



† *Designated as an Exemplary Final Project for 2014-15*

**Epigenetics: A Paradigm Shift
or Tweaking the Details?**

Emily Ray Barber

**Faculty Advisor: Jonathan Shaw
Department of Biology**

March 2015

This project was submitted in partial fulfillment of the requirements for the degree of Master of Arts in Graduate Liberal Studies in the Graduate School of Duke University.

Copyright by
Emily Ray Barber
2015

Abstract

We know that our lifestyle choices and experiences can influence our health: the food we eat, the environment we live in, and the social relationships we form can all have an impact on our development and health. But what if events that occur before we are even born, during our early stages of development, or even before we are conceived, determine our health in adulthood? What if our life experiences affect the health of our future, unborn descendants? Epigenetics is a field that gives us insight into the relationship between our surrounding environment and our genetic makeup, addressing the nature-nurture interaction.

Epigenetics is the study of the regulation of genes, specifically how genes are activated--or turned on--and deactivated--turned off. Environmental factors such as stress, nutrition, pollution, toxins, and many others, can affect the regulation of genes without altering the genome, and this dysregulation can lead to the development of disease states. Epigenetics describes how these environmental factors can become molecularly embodied in our cells. With this new field we must ask: to what extent (if at all) does epigenetics fundamentally change our way of thinking about human (1) mental and (2) physical health? Do new insights into epigenetic processes represent a paradigm shift, and what are the conceptual and/or practical implications of epigenetics in these fields? In order to answer these questions, I will review the research that has been done on the topic, present the current paradigms in fields associated with human health and disease, identify what constitutes a paradigm shift in science, and determine if epigenetics does in fact fundamentally change how we view human health and disease.

Ultimately, I determine that the aspect of epigenetics that provides the molecular mechanisms through which nature and nurture interact is merely an extension of the old paradigm. However, the idea that epigenetic changes can be transmitted through generations is novel and does constitute a paradigm shift in our thinking about human health and disease.

Contents

| | | |
|--------------|--|-----|
| Abstract | | iii |
| Introduction | | 1 |
| Chapter 1 | Mental Health Outcomes | 15 |
| | <i>Stress</i> | |
| | <i>Cognitive and Psychological Disorders</i> | |
| Chapter 2 | Physical Health Outcomes | 36 |
| | <i>Nutrition</i> | |
| | <i>Specific Health Outcomes</i> | |
| Chapter 3 | A Paradigm Shift or Tweaking the Details? | 45 |
| Conclusion | Looking Forward | 71 |
| References | | 74 |

Introduction to Genetics and Epigenetics

Health is a basic human right. This does not mean that every individual has the right to be healthy; rather it means that government is responsible for creating an environment where people can be as healthy as possible. This can include establishing healthy living and working conditions, providing good nutrition, and making the appropriate health services available (WHO, 2013). It is not news that the environment we are exposed to and how we treat our bodies affects our health, but the mechanisms through which these factors interact with our genetic makeup to produce health outcomes is just starting to become better understood. Additionally, it is a novel idea that these factors can also affect our future generations of offspring. Epigenetics is a field that is demanding more attention, as it explores this relationship between genes and environment.

A fundamental concept in biology is that the genome, comprising the nucleotide sequence, determines an organism's displayed characteristics, or *phenotype*. Evolutionary theory states that natural selection acts on variations in phenotype, and forms that are better adapted to the environment leave a disproportionate number of offspring (Darwin, 1859). Underlying an organism's phenotype is its *genotype*, the full complement of genes making up its genome and composed of deoxyribonucleic acid, or DNA. Changes in inherited information (DNA) occur at random, and mutation provides the raw material for evolution. It is widely accepted that the environment can affect phenotypes, and while non-genetic factors play a role in this variability, it is only the genetic factors that are heritable. The field of epigenetics, which presents a new form of inheritance, is currently challenging this concept. Epigenetics encompasses the biological mechanisms that can alter inherited information by modifying gene expression without altering the nucleotide sequence of the genome (Richards, 2006).

Modifications can occur at the DNA, nucleosomal, and chromosomal levels, and these changes can lead to modified phenotypes. These modifications can regulate genes by activating them -- turning them on --reducing activity, or deactivating them -- turning them off. What is particularly interesting is the “potential autonomy of epigenetic modifications from their genotypic context” (Richards, 2006, p. 395). In other words, epigenetic alterations function independently from the genotype. The stability and persistence of epigenetic variations, in combination with this autonomy, suggests an alternative inheritance system, one that demands more attention. And so we must ask: to what extent (if at all) does epigenetics fundamentally change our way of thinking about human (1) mental and (2) physical health? Do new insights into epigenetic processes represent a paradigm shift, and what are the conceptual and/or practical implications of epigenetics in these fields?

We should begin by discussing the foundation of molecular biology, as first understanding the basic structure of DNA, along with the cellular mechanisms of gene expression and inheritance, is critical to our understanding of epigenetics. Molecular biology studies the processes of replication, transcription, and translation, and how these biological processes relate to cellular function. Deoxyribonucleic acid, more commonly known as DNA, is the molecule that contains the genetic information necessary for all cellular development and functioning. DNA is a double-stranded, helical molecule. The “D” in DNA stands for deoxyribose, which makes up the backbone of DNA. The backbone consists of deoxyribose sugar groups separated by phosphate molecules. A chemical group called a base is attached to each sugar group, and the four bases involved are: adenine (A), cytosine (C), guanine (G), and thymine (T). DNA is a double-stranded molecule, so the base attached on one strand is paired with a base attached on the opposite strand. Certain bases pair with each other: A only binds to T, and C

only binds to G. Watson and Crick are credited with discovering the structure of DNA in 1953 (Watson and Crick, 1953). The central dogma of biology is the following:

DNA→RNA→Proteins

This means that DNA is transcribed to RNA, which is translated into protein, which ultimately determines the function of cells. Protein synthesis occurs when one strand of DNA serves as the template strand, and this strand is transcribed into RNA (or messenger RNA, mRNA). During translation, the mRNA becomes the template strand for the creation of a protein, which ultimately determines function.

Genes are segments of DNA that are heritable, or passed from parent to offspring.

Genes are organized and packaged into chromosomes, which also contain proteins. Genes have particular locations on chromosomes, known as loci, and there can be multiple variants at a locus, known as alleles. We inherit one allele from each parent, and if the allele is the same from each parent it is labeled as homozygous, and if it is different it is heterozygous. Humans have 23 pairs of chromosomes, one set from each parent.

Humans are composed of trillions of cells, all of which have specialized functions. It all starts with a single cell, called a zygote, which is formed when a sperm and an egg cell merge. The zygote splits into two separate cells, and continues to divide and split. As the cells divide, they become different from one another, and begin to form specialized cells for different tissues of the body, such as a liver cell or a blood cell. This process is known as differentiation, and explains how cells that differ so widely all started with the same genetic material. During differentiation, rather than losing genetic information that a specialized cell does not need, some genes are simply turned off while others are turned on. And differences in gene expression between genetically identical organisms (in terms of DNA sequence) need not be all or nothing – off or on. Genes can be “up-regulated” or “down-regulated” in different cells, or at

different times during development. Gene regulation is the process in which cells turn on or off certain genes. Throughout the life course each cell undergoes thousands of divisions, yet they maintain their identity (Masterpasqua, 2009). You may ask, how does this happen? Epigenetics is the answer.

Epigenetics did not gain major recognition until 1975 when two different papers were published on the methylation of DNA in bacteria. Riggs (1975) published "X inactivation, differentiation, and DNA methylation" (1975) and Holliday and Pugh published "DNA Modification Mechanisms and Gene Activity during Development" (1975). These researchers found that the DNA in bacteria could be methylated, the process in which a chemical methyl group is attached, and that this modification produced stable differentiated states that affected the function of genes despite the lack of genetic mutation (Burggren and Crews, 2014). Since this discovery, interest in the field has grown, and there is still much to be explored.

Terminology in the field of epigenetics has been a source of confusion, as a great deal of variability exists in the definition of epigenetics itself. There are many ways to define epigenetics, but the basic idea is that gene expression can be modified without altering the DNA sequence itself, and that these modifications can be heritable. Conrad Waddington (1905-1975) is the developmental biologist who coined the term epigenetics in his paper "The epigenotype." Waddington (1942) focused on how a gene can give rise to multiple phenotypes, and discussed the non-genetic inheritance of this information. Epigenetics is grounded in a developmental context, as epigenetic effects are particularly sensitive during certain stages of development. In simple terms, Burggren and Crews (2014) define epigenetic inheritance as, "the manner in which the environment affects the expression of the genome of the individual during its development AND the development of its descendants" (p.14). Bird (2007) defines epigenetics as "the structural adaptation of chromosomal regions so as to register, signal, or perpetuate

altered activity states” (p.398). Rapp and Wendel (2005) define epigenetics as “the alteration of phenotype, morphological or molecular, without change in either the coding sequence of a gene or the upstream promoter region” (p.82). Jablonka and Lamb (1995) define an epigenetic inheritance system (EIS) as “a system that enables a particular functional state or structural element to be transmitted from one cell generation to the next, even when the stimulus that originally induced it is no longer present. In other words, EISs are the systems that enable the transmission of the various phenotypic expressions of the genetic information in an individual. They underlie cell memory” (p. 80). At the foundation of epigenetics are epialleles, defined as “alleles that differ from each other in the patterns of methylation of DNA nucleotides of the gene, rather than stable nucleotide mutations” (Kalisz and Purugganan, 2004, p. 309). There are over 300 papers referenced in PubMed that contain the words “epigenetics” and “definition”; researchers are continually repeating, interpreting, and redefining epigenetics (Burggren, 2014).

Randy Jirtle, a former Duke professor and pioneer in the field of epigenetics, compares our genome and epigenome to a computer: if our genome (DNA sequence) is the hardware of the computer, then the epigenome is the software that tells the computer where, when, and how to work. All of our cells contain the same DNA, and it is the epigenome, the set of programs within the cell, that orders these cells to become a skin cell, a liver cell, a neuron, etc. In more scientific terms, the genome determines the structure of proteins, while the epigenome controls the function, including at what time, duration, and amount of protein synthesis occurs in each cell (Abdolmaleky et al., 2013). Dr. Jirtle predicts that someday there will not even be a field of epigenetics because this phenomenon encompasses all biological sciences, and you will not be able to conduct any research or conversation without including epigenetics (Jirtle, 2012).

While the term “epigenetics” was first coined more than half a century ago, it just recently started gaining attention, and the field has come on the scene quickly and in full force.

The New York Times has published articles titled, “Genes as Mirrors of Life Experiences” (Carey, 2010), “Why Fathers Really Matter” (Shulevitz, 2012), and “Inheriting Stress” (Gaisler-Salomon, 2014), while *The Economist* has published, “Methylated Spirits” (2009), “Grandma’s Curse” (2012), and “Poisoned Inheritance” (2013). The implications of the field are far-reaching, with applications in medicine, basic biology (particularly developmental, molecular, and evolutionary), the social sciences, public policy, and many other fields. Our understanding of human health and disease incorporates all of these disciplines, and with the explosion of research and new information the field presents, it is interesting to ask whether or not epigenetics represents a fundamental change in the relationship between genetics and organismal function, specifically focusing on human health. While human health is the focus in this paper, it is important to note that the majority of epigenetic studies have been done on plant and animal models.

