Published in final edited form as:

Clin Obstet Gynecol. 2013 September ; 56(3): 622–632. doi:10.1097/GRF.0b013e31829cb99a.

Epigenetic Mechanisms in Fetal Origins of Health and Disease

Amber Osborne-Majnik, Ph.D.¹, Qi Fu, M.D.¹, and Robert H. Lane, M.D., M.S.²

¹Division of Neonatology, Department of Pediatrics, University of Utah School of Medicine, Salt Lake City, Utah 84158

²Department of Pediatrics, Medical College of Wisconsin and Children's Hospital of Wisconsin, Milwaukee, Wisconsin 53201

Abstract

Early life environment predicts future health. The initial precedents and research focus for this concept arose out of historical events. However, this concept continues to be relevant as evidenced by the recent Chinese Famine and the evidence of racial disparities in the United States. The latter allows us to introduce the “life course model” and “weathering” as relevant epigenetic phenomena. We then review the molecular components of environmental epigenetics. We subsequently present glucocorticoid receptor biology as a paradigm that involves all of the components. Finally, we suggest that environmental epigenetics are a key component of the future of personalized medicine.

Introduction

Early life environment predicts future health. This is the core tenet of the Fetal Origin of Disease hypothesis. This prediction proves to be relevant in developed and developing countries, as well as extremes of the economic disparity continuum. Moreover, moving along the economic disparity continuum may actually strengthen the prediction in some circumstances. An aberrant *in utero* environment early in life increases susceptibility to the effects of a more affluent lifestyle later in life. Our recognition of this relationship between early life environment and future health has been a result of the rapid changes in our world brought forth by industry, technology, and population growth.

Humans have changed their environment more in the last 200 years in terms of diet and environmental exposures than all other epochs combined. The impact of these changes has significantly affected population health and disease. Reports exist revealing significant increases in multiple chronic pathologies ranging from autism to schizophrenia, from autoimmune disease to serious infectious disease, and from obesity to diabetes¹⁻³. The direct impact of these increases on individuals and population health is obviously significant. Moreover, indirect impact of these increases is also significant in terms of healthcare expenditures.

In the United States, healthcare expenditures increased to near 2.6 trillion dollars in 2010, which is 10 times the 256 billion dollars spent in 1980. Across the world, healthcare costs are also increasing. The Towers Watson survey of 237 leading medical insurers in 48 counties noted an average increase of 9.8% in 2011, and an increase of 9.6% is predicted this year. Surprisingly, regions of increased cost included Latin America and Asia-Pacific.

Corresponding Author: Robert H. Lane M.D. M.S., The Barri L. and David J. Drury Chair of Pediatrics, Chair, Department of Pediatrics – Medical College of Wisconsin, Pediatrician in Chief – Children's Hospital of Wisconsin, Children's Corporate Center, Suite 720, PO Box 1997. MS 720, Milwaukee, Wisconsin 54201-1997, P: 414-337-7770, F: 414-337-7771, rlane@mcw.edu.

Whereas advancing technology certainly contributes to the increase in healthcare cost in all regions, an increase in chronic pathologies and the associated morbidities must also contribute. As resources become more limited in terms of both micro and macro economies, the increased steal of resources by healthcare siphons investment away from key social concerns such as sanitation, nutrition, and education. Moreover, loss of investment in these concerns dramatically impacts pregnancy health and perpetuates an expanding disease cycle. The bottom line is that we cannot afford to fail in developing an understanding of the mechanisms involved in the “Fetal Origins of Disease” concept. The initial clues that epigenetics may be one of those mechanisms came from patterns observed in the following pioneering studies that set the stage for the field.

The Pioneering Studies the set the Standard for Studying the Fetal Origins of Disease

These pioneering studies include the early studies of Barker *et al.*, as well as two population cohorts, the Nurses’ Health Study, and the Dutch famine of 1944 – 1945. These studies have set the standard for the field in terms of the rigor, and it is this rigor that allowed the initial insights that epigenetics may play a role. These insights were based on three characteristics. The first characteristic is an early life experience of a differentiating environmental event (*e.g.* famine) followed experience of non-differentiating environment. The second characteristic is the appearance of a significant morbidity that is temporally separated from the early life experience. The third characteristic is a comparison cohort without the phenotype that is not genetically different. Rigor was embedded into these studies by controlling for confounding factors such as gestational age, maternal smoking, socioeconomic status, multiple births, ethnicity, lifestyle, medical history, parental occupation, parental diabetes, and physical active, among other things.

