues are sex-specific and give rise to sex-specific epigenomes (24, 67, 81–83). This can significantly improve our understanding of numerous neurodevelopmental disorders that are environmentally-contributed and exhibit a considerable sex bias in both prevalence and severity, such as depression, anxiety, schizophrenia, ADHD, and autism.

Last but not least, one of the greatest challenges in studying the role of environmentally-induced epigenetic dysregulation in humans is unavailability of the target tissue, especially in studies of neurobehavioral effects. Unlike the genome, the epigenome is cell-type specific. And, while the majority of human studies have analyzed associations between environmental exposures and epigenetic variation in peripheral tissues, including cord blood, whole blood samples, and saliva, it is not clear how well epigenetic patterns in these tissues correlate with these patterns in relevant brain regions. Therefore, it is of outmost importance to study these correlations in animals where both target and peripheral tissues of exposed animals are accessible. This can tremendously help our efforts to establish valid biomarkers that can predict neurobehavioral outcomes in humans.

In conclusion, the field of prenatal programming of psychopathology through epigenetic mechanisms is still in its infancy. However, this field holds great promise to advance our understanding of environmental contribution to human mental disease and to uncover new predictive biomarkers as well as preventive and therapeutic approaches for various neuropsychiatric disorders. Therefore, we should look forward to future advances in this field.

Acknowledgements. This work was supported by National Institute of Environmental Health Sciences Grant 5P01ES09600.

Conflict of interest statement

The authors stated that there are no conflicts of interest regarding the publication of this article.

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Received: August 11, 2013

Accepted: September 2, 2013