First, it is critical to identify what classifies something as a paradigm shift. Thomas Kuhn proposed that science advances through a series of revolutions, which leads to paradigmatic leaps. Kuhn (1962) identifies three ways that a new paradigm develops: there is a crisis of an existing theory; a new, alternative theory arises (not necessarily with strong supporting information); or there is a major technological advance (Vineis, 2010). By assessing the role that epigenetic regulation plays in health and disease, I will determine whether this rising field does represent a fundamental shift in our thinking about human health, and the implications of these findings.

It is evident that variations in disease susceptibility cannot be attributed solely to genetic variation, and epigenetics provides information about this missing piece of the puzzle surrounding disease predictability. Epigenetics places an emphasis on the role of the environment, not only during select critical periods, but also throughout the lifespan, in the

development of health and disease states. Additionally, epigenetics suggests that people need to be held more accountable for the decisions they make, as these choices can affect not only their own health but also the development and health of their future offspring.

Definitions

Epigenetic gene regulation occurs as a result of both internal and external cues; internally, hormones, cellular metabolic states, and neurotransmitters signal for gene regulation, while externally, many environmental cues such as nutrition, weather changes, drugs, toxins, etc., can alter gene regulation (Abdolmaleky et al., 2013). The epigenetic changes induced by environmental factors can be passed on to the next generation, potentially leading to the development of disease (Roseboom and Painter, 2014). There are particularly sensitive periods in an organism's development when they are extremely vulnerable to external factors. In humans, the epigenome is most vulnerable immediately following fusion of sperm and egg when the organism is just starting to develop, but the epigenome continues to change throughout an organism's lifetime. Developmental plasticity is a critical period in development when the system is sensitive to the environment and plastic. Following this critical period, the system becomes more fixed and loses its plasticity. From an evolutionary standpoint, this plasticity is advantageous because it allows systems to appropriately respond to the surrounding environment. "Developmental plasticity is defined as the phenomenon by which one genotype can give rise to a range of different physiological or morphological states in response to different environmental conditions during development" (Barker, 2007, p. 415).

Before diving into the link between epigenetics and health, it is important to fundamentally understand epigenetic mechanisms.

Mechanisms

As previously mentioned, epigenetic modifications can occur at the DNA, nucleosomal, and chromosomal levels, and these changes can lead to modified phenotypes. Murrell et al. (2005) defines epigenomics as the “effects of chromatin structure including the higher order of chromatin folding and attachment to the nuclear matrix, packaging of DNA around nucleosomes, covalent modifications of histone tails (acetylation, methylation, phosphorylation, ubiquitination), and DNA methylation” (p. R3). Therefore, the major epigenetic modifications that have been studied are DNA methylation, histone modification, RNA interference, and RNA editing. All of these modifications control the regulation of genes without actually changing the DNA sequence. Specifically, cytosine residues in the DNA can be methylated, histone proteins can be modified through methylation or acetylation, leading to chromatin remodeling, and, lastly, small RNA molecules can mediate regulatory processes (Bossdorf et al., 2008). These processes do not necessarily occur independently, but rather, they are continuously interacting.

It is necessary to break these molecular processes down into simpler terms. Epigenetic marks are identified as particular chemical attachments. The most common attachment is a methyl group, which is a carbon atom with three hydrogen atoms bonded to it (CH₃). When a gene has a methyl group attached, it is said to be methylated. But genes are not necessarily methylated or not methylated, there are degrees of methylation. Typically, the more methylated a gene is, the less active it is. At the extreme, the gene may be completely inactive, or unexpressed, and is said to be silenced. The process in which a stimuli (internal or external) increases the expression of a gene is known as up-regulation. DNA methylation is the best understood of the epigenetic mechanisms, and it occurs in most eukaryotes and prokaryotes. In eukaryotes, DNA methylation involves the addition of a methyl group to the 5-position of cytosines, specifically cytosines that are followed by guanines, known as CpG dinucleotides

(Gurdinguio et al., 2009). These CpG sites typically exist in the regulatory regions of genes, and tend to cluster together there (Bossdorf et al., 2008). As previously mentioned, this methyl addition to CpG sites typically induces gene silencing. The process in which a stimulus (internal or external) decreases the expression of a gene is known as down-regulation. In mammals, roughly 80% of all CpG sites are methylated (Eckhardt et al., 2004). Thus, most genes in the genome are silenced (not expressed) at any given time.

Genomic DNA is approximately two meters in length, yet it must fit into a nucleus whose size is six-microns. Therefore DNA must be compacted approximately 10,000 times. But this is a problem because how could it be accessed when it is compressed into such a small area? This is where chromatin-modifying enzymes come in (Lester et al., 2011). Chromatin is a protein-based structure that packages DNA (McGowan and Szyf, 2010), and it does this via a variety of nucleosomes. Each nucleosome contains an octamer of histone proteins surrounded by 146 base pairs of DNA (Dolinoy et al., 2007). Epigenetic modifications involve changes in chromatin-associated proteins, including linker histones, nuclear scaffold proteins, polycomb groups, and transcription factors (Eckhardt et al., 2004). Gene expression is controlled by histone modifications in three ways: first, histone modifications alter the structure of chromatin, which makes genetic loci either more or less accessible to the transcription machinery. Second, they respond to many biochemical signals by activating or repelling certain complexes. And lastly, histone modifications respond to what the organism is experiencing by mediating epigenetic changes in gene expression (Abel and Zukin, 2008).

Recall the replication-transcription-translation processes of molecular biology, and how cells divide and one of the DNA strands is copied. Typically the epigenetic attachments on the DNA strands, such as methyl groups, are wiped clean during this process, but recent research is showing that not all of these epigenetic marks are erased. The chemical attachments can be

copied and therefore be present on the new daughter DNA strand (Rothstein et al., 2009). In the production of egg and sperm cells, if the epigenetic marks are copied onto the new daughter cells then they will be passed on to the next generation, a surprising phenomena that deserves more attention. It is important to distinguish between different types of inheritance, which will be made clear in the next section.

Inheritance: Multigenerational vs. Transgenerational

One might overlook the importance of understanding the term “inheritance,” but this is critical to our understanding of epigenetics. Inheritance can refer to the transmission of information between generations through learning, imitation, communication, and teaching. This is known as cultural inheritance, and offspring learn behaviors from their parents and surroundings through these methods. Genetic inheritance is the other type of inheritance, and is commonly referred to as “Mendelian” inheritance. Mendelian inheritance encompasses the transmission of traits through meiosis (formation of egg and sperm) from parent to offspring, and DNA is identified as the sole unit of heredity (Burggren and Crews, 2014).

There are two types of epigenetic modifications: mitotic epigenetic modifications and meiotic epigenetic modifications. Mitotic epigenetic modifications can also be called context-dependent modifications, and these occur when an environmental stimulus invokes an epigenetic change, and this modification persists through generations as long as the initial stimulus is present. In this case, the epigenetic modifications are manifested in multiple generations because each generation is exposed to the same environment. If the stimulus is removed, then the epigenetic effects are reversed. For example, if epigenetic modifications occur because of a certain nutritional diet, the changes can exist in the following generation as

long as that same diet is still present. If the diet is changed, then the epigenetic modifications will be reversed. This effect is multigenerational, not transgenerational.

Epigenetic marks that are acquired throughout the lifespan were previously thought to be erased between generations, but recent evidence is showing that these marks can be passed on to the next generation through the germline, known as transgenerational epigenetic inheritance (Rachdaoui and Sarkar, 2014). Meiotic epigenetic modifications, or germline-dependent modifications, are epigenetic changes that are incorporated into the germline. They can result from a single exposure to an environmental event, and the epigenetic modifications continue to exist in subsequent generations despite the removal of the initial stimulus. The modifications have become “independent of the original causative agent” (Crews and Gore, 2014, p. 378). Germline-dependent modifications are the only modifications with truly transgenerational effects. Environmental effects can only be considered as transgenerational epigenetic effects if they persist for at least three generations for maternal exposure, and two generations for paternal exposure (Rachdaoui and Sarkar, 2014). It is expected that germline dependent epigenetic modifications would be expressed differently among males and females since this alterations are mediated through the germline and germ cells develop into sperm and ova (Crews and Gore, 2014). Therefore sex differences are an important characteristic of germline dependent epigenetic modifications. It is important to note that germline-dependent epigenetic modifications are not the same as genomic imprinting, which is when “genes are monoallelically expressed in a parent-of-origin dependent manner” (Crews, 2010). This phenomena will be discussed in detail in the next section.

X-chromosome Inactivation & Imprinting

There are two epigenetically regulated phenomena that have been studied in great detail: genomic imprinting and X-chromosome inactivation (Jirtle and Skinner, 2007). In the 1980s genomic imprinting was discovered, which is a form of gene regulation with a so-called parent-of-origin effect. Genomic imprinting is a normal part of development mediated by epigenetic mechanisms. In the somatic cells of mammals, typically each cell contains two copies of each autosomal gene, one from each parent. But there are some genes where one of the parental alleles is silenced, and this is known as genomic imprinting. If the inherited allele from the paternal side is imprinted, this means that it is silenced, and only the inherited maternal allele is expressed (Prickett and Oakey, 2012). This phenomenon is non-Mendelian in nature in that it does not involve the DNA sequence; instead it is an epigenetic process and works through DNA and histone methylation. This discovery was the first hint at the role of epigenetic processes in the transmission of disease between generations. “This silencing of one allele makes genetic imprinting especially susceptible to abnormal genetic expression. Again, DNA methylation and histone modification play essential roles in silencing the imprinted gene” (Masterpasqua, 2009, p. 196). An example of an imprinted gene is the insulin-like growth factor 2 (IGF2), which is important to normal fetal development and growth. IGF2 is only expressed from the paternal allele, while the maternal allele for this gene is silenced (Chao and D’Amore, 2008).

Imprinted genes are thought to be particularly vulnerable to epigenetic dysregulation, or impairment, by environmental factors because these gene expressions are allele specific. Environmental factors can include stress, nutrition, and toxins (Rachdaoui and Sakar, 2014). An example of a highly vulnerable tissue is the brain, as it contains the greatest number of imprinted genes of all the tissues (Prickett and Oakey, 2012). When the expression of imprinted genes is altered early in fetal development, these deviations can manifest as neurological and

developmental disorders. Research has demonstrated the effects of early life exposure to stress and nutrition on imprinted genes via epigenetic processes, and how these alterations can lead to neurobehavioral deficits and psychiatric disorders (Jirtle and Skinner, 2007; Prickett and Oakey, 2012).