An important and reasonable assumption for these studies was that poor fetal nutrition leads to low birth weight. Subsequently, low birth weight was used as a phenotypic marker of poor fetal nutrition or an abnormal environment. Out of all these cohorts, the reality and timing of poor fetal nutrition is best documented in the Dutch famine of 1944 – 45. The Dutch famine of 1944 – 45 lasted 5 months and daily rations dropped to 800 kcals/day from 1800 kcals/day. At the height of the famine, neither lactating nor pregnant women received supplemental rations.

The aggregate impact of the insult upon the population lead to the increased adult incidence of multiple chronic pathologies including schizophrenia spectrum disorders, antisocial personality disorder, affective disorder, obstructive airway disease, mortality at age 50, coronary artery disease, hypertension, dyslipidemia, obesity, and diabetes⁴⁻⁶. Among the most important observations from these studies is that the timing of the poor fetal nutrition is important in terms of predicting the later disease. Granted, specific prediction from the Dutch famine studies may not exactly replicate across all populations because of differences in genetics, the deprivation, or transgenerational environmental experiences. However, the concept that a population wide insult to early life environments significantly impacts a population’s health decades later appears to be conserved globally. This is evident in the Chinese famine.

Chinese Famine

The Chinese famine was truly a population wide insult whose echo we are just beginning to hear. The Chinese famine occurred from 1959 through 1961, and it was one of the largest famines recorded in human history. The Chinese famine provides us an opportunity to further our understanding of the Fetal Origins of Disease Hypothesis due to the

epidemiological differences versus the Dutch famine. Relative to the Dutch famine, the Chinese famine was 1) longer and less precisely defined; 2) superimposed upon a background of widespread chronic under nutrition; and 3) heterogeneous in terms of impact, with rural regions disproportionately affected. Relative to the Dutch famine, there are some similarities in terms of the population impact; namely, a prediction of insulin resistance and increased incidence of hypertension in the children of the famine^{7, 8}. However, differences exist also.

The Chinese famine affected the offspring of those mothers who endured its impact by reducing adult height and by affecting population neurodevelopmental outcomes⁷. The Dutch famine did not have these impacts. Moreover, though both the Dutch and Chinese famines affected the incidence of schizophrenia, the impact was different as a likely result of the heterogeneous nature of the Chinese famine. The Dutch famine increased the incidence of schizophrenia, as mentioned above. In contrast, the Chinese famine increased the incidence of schizophrenia in urban regions and decreased in incidence of schizophrenia in rural regions⁹. Finally, recent evidence suggests a transgenerational effect of the Chinese famine that appears to be more concrete than those suggested by the Dutch Famine. Grandchildren of the Chinese famine appear to be larger than those infants conceived after 1961. This is important because high birth weight predicts later life obesity and insulin resistance, similar to low birth weight. As alluded to in the introduction, the social and financial cost to China and the rest of the world is tremendous considering the tens of millions of individuals potentially affected across generations. Unfortunately, cross generational morbidity exists in the United States and is being revealed by racial disparities and deserves further investigation.

Racial Disparities

The high infant mortality in the United States occurs in large part due to the mortality rate of African-Americans, which is greater than twice the rate of non-Hispanic whites. This disparity occurs due to a long standing increased incidence of low birth weight (< 2500 grams) in African-Americans. An important concept has been proposed to build an infrastructure upon which to study racial disparities. This concept is the 'life course model'¹⁰.

The 'life course model' proposes that adverse pregnancy outcomes of African-American women occur because of a higher prevalence of multifactorial risk factors from conception to death. This model's inclusion of conception into its infrastructure allows for the maternal grandmother's life experience to impact upon pregnancy outcomes of the grandchildren. This is relevant because a maternal grandmother's exposure to neighborhood poverty during pregnancy predicts the risk for infant low birth and subsequently perinatal mortality among urban African-American women¹¹. Regression analysis suggests that this risk is independent of the mother's status. Moreover, 25% low birth weight infants born to non-low birth weight mothers can be statistically attributed to generational residence in low income neighborhoods. This same cannot be said for non-Hispanic white non-low birth weight mothers. The inclusion of a cross generational effect is particularly resonant with the historical African – American experience. Since the import of slavery in the early 1600s, no other racial or ethnic minority in the United States has endured such a monotonous period of discrimination, exposure to chronic stress, and lack of validation by the larger society, with the possible exception of the Native American.

The 'life course model's inclusion of the full life also allows for the concept of "weathering". "Weathering" is the concept that the cumulative impact of social disadvantages disproportionately affects African-American pregnancy outcomes. Examples of weathering include the following. First, the incidence of growth restriction leading to low

birth weight increases in African-American women enduring low income neighborhoods as maternal age increases. This phenomenon does not occur in non-Hispanic white women from similar neighborhoods¹². Second, the time spent homeless is a better predictor of perinatal mortality in African-American women as defined by low birth weight and preterm birth versus being homeless at the time of pregnancy¹³. This temporal separation between the early life event and a significant morbidity is the hallmark of an epigenetic event and is similar to those noted in the initial pioneering studies.

Two other observations support the role of epigenetics in the racial disparities evident in United States infant mortality data. The first observation has to do with African - American mothers who experience early life neighborhood poverty and subsequent low, modest, or high upward mobility. In those mothers who themselves were normal birth weight, upward mobility decreased the preterm birth rate by approximately 3%, 4%, and 6%, respectively¹⁴. However, this improvement in outcomes did not occur if the mother herself was low birth weight. In other words, the impact of maternal low birth weight is a better predictor of pregnancy outcome than social economic status¹⁴. This may be due to the neuroendocrine reprogramming that appears to occur in low birth weight infants, making the low birth weight mother extremely vulnerable to higher stress reactivity. The second is the initial observation that United States born non-Hispanic white women experience the same pregnancy perinatal mortality as that of foreign-born non-Hispanic white women who move to the United States. In this same initial study, United States born African American women experience greater rates of perinatal mortality than foreign born counterparts. Even sadder is a more recent study that demonstrates perinatal mortality is higher in women born in the United States regardless of race and individual risk factor levels than their foreign born counterparts¹⁵. This speaks toward the impact the environment in the United States has upon pregnancy health and further suggests the role of epigenetics.

Environmental Epigenetics

Epigenetic mechanisms underlie the regulation of gene expression in eukaryotes. Generally, epigenetic mechanisms allow cells to preserve memory and maintain distinctive transcriptional identities. Conservation and an “on – off” nature characterize epigenetic mechanisms used to guide embryonic and fetal development, as well as tissue specification. This is what we refer to as *Developmental Epigenetics*. Modulation and a ‘rheostat’ nature characterize epigenetic mechanisms used to provide a component of plasticity that allows for adaptation during times of early environmental stresses such as poor prenatal nutrition. This is what we refer to as *Environmental Epigenetics*¹⁶. A cell’s capability to sense, interpret, and act upon environmental stimuli by modifying gene expression is a basic survival skill. It is this capability that contributes to the “Fetal Origins of Disease” concept. The importance of modifying gene expression is evident by the amount of the genome that is dedicated to this effort, particularly when compared to the portion of the genome that encodes protein.

Only 2% of the human genome encodes protein coding genes¹⁷. The other 98% contains vast stretches of genome that consist of instruction or regulator elements that supervise the expression of those protein coding genes. These regions include the 5’ and 3’ regulatory regions of genes, intronic regions, and regulatory RNAs. 75% of the non-protein encoding DNA is actively transcribed. Moreover, the ratio of non-protein encoding DNA to protein encoding DNA correlates well with developmental complexity. Therefore, to understand the present state of the field of environmental epigenetics, an understanding key and regulatory components of the genome is necessary.

Regulatory Components of the Genome

The standard regulatory component of a gene that most people are aware of is the promoter. The promoter region of a gene contains the start site of transcription, and promoters are located on the same strand (cis-) as the DNA encoding protein. Promoters typically extend 100-1000 base pairs in length. Most clinicians are less familiar with enhancers and silencers.

Enhancers exert a positive effect on transcription and contain binding sites for transcription factors. Indeed, the interaction between enhancers and transcription factors appear to be the critical determinants of cell identity. In contrast to promoters, enhancers activate transcription regardless of location or orientation. Silencers share the same characteristics of enhancer with the obvious exception that they exert a negative effect on transcription. All of these regulatory components are formed through how the DNA interacts with its molecular scaffold.