Monozygotic Twin Studies

Studies on monozygotic twins play a key role in epigenetic research as they share a genotype but can display differing phenotypes, and disease discordance between two genetically identical individuals supports an epigenetic etiology (Waterland and Michels, 2007; Petronis, 2006; Wong et al., 2005). Monozygotic twins have been used to explore the role of the environment in the development of disease, but our understanding of how and why this discordance exists is still limited. Fraga et al. (2005) studied a large cohort of monozygotic twins, looking at both global and locus-specific differences in histone acetylation and DNA methylation patterns. In their early years, monozygotic twins do not display detectable epigenetic differences, but later in life major differences exist. Fraga et al. (2005) attributes this to both internal and external factors; factors such as time spent apart, lifestyle choices, and age likely determined epigenetic differences between individuals in each set of twins. In other words, these researchers suggest that with advancing age, monozygotic twins experience epigenetic drift in relation to each other. Additionally, “accumulation of epigenetic defects would probably occur at a faster rate than that corresponding to genetic mutations because their consequences in survival are probably less dramatic and cells have not developed a comparable amount of mechanisms to correct them” (Fraga et al., 2005, p. 10609). These results demonstrate the importance of environmental factors in determining phenotypes, as these individuals have the same genotype but differing phenotypes.

Now that we have a better understanding of what the field of epigenetics encompasses and the mechanisms through which these changes occur, we can move into epigenetics and human physical and mental health and disease. The next few sections will be a review of the research that has been done linking the environment and health via epigenetic mechanisms. I will start by looking into mental health through the exploration of the effects of stress on the epigenome, then will present specific mental health outcomes associated with epigenetic alterations. Next I will discuss physical health outcomes associated with epigenetic alterations, specifically assessing the role of nutrition. Following the review of current research, I will once again ask the question: does epigenetics represent a paradigm shift? Based on the research presented and current analysis of the topic I believe the answer will be clear.

Chapter 1: Mental Health Outcomes

Certain health conditions, and often diseases, tend to run in families. This is thought to occur through genetic mechanisms, such as a specific gene associated with a disease can be inherited across generations, or the disease results from the shared environment. Most often though, disease occurs as a result of both genetic and environmental factors. In addition to sharing genes, families often are exposed to the same environment, both physical and social, which includes lifestyle, housing, climate, and pollution (Roseboom and Painter, 2014). When discussing mental health, it is understood that mental illness results from a combination of heritable factors and environmental exposures, but the mechanisms by which these interact is not understood. The role of the social environment in the development of psychiatric disorders has been a major area of research for the past century. Of particular interest is stress, which serves as a major risk factor for psychiatric illness and can have an impact on behavioral and emotional development. Early life stress serves as not only a risk factor for psychiatric health, but also for physical health, such as cardiovascular disease and metabolic disorders, which will be discussed in detail later (Kinnally et al., 2011). As previously mentioned, the processes through which genes and environment interact are not completely understood, which is where epigenetics comes in. Genetic factors influence psychosocial outcomes, but what if psychosocial conditions could influence genetics? Epigenetics is suggesting this connection, and life experiences starting prior to conception seem to matter.

While human studies do exist, they are lacking in numbers compared with studies done with mice and rats, which have served as an excellent animal model when studying epigenetic effects. These animals can be carefully controlled, bred in large numbers, and tested for

physiological and neuroendocrinological profiles. Therefore, we can use these model systems to better understand the effects of preconception, prenatal and postnatal experience on development, specifically focusing on the neural systems that control emotional and stress related behaviors (Holmes et al., 2005). Early development is a critical period where epigenetic marks are established, and because of their stable nature they are maintained and often passed on to future offspring. I will explore the effects of stress at different stages of development, starting with preconception stress, moving to stress experienced during pregnancy, followed by early life stress. I will then discuss specific cognitive and psychological health outcomes.

During the perinatal period, gonadal and adrenal hormones affect the brain in a way that form and modify behavior, perception, and learning in individuals. Development is determined by the timing of hormone exposure along with the amount (Crews, 2010). During brain development and memory formation, regulation of gene expression is mainly controlled by epigenetic DNA modifications and chromatin remodeling (Abel and Zukin, 2008). The hippocampus is a region of the brain involved with learning and memory, and certain events can affect epigenetic processes in the neurons of this region. In response to stressful life events, the adrenal gland secretes glucocorticoid hormones. These hormones allow the organism to handle the stressful event in the best way possible by altering metabolic processes, along with other physiological processes (Lester et al., 2011). There are critical periods of brain development both pre- and postnatally where it is particularly sensitive to environmental factors. Synaptic and neuronal organization rapidly changes during these periods, and these processes are highly associated with epigenetic programming. In fact, epigenetic mechanisms play a critical role in the development of the nervous system, particularly in the regulation of synaptic plasticity. Therefore these critical periods of synaptic and neuronal organization are also characterized by

many epigenetic changes. Neuronal plasticity is greatest in the early stages of life, and the genotype and hormone activity determine how the individual will respond to future life events, and predispose the individual to future diseases (Crews, 2008).

Gräff and Mansuy (2008) define synaptic plasticity as “the ability of neuronal cells to strengthen or weaken their connections following neuronal activation” (p. 74). The idea that epigenetic mechanisms play a major role in the development of memory and cognition is substantially supported, and was first proposed by Francis Crick in 1984. Crick proposed that “memory might be coded in alterations to particular stretches of chromosomal DNA” (Crick, 1984). It was not until 2007 that DNA methylation was found to be a major brain process that controlled memory formation (Miller and Sweatt, 2007). Specifically, many studies have demonstrated the importance of histone acetylation and phosphorylation in memory and cognition (Gräff and Mansuy, 2008). Histone acetylation is a type of histone modification that is associated with transcriptional activation. Histone acetylation is catalyzed by histone acetyltransferases (HATs) and reversed by histone deacetylases (HDACs) (Lester et al., 2011). In learning and memory processes, HATS and HDACS are the best understood of the epigenetic mechanisms. Chromatin-modifying enzymes play a role in regulating gene expression, specifically in the formation of long-term memory.

Stress

The hypothalamic-pituitary-adrenal axis, or HPA axis, initiates a stress response that activates all of the body’s physiological systems when an organism encounters a stressor. When this happens, the “fight or flight” response kicks in and determines how the organism is going to respond. Some of the physiological responses associated with this system are the following:

heart rate elevation, skin perspiration, dilation of blood vessels, glycogen (which is the primary source of energy in the body), is broken down to glucose by the liver, the brain is aroused, and many other systems are activated that prepare the organism to respond to the threat appropriately (Francis, 2011). But this stress response does not apply only to single events, and if chronic stress occurs the stress response can be altered.

The HPA axis works to maintain homeostasis in the body when it is exposed to an adverse event. When an organism encounters a stressor, corticotropin releasing factor (CRF) is released, which initiates the HPA axis response to stress. The hypothalamus secretes corticotropin-releasing hormone (CRH) and vasopressin, and CRH stimulates the pituitary gland to release corticotropin (CT). CT stimulates the adrenal gland to release glucocorticoid stress hormones, such as cortisol (Zaiden et al., 2013). . When someone experiences a traumatic event or chronic stress, the HPA axis is overworked, and mental disease pathologies can result.

Preconceptual Stress

It is common knowledge that stress experienced by mothers during pregnancy can impact their fetus, but what if stress experienced by mothers prior to pregnancy could have an effect on their future offspring? And what about stress experienced by fathers, does that affect offspring? But stress is not encoded in an organism's DNA, so how is it passed on to offspring? Current research is suggesting that epigenetics is the answer.

Maternal Stress Prior to Pregnancy

Maternal stress not only during, but also before pregnancy can have an effect on offspring. Animals have been the primary model when researching stress responses, specifically

mice and rats. Shachar-Dadon et al. (2009) were interested in whether stress experienced by mothers prior to conception had a negative impact on progeny. The researchers first divided the female rats into two groups: rats that did not experience stressors (the control) and rats that were exposed to stressors for one week, termed preconceptional stress (PCS). Within the group of rats that were exposed to stressors, some were mated immediately following the week of stressors, while others were mated two weeks following the week of stressors. All of the offspring were raised normally into adulthood. The researchers reported that preconceptional stress in female rats altered the emotional, learning, and social behavior of their offspring, and these effects were sex-dependent and persisted into the offspring's adulthood. The female offspring were less sociable and more fearful, anxious, and cognitively impaired, while the male offspring were less fearful and sociable, and displayed more avoidant behaviors. What is particularly interesting about this study is the finding on the timing of adversity in relation to conception. The closer the proximity of the stress and conception, the greater the effects on the offspring, while the greater the time period between the adversity and conception, the more reduced the effects on offspring were. This study is one of many that support the idea that a woman's life experience prior to conception can be predictive of her future offspring's health outcomes (Shachar-Dadon et al., 2009). One might argue that the stressful experience altered the mother's ability to care for her future offspring, which negatively affected their behaviors, but what if studies exist that demonstrate that observable differences exist in the germline and cells of the offspring? The next discussed study did just this.

Corticotropin releasing factor type 1 (CRF1) is a key factor in the stress response, and therefore serves as an interesting focus of research when investigating epigenetic effects of stress. Zaidan et al. (2013) conducted a study on female rats where they exposed the subjects to a mild stressor for one week, which they term prereproductive stress (PRS), and then mated the

stressed females and their non-stressed controls with non-stressed males. Changes in CRF1 expression in both the brain and ova of the stressed female rats, along with the brain of the neonatal and adult offspring were studied. Additionally, the researchers observed behavioral changes in the adult offspring. The results demonstrated a slight increase in CRF1 messenger RNA expression in the frontal cortex of the mother's brain, and a significant increase in CRF1 mRNA expression in the mature oocytes of chronically stressed female rats. CRF1 expression was also increased in the neonatal offspring of stressed mothers, and these offspring demonstrated sex differences in CRF1 messenger RNA expression and behavior in adulthood. In adulthood, the offspring of stressed mothers displayed sex-specific behavioral patterns in anxiety and fear learning tests. The existence of altered CRF1 expression in the ova of stressed females demonstrates a potential mechanism of transgenerational transmission. Additionally, CRF1 expression was altered in the offspring at birth, suggesting that the stress effects were not a result of altered maternal nurturing (Zaidan et al., 2013).

The two previously mentioned studies focused on maternal stress in rats, but there are also relevant human studies. An interesting group of individuals to study is the offspring of people exposed to major traumatic events in history, such as the Holocaust or genocides in Rwanda, Nigeria, Cambodia, Armenia, and Yugoslavia. Research done on the offspring of survivors of these horrific events has shown that these groups of people display specific psychopathological symptoms (Gaisler-Salomon, 2014). One may ask if this is the result of poor parenting, as often survivors are left with their own set of psychological problems that could disrupt parenting, which then leads to disordered behavior in their offspring, which in turn negatively affects the following generation of offspring. Or, rather, is it that you can inherit stress effects from your parents biologically? There have been many studies in the field of biological psychology done to address this question.