Together, the DNA and scaffold are known as chromatin. The most basic level of chromatin structure consists of DNA (≈ 147 bp) wrapped twice around a protein core called a nucleosome. The nucleosome consists of two copies of 4 different histone proteins H2A, HB2, H3, and H4. Moreover, histone variants also appear to modulate gene expression. Genome wide profiling demonstrates the histone variant H3.3 enriches DNA of promoters and binding sites of regulatory complexes of actively transcribed genes.

Nucleosome position regulates access to DNA and thereby also modulates gene expression. Nucleosome positioning is a remarkably important determinant of gene expression based upon the conservation between yeast and human. Nucleosomes typically cluster around exons and intron-exon boundaries and avoid enhancer and promoter regions. The association of a nucleosome with DNA affects which transcription factors bind with the DNA. Pioneering transcription factors bind to their DNA response elements in the context of DNA tightly associated with a nucleosome. This makes pioneering transcription factors a particularly rich target for studies targeting the epigenetic mechanisms involving Fetal Origins of Disease. This is because the capability to bind to nucleosome associated DNA suggests pioneering transcription factors as an early initiator of re-programming from normal developmental patterns of gene expression.

Re-programming involves the use of multiple epigenetic tools. A core concept of environmental epigenetics is that a single tool does not determine expression alone, and that an understanding of how the different tools seamlessly integrate to generate normal and deviant patterns of gene expression is necessary to gain a full mechanistic understanding of Fetal Origins of Disease. That being said, there is value delineating the major components of the epigenetic toolbox, which consists of DNA methylation, histone covalent modifications, microRNAs (miRNA), and long non-coding RNAs (lncRNAs).

DNA Methylation

DNA methylation generally occurs on the cytosine of CpG dinucleotides. CpG dinucleotides are disproportionately located in CpG islands. CpG islands involve 200 or more bases with a GC content of 50% or greater. Moreover, these islands involve CpG frequencies of 60%. Approximately 2/3 of human gene promoters locate within CpG islands. Most promoters located within CpG islands stay unmethylated normally, though a small percent ($< 10\%$) become methylated during development. CpGs located in intragenic and intergenic regions are relatively rare and more likely to be methylated. Though DNA methylation within CpG islands often associates with gene silencing, initiation of silencing does not require DNA methylation. More likely, DNA methylation functions to maintain a repressed state of transcription over generations of replication.

DNA methylation also occurs within CpG shores. CpG shores contain a lower CpG density than CpG islands. CpG shores typically lie within 2 kb of CpG islands, and methylation of these regions usually negatively affects transcription, but appears to be more modulatory than DNA methylation of CpG islands. Finally, DNA methylation also clusters on DNA associated with nucleosomes, particularly those nucleosomes positioned at exon-intron and intron-exon boundaries.

Interestingly, two epigenetic phenomena related to DNA methylation have been recently reported. The first phenomenon is non-CG methylation on cytosines not adjacent to a guanine. Stem cells contain these methylated cytosines, which are clustered in the body of transcribed genes. Differentiation away from pluripotency appears to decrease this non-CG methylation, and restoration to stem cell status resurrects their appearance. The second phenomenon is 5-hydroxymethylation on cytosines. The distribution of cytosine 5'-hydroxymethylation demonstrates enrichment in transcribed regions, transcription start sites, and some enhancer regions. Moreover, similar to non-CG methylation of cytosines, 5-hydroxymethylation appears to participate in the maintenance of stem cell pluripotency.

Histone Code

The nucleosome core consists of the globular portion of the histone proteins mentioned above. The histone proteins also have N-terminal tails that extend from the nucleosome core that are subject to covalent modifications. These modifications impact chromatin function and structure. The impact of histone covalent modifications occurs through three mechanisms of action. The first mechanism of action neutralizes the positive charge of histone lysine residues and thus weakens charge dependent interactions between the nucleosome and DNA. Histone acetylation is the mostly widely studied of the histone covalent modifications that utilize this mechanism. Histone acetylation usually localizes to active chromatin. For example, enrichment acetylation of histone 3 lysine 27 (H3K27) and occupancy by the histone acetyl transferase p300 often characterize active enhancers. Cumulative charge neutralization may be the key characteristic of acetylation that modulates chromatin binding access rather than acetylation of specific lysine residues. Histone acetylation also appears to be important in terms of DNA replication and repair. Other histone covalent modification that affect charge driven interactions between the nucleosome and DNA include crotonylation, formylation, succinylation, malonylation, propionylation, and butyrylation.