A series of studies have demonstrated that the offspring of women who experience traumatic life events, such as the Holocaust or the attack on the World Trade Centers, have altered cortisol levels (Yehuda et al., 2005; Yeduda et al., 2007). Posttraumatic stress disorder (PTSD) is an anxiety disorder that can develop following a traumatic event. Symptoms can include avoidance behaviors, intrusive memories and flashbacks, negative mood and cognitions, and alterations in arousal and reactivity (American Psychiatric Association, 2013). It is important to note that not everyone that experiences a trauma develops PTSD. Yehuda et al. (2005) studied pregnant women who developed PTSD as a result of exposure to the attack on the World Trade Centers on September 11, 2001. These researchers were interested in the cortisol levels of the mothers and their infants, as decreased cortisol levels have been found to be associated with vulnerability to PTSD. Yehuda et al. (2005) observed decreased cortisol levels in both the mothers and infants who were exposed to the attack on the World Trade Centers during pregnancy when compared with controls. The most significant finding was that the low cortisol levels were most apparent in the infants whose mothers were exposed during their third trimester (Yehuda et al., 2005). Yehuda et al. (2007) did a similar study in the offspring of Holocaust survivors. These researchers found that in the offspring of parents who developed PTSD from exposure to the Holocaust, cortisol levels were lower, even though they were not directly exposed to the traumatic event (Yehuda, 2007).

Paternal epigenetic inheritance

In a New York Times article titled “Why Fathers Really Matter,” Judith Shulevitz (2012) discusses the political nature of pregnancy: once a pregnancy becomes public knowledge, everyone seems to have something to say. Women are continuously told what they cannot do during the pregnancy, but there doesn’t seem to be much of a focus on the father’s role in all of

this. But epigenetic research is suggesting that the life experiences of fathers prior to conception can have an effect on future offspring, an idea that seems far-fetched.

An example of this is a study done on male rats: Male rats exposed to stress early in life had altered DNA methylation patterns in sperm, which led to irregular behavior in offspring through adulthood (Franklin et al., 2010). Another study that demonstrated similar results was conducted by Dietz et al. (2011), who wanted to explore the paternal transmission of stress-induced vulnerability. These researchers used the chronic social defeat paradigm in male rats to do this. Adult male mice were placed in two groups: one group was exposed to chronic social defeat stress while the other group was labeled as the nondefeated control group. Both groups were mated with normal female mice. Both the male and female offspring of the defeated fathers displayed higher rates of depressive- and anxiety-like behaviors than the offspring of nondefeated fathers, though the abnormalities observed in the male offspring were more significant than the females (Dietz et al., 2012). This study demonstrated that both depressive- and anxiety-like behaviors could be transmitted from socially defeated fathers to their offspring.

In addition to paternal stress prior to conception, paternal age and nutrition prior to conception have also been discussed in epigenetic research. Paternal age at conception is a risk factor for the development of major psychoses in offspring, specifically schizophrenia. Schizophrenia is a psychiatric disorder characterized by disordered language, thought, perception, affect, and sense of self (American Psychiatric Association, 1994). Several studies have indicated that the offspring of men ages 35 and older have up to a three-fold increased risk of developing schizophrenia than those born to younger fathers. This is thought to be due to age-related DNA methylation changes (Rutten and Mill, 2009). Recent research has indicated the importance of paternal nutrition prior to conception, and this aspect will be discussed in detail in the section on nutrition.

Maternal Stress During Pregnancy

It is widely accepted that a mother's stress during pregnancy can have adverse effects on the developing fetus. During the gestational period, both the mother and her offspring are particularly vulnerable, and stressful events can negatively influence the health of both organisms. Not only does gestational stress disturb maternal care post-partum, but the brain plasticity and development of the offspring can be affected by the mother's experiences. In animal models, researchers have found that maternal care can affect the HPA stress response in offspring, and that this is mediated through changes in gene expression. Specifically, the glucocorticoid receptor (GR) gene *NR3C1* expression can be altered via epigenetic mechanisms (Liu et al., 1997; Francis et al., 1999). Oberlander et al. (2008) wanted to replicate these findings in humans, and therefore studied the effects of depression during maternal pregnancy on the developing fetus, and found that these effects appeared to be transmitted to the infants. These researchers studied the infants of three groups women who, during the third trimester, were (1) being treated for depression using serotonin reuptake inhibitors, (2) not being treated for their depression, or (3) not exhibiting symptoms of depression. The researchers found that maternal depression and anxious mood during the third trimester was associated with increased methylation of *NR3C1* at a specific binding site in her offspring at three months old. This increased methylation was also associated with increased salivary cortisol stress responses. The researchers concluded that the *NR3C1* is particularly sensitive to prenatal maternal depression and anxiety, and that their findings demonstrate a link between mood and an altered HPA stress response (Oberlander et al., 2008). Therefore, it is evident that the mental health of a pregnant woman is extremely important, both for her well being and the development and health of her baby.

Early Life Stress

Adverse environmental, physical, and psychosocial experiences in early life (following birth) can lead to psychological disorders in adulthood, including negative behavioral and emotional outcomes. Early life stress alters the behavioral responses of those affected, and has significant effects on the hypothalamic-pituitary-adrenal axis (Franklin et al., 2010). It is not new information that stress experienced early in life has long-term consequences. What is not understood is how exactly stressful exposures influences an organism, and why some organisms react in maladaptive ways to stressors. Studies on rodents, primates, and humans have demonstrated a link between poor maternal care and maladaptive behaviors in offspring (Franklin et al., 2010). The field of epigenetics suggests a plausible pathway by which these social interactions impact mental health (Toyokawa et al., 2012). Rodent studies have shown that maternal behaviors are predictive of offspring's health and behaviors: licking and grooming offspring typically leads to calm and brave offspring, while offspring that are neglected early on tend to be hyperactive and more nervous (Darnaudéry and Maccari, 2008; Henry et al., 1994; Zuena et al., 2008). It is thought that these differences are linked by patterns in the brain, specifically altered DNA methylation patterns of particular genes in the hippocampus. Research has demonstrated that these epigenetic mechanisms are maintained through adulthood, and play a role in the etiology of major psychiatric disorders.

Maternal separation and poor maternal care are risk factors for the development of mood and anxiety disorders (Franklin et al., 2010). The rat maternal separation (MS) model was created in order to assess the benefits and consequences of certain maternal behaviors on offspring. Throughout all of the variations of the MS, the outcomes are always similar: rat pups separated from their mothers are at a higher risk of developing social deficits along with depressive and anxiety-related behaviors in adulthood, and these effects are permanent and

long term, continuing across generations (Holmes et al., 2005; Mashoodh and Champagne). Franklin et al.'s (2010) study is an excellent example of this; they investigated the impact of early life stress and maternal factors on future generations, specifically the effect of maternal separation on multiple generations of offspring. The first generation (F1) was split into two groups: the control group and the MSUS group. MSUS stands for unpredictable maternal separation and unpredictable maternal stress, and this group was exposed to three hours of stress and separation from their mothers from day 1-14 postnatal. Then the F1 males (both stressed and controls) were mated with females that were not stressed, and then removed from these pregnant females. The male offspring produced (F2 generation) along with controls were bred with nonstressed females to produce the F3 generation. Franklin et al. (2010) found that chronic maternal separation during early postnatal life lead to depressive-like behaviors in adulthood, along with abnormal responses to normal and aversive environments. Additionally, these depressive-like behaviors and altered responses to stressful environments were expressed in the next generation, the F2 generation, despite the fact that this generation was raised in a normal setting without stress or maternal separation. The results demonstrated these effects were transmitted through multiple generations, even when offspring were raised in a normal environment. The researchers also found that the effects were sex-specific: males that were subjected to maternal separation and maternal stress transmitted these effects through the germline and their male offspring exhibited depressive behaviors. The researchers examined the DNA methylation levels in the promoter of several candidate genes in the sperm of males from the first generation exposed to maternal stress and separation. The candidate genes chosen were either associated with the epigenetic regulation of gene expression or depression and emotional behavior. For example, they looked at the gene that codes for MeCP2, methyl CpG binding protein 2, which is a transcriptional regulator that binds methylated DNA. MeCP2 is

involved in the etiology of the cognitive disorder Rett Syndrome, which will be discussed in detail later. MeCP2 is also involved in the stress response, and when expression of MeCP2 is decreased, the stress response increases (Franklin et al., 2010; Urdinguio et al., 2009). In Franklin et al.'s (2010) study, in the sperm of the F1 MSUS males, at the transcription initiation site of MeCP2 there was an increase in the methylation of the CpG island. On the other hand, the stress hormone receptor CRFR2 (corticotropin releasing factor receptor 2) had decreased methylation patterns at the CpG island. These results indicated that DNA methylation was altered in multiple directions at different sites specific to genes, and suggests that transmission of stress vulnerability through multiple generations can be mediated through the male germ line. "These provocative findings suggest that environmental perturbations can lead not only to lifelong behavioral adaptations for the individuals who experience such challenges, but might also alter behavioral responses of future generations who were not directly exposed to the trauma per se" (Dietz et al., 2011).

Cognitive and Psychiatric Disorders

Over the past century, a great deal of research has linked the social environment with incidence of many mental illnesses. Examples of these relationships on a large scale include the following: natural disasters such as hurricanes and earthquakes are associated with the development of posttraumatic stress disorder (PTSD), schizophrenia incidence is higher among inner city environments compared with more rural areas in Western societies, and increased labor stress places people at a greater risk of major depressive disorder (MDD). On a smaller scale, social factors and relationships can lead to the development of eating disorders (Toyokawa et al., 2012). Additionally, there are significant gender differences among psychiatric

disorder incidence rates: women are at a higher risk of developing eating disorders, obsessive-compulsive disorder, posttraumatic stress disorder, major depressive disorder, anxiety and panic disorders, and Alzheimer's disease and dementia, while men are at a higher risk of autism, schizophrenia, and other early onset disorders (Crews, 2010). We see a link between particular environmental events and mental health outcomes, but the pathophysiologic pathways that create this linkage are not well understood. This is where epigenetic mechanisms come in, as the mediators between these events and health outcomes. We have already discussed how psychiatric disorders result from a combination of both environmental and genetic factors (Sweatt, 2009), and how stressors present during early development can have a serious impact on emotionality and stress responsivity, leading to the development of disease. Toyokawa et al. (2012) asks how exactly the social environment "gets into the mind"? How do environmental factors affect individuals and ultimately result in pathophysiological outcomes? Most epigenetic research has focused on its role in cancer, but recent evidence is suggesting an important link between epigenetic regulation and mental health outcomes.

Cognitive dysfunctions

"Environmental influences continuously bombard neurons, and in principle, the inputs are transduced to the nucleus, activating epigenetic mechanisms and thus reprogramming gene activity" (Balazs et al., 2011, p. 1182).

Epigenetic regulation is very important to normal nervous system development, therefore disorders of synaptic plasticity and cognition are often characterized by epigenetic dysregulation. Specifically, epigenetic mechanisms have been found to be associated with many common neurodevelopmental, neurodegenerative, and psychiatric illnesses. Some of these disorders and diseases include mood disorders such as depression and anxiety,

neurodegenerative disorders such as Parkinson's disease (PD) and Huntington's disease, and lastly neurodevelopmental diseases including Rubinstein-Taybi syndrome (RTS), Fragile X syndrome, and Rett syndrome (RS). Cognitive dysfunctions are very difficult to understand because of the complex interactions between many factors. Studying patients with cognitive disorders has given insight into these interactions, and have identified epigenetic mechanisms as the underlying theme. That being said, the reversibility of these mechanisms is promising for future prevention and treatment plans.

Neurodevelopmental Disorders

Fragile X Syndrome (FXS)

Fragile X syndrome (FXS) is a common heritable disease that occurs about 1 in 8000 women and 1 in 4000 men in the United States (Gräff and Mansuy, 2008). This disease is characterized by chromosome instability, and symptoms involve intellectual disabilities, including mental retardation and learning deficits. At the 5'-end of the gene encoding fragile X mental retardation protein 1 and 2 (FMR1, FMR2), the trinucleotide repeats CGG (in FMR1) and CCG (in FMR2) are abnormally expanded in individuals with FXS (Ashley et al., 1993; Gecz et al., 1996). These trinucleotidic repeats within the genes result in an increase in methylation and therefore gene repression. These alterations lead to nervous system flaws and result in cognitive impairment (Gräff and Mansuy, 2008).

Rett Syndrome (RS)

Rett syndrome is another cognitive dysfunction characterized by intellectual disabilities. Rett syndrome is an X-linked developmental disease and is one of the most common causes of mental retardation among women. In individuals with Rett syndrome, methyl CpG binding

protein 2 (MeCP2) is disrupted, and this protein binds to methylated DNA (Gurdinguio et al., 2009). Girls with Rett Syndrome develop normally in their first few months, but begin showing signs of the disease in months 6-18. They begin to lose functioning in their hands, and the disease progresses to an autistic period, followed by a loss of cognitive and motor functioning. Additionally, these individuals digress in their abilities to socially interact, often developing neurological and psychological symptoms. Eventually they become severely mentally disabled, with motor deficiencies including tremors and ataxia. Health problems including apnea, which is a cessation of breathing, hyperventilation, and seizures can also occur. While it is more common among women, men can also have Rett syndrome, and typically these cases are even more severe (Gurdinguio et al., 2009; Tsankova et al., 2007). Recent studies with mice demonstrate that the reversal of an MeCP2 deficit can reverse many of the symptoms (Guy et al., 2007). These findings suggest that Rett syndrome can be treated in humans by reversing the mutation in MeCP2, even after symptoms are present (Tsankova et al., 2007).

Rubinstein-Taybi Syndrome (RTS)

Rubinstein-Taybi Syndrome (RTS) is a rare congenital (from birth) disorder, and individuals with this syndrome have skeletal abnormalities, a short stature, particular facial features, and exhibit mild to severe mental retardation. Rubinstein-Taybi syndrome accounts for approximately 1 in 300 patients with mental retardation (Sweatt, 2009). People with this disease can have deficiencies in postnatal growth, followed by extreme weight gain around puberty. This disorder occurs because of autosomal mutations in the gene that codes for CBP, which stands for CREB binding protein. (Gräff and Mansuy, 2008; Tsankova et al., 2007) and the dysfunction in this syndrome revolves around a histone acetyltransferase (HAT) (Gurdinguio et

al., 2009). Therefore, a potential treatment strategy could be the use of histone deacetylase (HDAC) inhibitors to reverse the cognitive deficits, (Abel and Zukin, 2008).

These studies have demonstrated that a disruption of epigenetic mechanisms is associated with cognitive dysfunctions, and the reversibility of these alterations demonstrates the potential for future therapy.

Neurodegenerative Disorders

Neurodegenerative disorders include Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS), and these three diseases affect several million people worldwide (Migliore and Coppede, 2010). Additionally, Huntington's disease is a progressive neurodegenerative disorder characterized by major impairments.

Alzheimer's disease (AD)

Alzheimer's disease (AD) is a progressive neurodegenerative disease, and it a form of dementia that interferes severely with everyday life by affecting memory and cognitive functioning. The prevalence of AD is very high, with approximately 1 in 100 cases in the US (Gräff and Mansuy, 2008). Gene mutations account for only about 5% of Alzheimer's cases (Balazs et al., (2011). Aging is a major risk factor for Alzheimer's disease, and methylation changes have been observed in aging tissues (Balazs et al., 2011), suggesting epigenetic influences. Additionally, studies have shown that Alzheimer's is characterized by abnormal CBP (CREB binding protein) activity, which possess histone acetyltransferase (HAT) activity. Histone acetylation modifies the structure of chromatin, thereby altering transcription. Therefore, the application of an HDAC is a potential treatment option.

One group of researchers examined a rare set of monozygotic twins that were discordant for Alzheimer's disease (AD) because of the potential to reveal the role of epigenetic mechanisms in the development of AD, as these modifications can lead to differing disease states in monozygotic twins. One twin was identified as neurologically normal and non-demented (ND) and died at age 79 from prostate cancer, while the other was diagnosed with AD at age 60, and his cognitive functioning progressively declined over the next 16 years until his death at age 76. Both men had identical education backgrounds and were chemical engineers, but the AD twin was constantly exposed to pesticides at work, while the non-AD twin had a different work environment where he was not exposed to pesticides. The researchers observed significant DNA methylation pattern differences between the two men: the AD twin had much lower levels of DNA methylation in temporal neocortex neuronal nuclei. These results support their hypothesis that epigenetic mechanisms may mediate the effects of life experiences on Alzheimer's disease risk. In this case, exposure to toxic substances (pesticides) was the differing environmental exposure. These results also explain why genetically similar individuals can vary in Alzheimer's disease states (Mastroeni et al., 2009).

Huntington's disease (HD)

Like Parkinson's disease, Huntington's disease (HD) is a progressive neurodegenerative disease characterized by major cognitive, psychiatric, and motor deficits. Motor deficits include uncontrollable movements (Gräff and Mansuy, 2008). Huntington's disease is an autosomal dominant trait, which means that this disease results from a single gene mutation, and it is characterized by a late-onset, meaning that the symptoms begin later in life. HD is caused by a mutation in the *huntingtin (htt)* gene, resulting in a high number of CAG repeats, creating a polyglutamine extension of the huntingtin protein, ultimately dysregulating

transcriptional activity (Gräff and Mansuy, 2008). The motor deficits and cognitive decline are associated with decreased histone methylation and increased histone acetylation, which suggests the importance of histone modifications in this disease. Recent mouse models demonstrate that these alterations can be reversed through the use of HDAC inhibitors, suggesting a potential future therapy for humans (Ferrante et al., 2003; Hockly et al., 2003; Gräff and Mansuy, 2008).

Psychiatric Disorders

Psychiatric disorders include schizophrenia (SZ), bipolar disorder (BD), and major depressive disorder (MDD). Bipolar disorder and major depressive disorder are episodic in nature, meaning that they may occur and remit spontaneously. This characteristic signifies that these diseases are not entirely genetic because typically, purely genetic diseases persist throughout the life course. Genome-wide studies demonstrate the existence of hundreds of mutations in genes associated with these disease states, but the contribution is roughly only 1-2% of the cause (Abdolmaleky et al., 2013).

Monozygotic twin discordance, parent-of-origin effects, sex effects, and late age of onset are a few characteristics of psychiatric disorders that also coincide with epigenetic dysregulation (Ptak and Petronis, 2010). It is accepted that schizophrenia and bipolar disorder have a genetic basis, but no single gene has been found to be the cause of these illnesses. Patients with psychiatric disorders, along with animal models of these disease states, have specific pathophysiological changes in the brain that include “gross differences in the sizes of specific brain regions, alterations in the morphology of subpopulations of neurons, neurochemical changes at the synaptic cleft, alterations in intracellular signaling” (Tsankova et al., 2007, p.355), and most importantly to us, changes in the regulation of gene expression.

Schizophrenia (SZ)

Schizophrenia is the most disabling mental disorder, characterized by major impairments in fundamental human functioning. The brains of schizophrenic individuals are abnormal in both structure and function, and these irregularities result in impaired cognitive and emotional functioning. Language, thought, affect, perception, and sense of self are all human attributes that are affected (American Psychiatric Association, 1994). Schizophrenia is characterized by delusions, hallucinations, erratic behavior, and inappropriate emotional responses. Over time, social and occupational functioning deteriorates (Gräff and Mansuy, 2008). Schizophrenia has a 1% prevalence rate worldwide (Gräff and Mansuy, 2008), and in Western countries rates are higher in inner city and urbanized areas (Pedersen and Mortensen, 2006). Explanations for these higher rates in urban areas include nutritional intake, social isolation, and drug abuse (Toyokawa et al., 2012).

In research exploring epigenetic mechanisms in mental health, schizophrenia has shown the strongest associations between specific gene irregularities and disease outcomes. There is a gene labeled as *RELN* that codes for the protein reelin, and this gene contains many CpG dinucleotide repeats in the promoter region, making it highly susceptible to epigenetic regulation, specifically by DNA methylation. Hypermethylation of the DNA decreases the expression of the *RELN* gene, and evidence has shown a link between a downregulation of the protein reelin and schizophrenia, suggesting the role of epigenetics in the etiology of this disease (Tsankova et al., 2007). For example, Abdolmaleky et al. (2005) showed that DNA hypermethylation-mediated hypo-expression of *RELN* in the frontal lobe was associated with schizophrenia. Additionally, histone acetylation has also been linked to modification of *RELN* expression (Gräff and Mansuy, 2008). Because of the complex nature of the disease, it is highly

likely that epigenetic regulation of other genes is involved in disease causation, but these genes have yet to be discovered and therefore more research in this area must be done.

In addition to the *RELN* gene, the dopamine receptor subunit 2 (DRD2) (Petronis et al., 2003) and the catechol-o-methyltransferase (*COMT*) gene (Abdolmaleky et al., 2006) have also been shown to have a relationship with schizophrenia. Catechol-*O*-methyltransferase (*COMT*) is a gene that regulates homeostatic levels of dopamine, and in the nervous system, dopamine is a neurotransmitter that plays a critical role in our mental health. Abdolmaleky et al. (2006) studied how methylation patterns in the promotor region of membrane-bound catechol-*O*-methyltransferase (*MB-COMT*) has an effect on activity, and how this along with the *COMT*Val158Met polymorphism has an effect on risk for schizophrenia and bipolar disorder. These researchers studied 115 post-mortem brain samples of the frontal lobe. They found that the *MB-COMT* promoter DNA is hypomethylated in schizophrenic and bipolar disorder patients when compared with controls, specifically in the left frontal lobes. Their findings suggest that “*MB-COMT* over-expression due to promoter hypomethylation and/or hyperactive allele of *COMT* may increase dopamine degradation in the frontal lobe providing a molecular basis for the shared symptoms of schizophrenia and bipolar disorder” (Abdolmaleky et al., 2006). Therapeutic agents that could reactivate gene expression could potentially serve as treatment options for patients diagnosed with schizophrenia. Examples include inhibitors of DNMT1 (DNA methyltransferases) or HDACs (Tsankova et al., 2007).