In contrast, the second mechanism of action is the modulation of affinity of chromatin regulating and transcription complexes to chromatin without affecting the charge driven interactions between nucleosomes and DNA. Histone methylation is the most widely studied histone modification that utilizes this action. For example, monomethylation (but not trimethylation) of histone 3 lysine 4 (H3K4) enrich active enhancer regions. Trimethylation of H3K27 enriches the flanking regions of poised enhancers. Trimethylation of H3K4 enriches promoters, regardless of activity state.

The third mechanism of action affects both charge and affinity. The most well studied histone covalent modification that does both is histone phosphorylation. The phosphate of the DNA backbone is normally negatively charged, so the addition of a phosphate to a histone creates charge repulsion between the nucleosome and DNA. Moreover, histone phosphorylation alters the affinity of chromatin regulating complexes for their targets. For example, phosphorylation of a serine adjacent to a methylated lysine reduces the affinity of methyl binding proteins.

Finally, as a corollary to the above core concept that a single epigenetic tool does not determine gene expression, a single histone covalent modification does not determine gene

expression. The existence of 51 distinct ‘chromatin’ states based upon varying combinations of histone covalent modifications has been described. Indeed, the aggregate of histone modifications within a nucleosome, a gene, and a wider region modulate gene expression. How wide a region is still indeterminate but likely encompasses the majority of genome because of the concept of interchromosomal interactions through chromatin looping. Chromatin looping brings distal regulator elements into close contact with gene promoters. Our understanding of this phenomenon is still quite conceptual.

Micro RNA (miRNA)

miRNAs consist of small ≈ 21 bp non-coding RNAs that mediate post transcriptional regulation of expression. This mediation occurs through miRNA interactions with regions of complementary RNA in the 3' untranslated region (3' UTR) of target genes. These interactions mediate repression of target transcript expression by negatively regulating mRNA stability and / or protein translation. For the latter, miRNAs inhibit initiation and elongation steps of translation. miRNA regulation facilitates key developmental processes such as cell proliferation, programmed cell death, and cell line differentiation. The importance that evolution places on miRNA regulation is evident through the observation that up to 60% of our genome is regulated by miRNAs, and each miRNA likely modulates the expression of 200 target genes.

Long non-coding RNA (lncRNA)

lncRNAs consist of a diverse class of transcripts that are greater than 200 nucleotides in length and lack a functional open reading frame. lncRNAs locate throughout the genome, and have been identified with both intergenic and intragenic regions. Indeed, the transcription of lncRNAs drives the complexity of the human transcriptome, though low transcript levels compared to protein encoding transcripts make some lncRNAs difficult to detect. This low level of expression suggests that lncRNAs modulate levels of gene expression that require fine control.

lncRNAs regulate gene expression through multiple mechanisms. lncRNAs bind to other RNAs and thereby can act as sensors of mRNA, miRNA and other lncRNAs transcripts. lncRNAs also bind to chromatin regulating proteins and transcription factors. In addition, they may act as scaffold molecules to deliver regulatory proteins, and lncRNAs may exert translational control either directly or through the regulation of mRNA stability. This interaction between lncRNAs and protein represents an interface of molecular communication between the transcriptome and the proteasome, which may be a key locus of control when environments are changing and demanding adaptation to ensure survival.

Glucocorticoid Biology

Adaptation requires the full toolbox of environmental epigenetics. Though innumerable examples exist of isolated observations on how fetal environment epigenetics and leads to later life disease, the impact of poor fetal nutrition upon the glucocorticoid receptor and glucocorticoid homeostasis provides a useful paradigm. This paradigm will allow us to demonstrate concepts that are applicable to multiple prenatal environments, genes, and systems. The paradigm components can be divided into 1) the impact of poor fetal nutrition upon the glucocorticoid receptor gene; and 2) the impact of poor fetal nutrition upon multiple tissues and other components of glucocorticoid homeostasis.