The previously discussed cognitive and psychiatric illnesses are all linked with epigenetic alterations. There is substantial evidence indicating the environment’s role in these changes, whether internal or external factors, and these agents can include stress, nutrition, pollution, etc. The organism’s systems receive the information from the environment, adapt to it, and

while these changes may help the organism survive in the short term, they can become detrimental in the long term, in some cases impacting multiple generations of progeny. It is generally accepted that this epigenetic reprogramming can occur in both the maternal and paternal germline and therefore be passed through generations, but what is not understood is the extent to which these alterations affect the organism, and how often these modifications occur (Tollefsbol, 2014). With this new information comes an increased interest in developed methods to prevent, diagnose, and treat diseases that could be transgenerationally inherited. Genetic marks cannot be reversed, but epigenetic marks can, and there are variety of therapeutic agents that can potentially do this, including hormones, enzymes, vitamins, nutrients, and drugs (Abdolmaleky et al., 2013). DNA methyltransferase (DNMT) and histone deacetylase (HDAC) inhibitors are currently being tested in clinical trials as therapy for epigenetically based diseases (Tollefsbol, 2014).

Chapter 2: Physical Health Outcomes

Nutrition

Many experimental studies have demonstrated that adverse phenotypes later in life can be explained by epigenetic changes early in life. Of particular interest is the maternal diet during pregnancy, as this can have major impacts on the embryo's development and future health. One of the most famous epigenetic studies on nutrition was conducted by Waterland and Jirtle (2003) on agouti mice. These researchers demonstrated the effects of maternal diet during pregnancy on DNA methylation of certain genes in mice, which produced altered phenotypic outcomes in offspring such as body weight, coat color, and blood pressure. The researchers provided the mice with a diet rich in folic acid, vitamin B₁₂, choline, and betaine, which are considered methyl donors, and this supplementation induced an increase in methylation, which resulted in a shift in the distribution of coat color (Waterland and Jirtle, 2003). On the other hand, paternal diets are also important, which is not an intuitive thought. The Economist published an article titled "Poisoned Inheritance" (2013) that addresses the importance of paternal diets. Women are encouraged to eat leafy, green vegetables during pregnancy because they contain the important vitamin B9, or folate, which is necessary for proper neural tube development of the embryo. But now studies are looking into the diets of fathers, and finding that the fathers' consumption of folate is equally important to their future offspring's development. If the proper amount of folate is not consumed when sperm is forming, the offspring formed can have serious deficiencies. Some of the more serious outcomes included head, spine, and limb deformities (Poisoned Inheritance, 2013). And so you might ask how exactly the amount of folate in a father's diet is influencing his offspring? What is the mechanism by which this occurs? Epigenetic modification is the answer. Folate acts by

regulating DNA methylation, and as previously mentioned epigenetic modification functions through methylation. In addition to the effects of maternal and paternal diet on the first generation of offspring, epigenetic research demonstrates that multiple generations can be affected, and these findings will be discussed in detail below.

Specific Health Outcomes

Cardiovascular Disease

In the field of research linking epigenetics and disease pathologies, cancer has been the major focus. In 2010, a PubMed database search of “epigenetics and cancer” revealed over 10,000 results, while “epigenetics and cardiovascular” only revealed one tenth of this number (Ordovás and Smith, 2010). But cardiovascular health demands more attention, as cardiovascular disease (CVD) is the leading cause of death worldwide (Zullo et al., 2014,). The World Health Organization (WHO) (2015) defines cardiovascular disease (CVD) as a group of disorders that affect the heart and blood vessels. Some of the diseases of this group include coronary heart disease, which affects the blood vessels that supply the heart, cerebrovascular disease, which affects the blood vessels that supply the brain, and peripheral arterial disease, which affects the blood vessels that supply the arms and legs. The events most commonly associated with CVD are heart attacks and strokes. Over the past few decades, CVD mortality rates have decreased, but not because of prevention strategies; rather, we have been able to develop treatment plans and improve quality of care. Until recently, coronary heart disease, along with many other diseases, were understood to be caused by immediate risk factors and fetal programming. The most common risk factors associated with CVD risk include physical inactivity, tobacco use, alcohol use, and unhealthy diet (WHO, 2015). Risk factors have been the

primary focus of preventative care research, as identification of these factors has created a target for therapy. Knowing the risk factors associated with CVD, researchers and physicians can attempt to reduce mortality and morbidity of the disease by decreasing exposure to these risks. Examples include increasing physical activity, sustaining a healthy diet, and quitting smoking. But these risk factors, both genetic and environmental, cannot account for the variability in overall CVD risk, and we are therefore looking for other factors that can explain individual differences. Epigenomics has become a promising area of research in the development of cardiovascular disease. Once we expand our knowledge base in this field, we will be able to develop more effective preventative strategies and therapeutic interventions in the treatment of disease.

If unhealthy lifestyles and genetic factors do not account for the higher presence of CVD in certain geographical areas along with the changing incidence, then what does account for these differences? Geographical studies gave the first insight into the idea that adult disease could be programmed in utero. The Barker hypothesis, also known as the “fetal origins hypothesis”, was developed following influential epidemiological studies conducted by David Barker and colleagues based on information provided by birth and death records. This information indicated a strong correlation between birth weight and rates of ischemic heart disease, also known as coronary heart disease (CHD) or coronary artery disease (CAD) (which as previously mentioned is a disease of the blood vessels that supply the heart), related death, all of which appeared to be related to the prenatal environment (Barker and Osmond, 1986;Dover, 2009; Wadhwa et al., 2009). This hypothesis posits that *in utero*, undernutrition alters the body’s structure, function, and metabolism, and these changes can lead to CHD decades later. This phenomenon is known as fetal programming, which refers to the long-term (or permanent) effects of a stimulus present during a critical period (Lucas, 1991). This “fetal programming” of

disease has been a popular research topic in diseases ranging from diabetes (Hales et al., 1991) to schizophrenia (Hultman et al., 1999). The Barker hypothesis was recently renamed the “developmental origins of health and disease” (DOHaD), as it was discovered that developmental plasticity persists into the postnatal period (Waterland and Michels, 2007). The “developmental origins hypothesis” indicates that environmental exposures (including nutrition) during critical periods of development (both prenatal and postnatal) influence developmental pathways, leading to changes in metabolism and disease susceptibility, and that these early alterations are permanent (Waterland and Michels, 2007). Permanent means that these early alterations persist throughout the lifespan: “Once established during development, epigenetic mechanisms are in most cases maintained with high fidelity throughout life” (Waterland and Michels, 2007, p. 374). Substantial research on both human and animal models supports this hypothesis, but the biological mechanisms that make these changes in structure, function, and metabolism occur are poorly understood.

While Barker may have been to first, he certainly was not the last to reveal how environmental factors during prenatal and postnatal life can have effects on metabolic pathways, signals within cells, and interactions between cells (Dover, 2009). These effects are not limited to the cellular level; they place individuals at an increased risk of developing a childhood and adult disorders. Therefore the chain of events is as follows: systems of the body respond to the environment in utero by changing structure, function and metabolism. In particularly adverse environments, such as famine, specific patterns of interaction between genes are established in order to control cellular and organ functioning to survive the adverse environment. But, these changes can place the individual at increased risk of developing disease in adult life if the postnatal environment does not match that of the prenatal life (Dover, 2009).

Throughout history, populations of people have encountered periods of severe environmental change, such as famine or plague, that result in major adverse effects on health. Therefore, these periods serve as excellent sources of information when investigating how people respond to adverse environments, and what the long-term consequences are. An example is the Dutch Hunger in WWII during the 1944-1945 winter. This occurred because the Dutch government called a national railroad strike in order to prevent German military initiatives, but in response the Germans cut off all food supply to the western part of the Netherlands. This resulted in widespread starvation, with people surviving on as little as 500 calories per day. Researchers have taken a particular interest in this event to explore the effects of famine on development, and discovered a variety of health outcomes (Heijmans et al., 2008; Roseboom et al., 2006). People exposed to this famine *in utero* had higher rates of adverse metabolic outcomes, and mental health phenotypes, such as decreased cognitive functioning, more than half a century later. These outcomes were sex-specific, meaning they depended on the sex of the individual, and the timing of exposure to the famine was also important. Specifically, the younger the embryo was, the more vulnerable it was; therefore, those who experienced the famine closer to conception were affected to a greater extent. More than half a century later, the individuals whose mothers experienced the famine early in fetal development were at an increased risk of dyslipidemia, obesity, and deaths related to cerebrocardiovascular events, and these effects were also sex-specific. Women had greater body mass index (BMIs) and waist circumference, while men had high cholesterol and HDL (high density lipo-protein) levels, which are associated with cardiovascular health (Ordovás and Smith, 2010). Heijmans et al. (2008) also studied individuals that were exposed to the Dutch hunger, and found that people exposed to this famine *in utero* displayed altered DNA methylation patterns in genes that are linked to metabolic and growth related diseases, (recall the imprinted IGF2 gene), when

compared with controls not exposed to the famine (but were biologically related and the same sex), or exposed to the famine later in gestation. These DNA methylation changes were persistent, as they were observed in adulthood. Roseboom et al. (2006) found that *in utero*, individuals who experienced famine during specific trimesters had specific health outcomes later in life. Those who were exposed to the famine in the first trimester experienced poor cardiovascular health later in life. Those exposed during the second trimester had problems with lung and kidney function, and with exposure in the third trimester, individuals tended to have glucose intolerance later in life.

These approaches to CHD identify undernutrition *in utero* as the major determining factor in disease development, but epigenetic inheritance suggests an additional component. What if the determining factors of CHD could originate in our ancestors, rather than at the start of our lives? What if our risk for developing disease is predetermined even prior to conception because of what our ancestors experienced, and not because of Mendelian inheritance? Epigenetics encompasses the importance of nutrition during development, but also stresses the importance of our ancestors' history.

In addition to effects of *in utero* exposure, there are other critical windows following birth where adverse environments can have effects on future offspring of those exposed. In the growth velocity of children, the slow growth period (SGP) is a time period right before the prepubertal peak, approximately ages 8-12. This period has been significant to the field of epigenetics, as studies have demonstrated a link between nutrition during this period and health outcomes later in life and in future generations, as metabolic functioning is particularly vulnerable during this time (Pembrey et al., 2006). For example, food availability during the SGP of the paternal grandfather has an effect on the life expectancy of his grandchildren; food scarcity lengthens the life expectancy while food abundance shortens it. In regards to

cardiovascular mortality, children whose fathers had a limited access to food during their SGP are at a decreased risk. During the SGP of paternal grandfathers, those who had access to an abundance of food were at a much higher risk of developing diabetes; specifically, the offspring had a fourfold excess mortality due to diabetes. On the other hand, fathers who are exposed to an abundance of food during their SGP had a lower risk of diabetes; thus, food availability during the SGP seems to serve as a protective factor against diabetes in offspring (Kaati, 2010). These effects are transgenerational, as the modifications were transmitted through the germline and persisted despite the disappearance of the initial environmental factor (food availability).

In northeastern Sweden, there is a small, secluded town known as Överkalix. This area has been particularly interesting to study because of food availability trends based on its location, which was cut off from the rest of the world during the early twentieth century. Several groups of researchers have demonstrated a relationship between the availability of food during the grandpaternal's (F0) SGP, and cardiovascular and diabetes mortality rates in the grandoffspring generation (F2) (Kaati et al., 2002; Bygren, 2013). When harvests were successful, people over-ate, and when they were not, people starved. Looking at pre-pubescent boys who lived in this region during times of starvation or overabundance of food, researchers found that the offspring of boys who ate too well had higher rates of diabetes, while the offspring of boys who starved had lower rates of heart disease. These results were persistent across at least two generations. As previously discussed, epigenetic effects have been found to be sex-specific. In this case this means that the effects of the paternal grandfather's food supply were only on the mortality of sons, while the paternal grandmother's food supply only had effects on the granddaughter's mortality. According to Kaati (2010), "This indicated the existence of a direct biological transgenerational effect, an epigenetic inheritance with the sex-

specific patterns of transmission suggesting a direct role for the Y chromosome and possible X chromosome” (p. 63).

Diabetes

Positive natural selection pertains to the selection of beneficial traits in a population. A trait that is beneficial to the organism by increasing survival and reproductive rates is passed on to offspring and is said to be under positive selection. But a phenotype that is beneficial under certain circumstances may be detrimental under others. This is known as the “thrifty gene hypothesis”, and was proposed by James Neel in 1962 (Neel, 1962). The hypothesis was developed to explain why some populations of people are more at risk of developing diabetes than are others. Neel hypothesized that people with diabetes have certain genes with allelic variations that historically allowed them to effectively utilize food, but in our modern day world these genotypes are harmful. These “thrifty” genes would be helpful in times of food scarcity, but in our modern developed world they predispose individuals to obesity and diabetes (Ordovás and Smith, 2010).

Diabetes is a major global health problem, with approximately 347 million people diagnosed with diabetes worldwide in 2010 (Danaei et al., 2011). It is predicted that by 2030, diabetes will become the seventh leading cause of death. This disease is characterized by high blood glucose levels, which occurs because the body either does not produce enough insulin, and/or the surrounding tissues are unable to respond properly to insulin. What makes this disease so alarming is its relationship to other diseases; specifically, it places individuals at a much higher risk of developing other high mortality diseases. These include hypertension, cardiovascular disease, and many cancers. When compared with the healthy population, the risk of mortality doubles among patients with diabetes (Jiménez-Chillarón et al, 2014).

In the late 1970s, Singhal et al. (2003) started a clinical trial where preterm babies were fed either a normal diet or a diet high in protein and carbohydrates. The diets were assigned randomly, and were administered for four weeks, or until the baby reached 2000 grams. Following the assigned diet for four weeks, the babies were fed however their parents decided. Twenty years later, the insulin levels of these people were assessed. Babies who were fed high fat and carbohydrate diets had increased pre-insulin levels, and therefore were at an increased risk of developing type II diabetes (Dover, 2009). As previously mentioned, this is an example of the thrifty gene hypothesis. Singhal et al. (2003) discusses how in adverse environments, the fetus responds by diverting the limited blood or nutrient supply to the more essential organs at the cost of other organs. In this case, tissue insulin levels are altered in a way that is adequate to regulate glucose homeostasis if the individual remains in a nutrient-poor environment, but harmful and places the individual at an increased risk of developing non-insulin-dependent diabetes later in life if exposed to an excess of nutrients. This study demonstrates that low nutrient diets along with slow growth in early postnatal life actually serve as protective factors against the development of harmful metabolic disease later in life (Singhal et al., 2003). These findings suggest that we need to reevaluate the current recommendations for the prenatal and postnatal diet. Nutrition is a controllable factor, and therefore would serve as an excellent target of therapy.

Chapter 3: A paradigm shift or tweaking the details?

I have now discussed how environmental factors, specifically stress and nutrition, influence the epigenome, and how these epigenetic alterations can be passed through generations, ultimately influencing offspring development and disease. Both stress and nutritional environments can influence the development of a range of mental and physical health outcomes, including but not limited to: neurodevelopmental disorders such as Fragile X Syndrome and Rett Syndrome, neurodevelopmental disorders such as Alzheimer's disease and Parkinson's disease, psychiatric disorders such as depression and PTSD, and physical diseases such as cardiovascular disease and diabetes. There is a great deal of evidence that demonstrates the role of epigenetic mechanisms in disease states, and therefore it is clear that the field of epigenetics plays a major role in both the biological and medical fields, but does this mean we are currently experiencing a paradigm shift? Prior to determining whether or not epigenetics represents a paradigm shift in our understanding of human health and behavior, it is first necessary to define what is meant by a scientific paradigm, and what constitutes a shift. Thomas Kuhn was a very influential person in this field, and his terminology and ideas are still widely accepted today.

The Nature of Scientific Revolutions

Thomas Kuhn published *The Structure of Scientific Revolutions* in 1962. As a graduate student in theoretical physics, Kuhn was nearing the end of his graduate career when he took a course exploring the history of science, and this course drastically changed his career path from

physics to the philosophy and history of science. Kuhn was puzzled with the differences between social scientist communities and natural scientist communities. "Particularly, I was struck by the number and extent of the overt disagreements between social scientists about the nature of legitimate scientific problems and methods. Both history and acquaintance made me doubt that practitioners of the natural sciences possess firmer or more permanent answers to such questions than their colleagues in social science. Yet, somehow, the practice of astronomy, physics, chemistry, or biology normally fails to evoke the controversies over fundamentals that today often seem endemic among, say, psychologists or sociologists. Attempting to discover the source of that difference led me to recognize the role in scientific research of what I have called "paradigms." These I take to be universally recognized scientific achievements that for a time provide model problems and solutions to a community of practitioners." (Kuhn, 1962, p. x). Prior to Kuhn's publication, the view was that science progressed steadily, accumulating new ideas over time. In *The Structure of Scientific Revolutions*, Kuhn argues that science does not progress linearly, rather it is characterized by periodic revolutions, which he labels as paradigm shifts. Kuhn's main objective of his publication was to change the way different communities of people perceive and evaluate the same data. Kuhn was successful as his ideas were widely accepted, and are currently used today.

There are five main steps in the Kuhn Cycle. The first step is *normal science*, which incorporates research with a great deal of support that is based on a past scientific achievement, and this stage represents the foundation of that particular field. The next step is *model drift*, which occurs when the understanding of the field begins to drift because of new evidence that does not fit into the current model. Next is the *model crisis*, where the model is disrupted because there is a great deal of evidence that conflicts with the current model, and the current model can no longer serve as the appropriate model in the field. Following the

model crisis, a *model revolution* occurs, where new models are proposed that attempt at explaining the new evidence. This is termed as a revolution because of its extreme change from the old model. Ultimately, the paradigm change occurs, where a single new paradigm emerges and the field begins to function with the new model. When the paradigm change ends, the new model becomes the new normal science (Kuhn, 1962). Now that we understand what exactly a paradigm shift entails, let's look at the current paradigm across biological science.

The Current Biological Paradigms: Molecular, Medical, Developmental and Evolutionary

In biology, the current paradigm can be labeled as a combination of the central dogma and genetic determinism. Recall that the central dogma is the following:

DNA → RNA → Proteins

DNA is transcribed to RNA (or mRNA), which is translated into proteins, which ultimately determine the function of cells. In simple terms, genetic determinism means that genes control our behavior. DNA is the basic unit of heredity, and all genetic information is stored in the DNA sequence. A single genotype can give rise to multiple phenotypes. Current biologists agree that individual variations in phenotype can be the result of genome-environment interactions (Kitcher, 2001), and while the environment can influence phenotypes, these phenotypes are not passed on to future generations. While genetic determinism acknowledges that gene-environment interactions exist, it does not have an explanation for how exactly they occur.

Developmental biology follows the same central dogma: development occurs because of protein signals, which are created from the basic DNA sequence. The current paradigm in evolutionary biology is known as the Modern Synthesis (MS), which identifies the gene as the material

foundation of all evolutionary change, and states that the environment cannot influence the organism at the level of the gene. The evolutionary framework will be discussed in detail below.

The fields of medical science have adopted the same genetic view of life, approaching their field with a focus on specific genes in the development of disease. In biomedical research, the current paradigm is the following: genes cause disease, genes cause aging and health, and therefore developments in genetic research will successfully eliminate disease and extend the life span (Strohman, 1993). This approach in medicine is reflected in the early confidence people had in the Human Genome Project (HGP), which sought to map the entire human genome and therefore identify which specific genes are associated with particular health outcomes. But as we know, most of the common, detrimental health outcomes are the result of many interacting factors, and therefore cannot be explained using the single gene-outcome interaction.

Discussing the current paradigms in biological and medical sciences, it is clear that genetics is not the whole story in the development of health and disease.

A Paradigm Shift in Scientific Thought

In the past decade, both North America and Europe have exploded with publications on epigenetics. Epigenetic projects have been funded, such as The Human Epigenome Project (HEP), which “aims to identify, catalog and interpret genome-wide methylation patterns of all human genes in all major tissues” (Eckhardt et al., 2004). New journals specifically about epigenetics have been created, such as *Epigenomics* and *Epigenetics*. Centers for epigenetic research have been established at universities, old textbooks are being revised and new ones written, and thousands of articles on the field have been published (Allis et al., 2007; Gilbert & Epel, 2008; Landerdecker & Panofsky, 2013). Haig (2012) identifies a tenfold increase in the number of papers with the word “epigenetics” in the title. It is an understatement to say that

the interest in epigenetic research is growing, and with any new field, there is both support and criticism. The following sections will identify the current discussions, and ultimately determine if epigenetics represents a fundamental change in our thinking about health and disease.

Supporting the Epigenetic Revolution

The nature versus nurture debate was one of the most prominent in biomedical science in the twentieth century, and was “resolved” when researchers agreed that both nature and nurture play critical roles in development and that phenotypes result from a combination of both factors (Petronis, 2010). I highlight the word resolved because the scientific community agreed that these factors interacted, but did not understand how this interaction produces developmental and health outcomes. Sweatt (2013) states, “It is now clear that there is a dynamic interplay between nature and nurture. That mechanistic interface is epigenetics” (p. 624). Epigenetics is accounting for the differences among individuals that cannot be explained by the genetic code alone. Let’s first look at the major ways in which epigenetics differs from the current paradigm of genetics (Vineis, 2010; Dupras et al., 2014; Rothstein, 2013):

1. Epigenetics refers to functional, not structural, changes in the DNA.
2. Epigenetic changes are heritable.
3. Epigenetics is dependent on the environment.
4. Epigenetic changes are dynamic and reversible, as opposed to genetic changes, which are both rare and irreversible.
5. Epigenetics explains the mechanisms through which the gene-environment interaction occurs.

6. In epigenetics, continuous quantitative changes occur, which suggests that nature functions in degrees, rather than leaps like mutations. Additionally, epigenetic modifications occur at a much higher frequency than genetic mutations.
7. Susceptibility to epigenetic modification depends on the timing of exposure to an environmental agent during development, along with the dosage of the agent.
8. Epigenetic changes can be tissue-specific, meaning they only occur in one type of tissue, so differences among cell types can exist within the same organism. Genetic changes in an individual organism tend to be consistent throughout all of the tissues.
9. Epigenetic changes can be species specific, meaning an environment agent can produce specific epigenetic modifications in one species but not another.

Now that we have clearly identified the ways in which epigenetics differs from genetics, let's look at current discussions on epigenetics as a paradigm shift.