Glucocorticoid Receptor

The mammalian glucocorticoid receptor (GR) gene allows for nimble adaptation based upon its complexity. Adaptation and differential expression of GR depends upon transcriptional control of the complex 5' UTR¹⁸. The human GR contains 8 translated exons and 9 untranslated alternative exons. These alternative first exons, with each being preceded with its own promoters, locate in one of two promoter regions that are located 5 and 30 kbs upstream. The distal promoter controls alternative first exons 1A and 1I. The proximal promoter controls exons 1B, 1C, 1D, 1E, 1F, 1H, and 1J. Exons 1D to 1H happen to be in a CpG island. The use of these alternative exons gives the cell fine control over GR expression levels because the specific usage of alternative first exons is important for translational regulation¹⁹. Indeed, GR mRNA and protein are expressed by almost all cell types, but mRNA and protein expression differs widely between cell types.

The 3' UTR of the GR gene also affects differential gene product expression. The variable 3' region encodes splice variants that carry out different function. The most widely studied of these splice products are GR α , GR β , and GR-P. Alternatively spliced 3' exons of 9 α and 9 β produce GR α and GR β , respectively. GR-P lacks both exons 8 and 9. GR α is the most active isoform in terms of glucocorticoid signaling, and GR β appears to function in a dominant negative fashion. GR-P with its truncated ligand binding domain likely enhances GR α activity.

Human GR α mRNA further incorporates more fidelity of expression by using at least 8 alternative translation sites. Each GR α isoform initiates different patterns of gene expression in response to dexamethasone as determined by microarray. Ultimately, the combination of splice and translational variants expressed form up to 256 different combinations of homo – and heterodimers with varying cellular location and transcriptional activities. Though not as widely studied, the rat GR gene appears to be similarly organized. The conservation of the rat GR gene organization relative to the human allows the study of how poor fetal nutrition affects GR epigenetics.

Poor fetal nutrition and fetal stress leading to low birth weight affect hippocampal GR DNA methylation and histone modifications in the rat. Rodent models of low birth weight utilizing poor fetal nutrition often find altered hippocampal GR mRNA expression. Hippocampus is often studied in these models because it is particularly vulnerable to prenatal insults, and the hippocampus is involved in feedback circuitry with the HPA axis. In a study involving a model of prenatal stress, GR expression was selectively decreased in the dentate gyrus and CA3 region of the hippocampus. These changes in GR mRNA levels were associated increased DNA methylation at multiple sites within the exon 1 promoter, which is the primary CNS promoter in the rat²⁰. In one detailed study, low birth weight changed postnatal levels of three alternative exon 1 transcripts (including exon 1 γ) and four 3' transcripts (including GR α and GR β) in a sex specific manner²¹. These changes in mRNA levels were associated with concurrent changes in the multiple acetylation and methylation histone covalent modification in multiple promoters and 3' regions.

lncRNAs and miRNAs also regulate GR expression. Recent studies involving cell culture found that poor nutrition and lack of growth factors increases the growth arrest-specific 5 (GAS5) lncRNAs. Gas5 binds to the DNA-binding domain of the glucocorticoid receptor, functions as a riborepressor, and thereby deregulates glucocorticoid homeostasis²². Finally, miRNAs miR -18 and miR-24 regulate GR levels in neuron cell culture²³. miR-124 expression appears to be specific to the brain, and expression of this miRNA increases GR protein levels neuronal cell culture. In terms of miR-18, chronic stress in adult rats increases hippocampal miR-18 expression *in vivo*²⁴. These studies poise future investigators to dive

even deeper into impact of poor fetal nutrition upon miRNA regulation of CNS GR expression.

Glucocorticoid Homeostasis

The impact of poor fetal nutrition upon glucocorticoid homeostasis is most widely studied through two approaches. The first approach involves studying hypothalamic-pituitary-adrenal axis reactivity. The aggregate result in both humans and rodents is a resetting of the postnatal HPA axis that is characterized by chronic hyperactivity. These studies include many components of the central hypothalamic – pituitary axis such as hippocampus, hypothalamus, and adenopituitary. The second approach involves the placenta, which is a significant determinant of the *in utero* fetal nutritional and hormonal milieu.

Placental expression of genes involved in glucocorticoid homeostasis change in response to environmental stresses such as altered fetal nutrition. For example, when 480 placentas were assessed for DNA methylation at GR gene exon 1F, an association became evident between differential methylation and large for gestational age²⁵. Another gene whose expression is vulnerable to these stresses is placental 11- β hydroxysteroid dehydrogenase type 2 (11 β HSD2). In animal models, aggregate results demonstrate placental decreased expression. Recent human studies demonstrate similarly that decreased 11 β HSD2 expression accompanies decreased mRNA levels and increased promoter DNA methylation in lower birth weight and ponderal index (per g / cm³ × 100)² infants²⁶. The ponderal index is often used as a measure of relative growth restriction. Moreover, the increased methylation of the 11 β -HSD2 promoter predicted reduced scores of quality of movement on the NICU Network Neurobehavioral Scales²⁶. Similarly, methylation of the GR promoter in placentas of genetically susceptible infants may also predict neurodevelopmental issues²⁷. This last two studies hint at the possibility of epigenetics being used as a component of personalized medicine by function as an environmentally sensitive single nucleotide polymorphism that predicts future outcomes.

Personalized Medicine

Assessing GR epigenetics and expression in lymphocytes potentially presents an opportunity to develop biomarkers that not only predict disease, but also permit bench marking intervention. This is particularly true since > 75% of the alternative first exons falling into CpG islands that are conserved across phyla. Studying GR expression in leukocytes, which is an accessible tissue, is also relevant since approximately 20 % of leukocytes are positively or negatively regulated via glucocorticoid signaling²⁸. In a study of 26 healthy adults, the GR promoter regions of leukocytes were extensively methylated allowing for the possibility of interpretation once we learn to ‘crack the code’.

Epigenetics represents the key to unlocking the door of ‘personalized’ medicine, and we are excited about the potential. Though we have focused upon poor fetal nutrition in this review, multiple environmental exposures within the perinatal period predispose infants towards adult diseases such as diabetes, obesity, and cardiovascular disease. By in large, most animal model and human environmental epigenetic studies demonstrate that epigenetics ‘happens’, but not how it happens or what it means. As we increase understanding of the ‘meaning’ of environmental epigenetic responses to environmental exposures, we hope to identify those individuals most predisposed towards adult diseases.

Acknowledgments

Support: This effort was support in part by DK081756 and HL110002 (RHL)

References

1. Okkels N, Vernal DL, Jensen SO, et al. Changes in the diagnosed incidence of early onset schizophrenia over four decades. *Acta psychiatrica Scandinavica*. 2013; 127(1):62–68. [PubMed: 22906158]
2. Baker MG, Barnard LT, Kvalsvig A, et al. Increasing incidence of serious infectious diseases and inequalities in New Zealand: a national epidemiological study. *Lancet*. 2012; 379(9821):1112–1119. [PubMed: 22353263]
3. Webber LS, Bedimo-Rung AL. The obesity epidemic: incidence and prevalence. *The Journal of the Louisiana State Medical Society : official organ of the Louisiana State Medical Society*. 2005; 157(Spec No 1):S3–11. quiz S11. [PubMed: 15751904]
4. de Rooij SR, Painter RC, Phillips DI, et al. Hypothalamic-pituitary-adrenal axis activity in adults who were prenatally exposed to the Dutch famine. *Eur J Endocrinol*. 2006; 155(1):153–160. [PubMed: 16793962]
5. Roseboom TJ, van der Meulen JH, Osmond C, et al. Plasma lipid profiles in adults after prenatal exposure to the Dutch famine. *Am J Clin Nutr*. 2000; 72(5):1101–1106. [PubMed: 11063435]
6. Susser E, Neugebauer R, Hoek HW, et al. Schizophrenia after prenatal famine. Further evidence. *Arch Gen Psychiatry*. 1996; 53(1):25–31. [PubMed: 8540774]
7. Huang C, Li Z, Wang M, et al. Early life exposure to the 1959-1961 Chinese famine has long-term health consequences. *J Nutr*. 2010; 140(10):1874–1878. [PubMed: 20702751]
8. Xiao X, Zhang ZX, Cohen HJ, et al. Evidence of a relationship between infant birth weight and later diabetes and impaired glucose regulation in a Chinese population. *Diabetes Care*. 2008; 31(3):483–487. [PubMed: 18070988]
9. Song S, Wang W, Hu P. Famine, death, and madness: schizophrenia in early adulthood after prenatal exposure to the Chinese Great Leap Forward Famine. *Social science & medicine*. 2009; 68(7):1315–1321. [PubMed: 19232455]
10. Lu MC, Halfon N. Racial and ethnic disparities in birth outcomes: a life-course perspective. *Maternal and child health journal*. 2003; 7(1):13–30. [PubMed: 12710797]
11. Collins JW Jr, David RJ, Rankin KM, et al. Transgenerational effect of neighborhood poverty on low birth weight among African Americans in Cook County, Illinois. *American journal of epidemiology*. 2009; 169(6):712–717. [PubMed: 19179359]
12. Love C, David RJ, Rankin KM, et al. Exploring weathering: effects of lifelong economic environment and maternal age on low birth weight, small for gestational age, and preterm birth in African-American and white women. *American journal of epidemiology*. 2010; 172(2):127–134. [PubMed: 20576757]
13. Stein JA, Lu MC, Gelberg L. Severity of homelessness and adverse birth outcomes. *Health psychology : official journal of the Division of Health Psychology, American Psychological Association*. 2000; 19(6):524–534.
14. Collins JW Jr, Rankin KM, David RJ. African American women's lifetime upward economic mobility and preterm birth: the effect of fetal programming. *American journal of public health*. 2011; 101(4):714–719. [PubMed: 21330589]
15. Collins JW Jr, Soskolne GR, Rankin KM, et al. Differing First Year Mortality Rates of Term Births to White, African-American, and Mexican-American US-Born and Foreign-Born Mothers. *Maternal and child health journal*. 2012
16. Joss-Moore LA, Lane RH. Epigenetics and the developmental origins of disease: the key to unlocking the door of personalized medicine. *Epigenomics*. 2012; 4(5):471–473. [PubMed: 23130826]
17. Lander ES, Linton LM, Birren B, et al. Initial sequencing and analysis of the human genome. *Nature*. 2001; 409(6822):860–921. [PubMed: 11237011]
18. Turner JD, Alt SR, Cao L, et al. Transcriptional control of the glucocorticoid receptor: CpG islands, epigenetics and more. *Biochem Pharmacol*. 2010; 80(12):1860–1868. [PubMed: 20599772]
19. Pickering BM, Willis AE. The implications of structured 5' untranslated regions on translation and disease. *Seminars in cell & developmental biology*. 2005; 16(1):39–47. [PubMed: 15659338]

20. Oberlander TF, Weinberg J, Papsdorf M, et al. Prenatal exposure to maternal depression, neonatal methylation of human glucocorticoid receptor gene (NR3C1) and infant cortisol stress responses. *Epigenetics*. 2008; 3(2):97–106. [PubMed: 18536531]
21. Ke X, Schober ME, McKnight RA, et al. Intrauterine growth retardation affects expression and epigenetic characteristics of the rat hippocampal glucocorticoid receptor gene. *Physiol Genomics*. 2010; 42(2):177–189. [PubMed: 20388836]
22. Kino T, Hurt DE, Ichijo T, et al. Noncoding RNA gas5 is a growth arrest- and starvation-associated repressor of the glucocorticoid receptor. *Science signaling*. 2010; 3(107):ra8. [PubMed: 20124551]
23. Vreugdenhil E, Verissimo CS, Mariman R, et al. MicroRNA 18 and 124a down-regulate the glucocorticoid receptor: implications for glucocorticoid responsiveness in the brain. *Endocrinology*. 2009; 150(5):2220–2228. [PubMed: 19131573]
24. Uchida S, Nishida A, Hara K, et al. Characterization of the vulnerability to repeated stress in Fischer 344 rats: possible involvement of microRNA-mediated down-regulation of the glucocorticoid receptor. *The European journal of neuroscience*. 2008; 27(9):2250–2261. [PubMed: 18445216]
25. Filiberto AC, Maccani MA, Koestler D, et al. Birthweight is associated with DNA promoter methylation of the glucocorticoid receptor in human placenta. *Epigenetics*. 2011; 6(5):566–572. [PubMed: 21521940]
26. Marsit CJ, Maccani MA, Padbury JF, et al. Placental 11-beta hydroxysteroid dehydrogenase methylation is associated with newborn growth and a measure of neurobehavioral outcome. *PLoS One*. 2012; 7(3):e33794. [PubMed: 22432047]
27. Bromer C, Marsit CJ, Armstrong DA, et al. Genetic and epigenetic variation of the glucocorticoid receptor (NR3C1) in placenta and infant neurobehavior. *Developmental psychobiology*. 2012
28. Galon J, Franchimont D, Hiroi N, et al. Gene profiling reveals unknown enhancing and suppressive actions of glucocorticoids on immune cells. *Faseb J*. 2002; 16(1):61–71. [PubMed: 11772937]