Vineis (2010) references Kuhn's *Structure of Scientific Revolutions* when identifying three ways in which a new paradigm arises: a crisis in the existing theory can occur, a new theory can be presented, or new technological advances can develop. Vineis (2010) suggests that in epigenetics, all three of these events have occurred, resulting in a shift from genetics to epigenetics. Initially, the theory of epigenetics was presented by Conrad Waddington when genetics was booming, then the field of genetics showed signs of crisis (in that it could not account for the variability of phenotypes), and finally technological advances were made that allowed researchers to explore epigenetics in both animal and human models. Because of the recent launch of genome-wide association studies, it may not seem like the field of genetics could be experiencing a crisis, but there are two major problems with the theory surrounding

the field: the gap in our knowledge on the relationship between our genes and the environment, and the inability to account for differences among species with very similar DNA sequences. Strohman (1993) also suggests that the medical sciences are in crisis, specifically that the current genetic paradigm is not sufficient to explain complex phenotypes. While the current genetic perspective has led to great developments in our understanding of biological mechanisms, it does not assess the organism as a whole, and therefore we cannot effectively research the organism in the context of preventing and treating developmental and health abnormalities. The complexity of living systems is currently challenging us and demanding more attention, and epigenetics is filling these gaps and providing a sound explanation (Vineis, 2010).

In addition to Vineis' (2010) discussion on how we are currently experiencing a paradigm shift, there have been many other publications on the epigenetic revolution. Here are some quotes across multiple disciplines supporting this idea:

In developmental biology:

“The paradigm that genetics is the primary factor to regulate developmental biology is limited and ignores the plasticity to respond rapidly to environment, nor does it explain abnormal development and disease etiology in the absence of genetic alterations. Epigenetics provides an additional molecular mechanism to complement genetics in the regulation of development. Therefore, the paradigm shift is that layers of molecular control and cascades of both epigenetic and genetic factors or processes are involved in regulating developmental biology” (Skinner, 2011, p. 52).

In molecular biology:

“The main idea is that epigenetic stability, or instability-that is, both rigid and plastic epigenetic regulation of genomes- can largely replace the genetic and environmental components in traditional models, and that inherited or acquired epigenetic regulation

or misregulation can be a core unifying molecular mechanism of complex, non-Mendelian, traits and diseases” (Petronis, 2010, p.722).

Thomas Kuhn asserts that the shift from a paradigm in crisis to a paradigm shift is not a cumulative process, or one that is merely an extension of previous ideas. Rather, it is reconstruction in which old ideas are viewed through a new lens: “a reconstruction that changes some of the field’s most elementary theoretical generalizations as well as many of its paradigm methods and applications” (Kuhn, 1962, p. 84). During the shift, an overlap exists between the old and new paradigm, and ultimately, the field changes their view, methods, and goals.

In some ways epigenetics represents a paradigm shift, while in others it does not. We have known for a long time that the environment interacts with our genes, but what we have not understood until now is how this interaction occurs. Epigenetics fills this previous gap in our knowledge, and explains the way in which environmental factors are molecularly embodied in our cells. So in this sense, molecular biology is not undergoing a paradigm shift, rather we are experiencing an expansion of information and knowledge. We can identify this aspect of epigenetics that explains the previous gap in our knowledge on the interaction between genes and the environment with molecular mechanisms as part of what Kuhn labeled “normal science”. Normal science entails the “steady, day-to-day, painstaking accumulation of experimental data, accredited facts and new discoveries” (Goldstein, 2012). Developments in science are continuously occurring, and can be extremely significant, but not necessarily suffice a paradigm shift. The information that epigenetics provides in the aspect of the nature-nurture interaction is an example of this. While these findings are significant and will change research in many fields, it cannot be labeled as a paradigm shift. Though Kuhn’s focus was on major

revolutions in science, he did acknowledge the importance of normal science, recognizing that most discoveries do in fact occur during normal science periods (Goldstein, 2012).

What *is* novel about epigenetics is that alterations in the epigenome can be passed through generations, and this makes epigenetics a paradigm shift across many fields, all of which contribute to our understanding and treatment of human health and disease. The research conducted on epigenetics in molecular and developmental biology provides evidence that undermines the current paradigm in biomedicine that identifies genetic determinism as the central focus of our current healthcare system (Strohman, 1993). The current paradigm shift indicates that many disease states are programmed long before an organism experiences any signs and symptoms of that disease, and sometimes even prior to conception. This shift places more responsibility on the ancestors of organisms, as events in their lifetime can be embodied in the epigenome of their progeny. Knowing epigenetic changes can be induced by specific environmental events and also reversed can serve as a potential treatment opportunity, as specific organisms with adverse ancestral history can be targeted for epigenetic therapy.

The view of the living system is changing from a genetic to an epigenetic one; the focus is shifting from the gene sequence itself to the expression of genes. Researchers have been searching for variation in the DNA sequence when phenotypic individual differences are observed, but epigenetics questions *how* individual differences exist when they have seemingly identical genomes. Herbert Butterfield, a historian, described the paradigm shift process as “picking up the other end of the stick”, and one that involves “handling the same bundle of data as before, but placing them in a new system of relations with one another by giving them a different framework” (Butterfield, 1949). This quote perfectly sums up what epigenetics is doing in some aspects of biology.

Epigenetics has an effect on a variety of areas: “cellular differentiation, tissue development, environmentally induced disease etiology, transgenerational epigenetic inheritance, and the general systems biology of organisms and evolution” (Skinner, 2011, p. 51). But biology and medicine are not the only fields that epigenetics is impacting, there are many other disciplines that are becoming aware of the impact epigenetics has on the fundamental principles of their field. Examples include evolution, bioethics (Dupras et al., 2014), human geography (Guthman and Mansfield, 2012), political theory (Hedlund, 2012), legal theory (Rothstein et al., 2009), and philosophy of identity (Boniolo and Testa, 2011). Below we will look at epigenetics from an evolutionary standpoint. Evolution is fundamental to our understanding of all basic sciences, and medicine is built off of these disciplines. Evolution is extremely important to consider because all biological systems continue to evolve, and taking an evolutionary perspective helps identify the roots of disease.

Evolutionary Aspects

“Nothing in biology makes sense except in the light of evolution.”

–Theodosius Dobzhansky, 1973

Epigenetics is receiving a great deal of attention in medicine and development, but people are more reluctant to believe these alternative inheritance systems play a major role in evolution. Evolutionary biology has not undergone a paradigm shift since Darwin’s discoveries; instead, new ideas have been added to the evolutionary paradigm without discarding the previous explanations. The current framework in evolutionary biology is known as the Modern Synthesis (MS), which was first developed in the 1940s as an integration of Darwin’s theory of evolution and Mendelian genetics (which identified the gene as the unit of inheritance). The MS was originally comprised of three major ideas: germ-line genes carry information through

generations, random combinations of alleles result in variation and novel variations arise from mutations, and phenotypes that make organisms better adapted for the environment are selected (Jablonka and Lamb, 2005). Over time, small changes were made to the Modern Synthesis as molecular developments occurred, such as the understanding of the structure of DNA and its replication, but it is generally what we use today.

In the MS, genes are identified as the material foundation of all evolutionary change, and are not influenced by the environment. However, the environment does have a major impact on organisms, but not at the level of the genotype, as phenotypic changes produced by the environment cannot be passed on to future generations (Jablonka and Lamb, 1995). Many argue that this paradigm is no longer sufficient for the field, and it needs to be extended. Pigliucci (2007) states that there are four major components missing from the Modern Synthesis: development, ecology, “-omics” revolution, and important biological phenomena such as phenotypic plasticity, evolutionary capacitance, and epigenetic inheritance. Pigliucci maintains that evolutionary theory began as a theory of form, shifted to a theory of genes, and now needs to be extended back to a theory of form, identified as the Extended Evolutionary Synthesis (EES).

The molecular, physiological, behavioral, cellular, and morphological phenotype of an organism can be modified by the environment through epigenetic mechanisms, effecting epigenetic inheritance (Burggren and Crews, 2014). Recall that the three main mechanisms that can control gene expression are DNA methylation, histone modifications, and regulatory processes mediated by small RNAs. Methylation is of particular interest to evolution because it has a direct impact and persists in the long term (Rapp and Wendel, 2005). There is a great deal of evidence that illustrates how phenotypic variation induced by the environment can be transgenerationally inherited. Microevolution, small-scale evolutionary change, is driven by

heritable phenotypic variation among populations, which suggests the potential role epigenetics has played in the history of our evolution.

Richards (2006) proposes the question, “Does the existence of inherited epigenetic alleles that are to some degree independent of genetic variation necessitate a modification of our models of evolutionary change?” (p.398). In other words, does the presence of epigenetic inheritance systems require us to reevaluate our thinking about evolution? The truth is that the field is just beginning, and much more research needs to be done, yet there are many arguments for the critical role ESIs have played in evolution that demand attention.

The role of epigenetic inheritance in evolution

Modern biologists agree that the environment is in fact important, but its role is not in the generation of heritable variation, but in the selection of traits. Recently, however, there have been three major changes in perceptions of the nature of heritable variation. First, studies on bacteria and unicellular eukaryotes have shown that some mutations are adaptive in response to the surrounding environment, and therefore are not random. Second, the genome is no longer viewed as a static, passive entity; instead, it is labeled as flexible and dynamic, and both an information carrier and a response system. Third, DNA is no longer viewed as the only heritable information; other elements are being transmitted through generations that do not lie in the basic DNA sequence. These three changes in attitude led Eva Jablonka and Marion Lamb (1995;1998A;1998B;2005) to explore environmentally induced variation, specifically epigenetic inheritance systems (EISs) for some answers.

Before Darwin’s theory of evolution, Jean-Baptiste Lamarck proposed a theory of evolution that incorporated a belief in the inheritance of acquired characteristics, also labeled as *soft inheritance*. He believed that an organism could pass on characteristics acquired over the

life course to their offspring. The famous example of Lamarckism is the neck of the giraffe: he believed that over the life course a giraffe's neck would stretch and lengthen to reach high leaves, and that this elongated neck could be passed on to its offspring. While Lamarck's theory of evolution was discredited, it seems to be making a comeback in the field of epigenetics, as the soft inheritance aspect of epigenetics has a Lamarckian air to it. In 1995, Jablonka and Lamb published a book called *Epigenetic Inheritance and Evolution: The Lamarckian Dimension*, in attempt to re-evaluate the role of the inheritance of acquired characteristics in evolution. One might initially assume that Jablonka and Lamb are anti-neo-Darwinism and supporters of neo-Lamarckism, but they assert that their views are both neo-Darwinist and neo-Lamarckist. Jablonka and Lamb accept the central tenet of Darwin's theory that natural selection acts on the heritable variations that affect fitness, but question the nature of the variations, and argue that this part of the theory is lacking. The current neo-Darwinian theory relies on two basic assumptions: in heritable material all variations are random, and all hereditary variations lie in DNA base sequences. Jablonka and Lamb (1995) question both of these assumptions, and argue that we need to reevaluate our thinking about genetics and evolution.

Jablonka and Lamb (1998A) investigated the role of epigenetic inheritance systems (EISs) in the evolution of multicellularity, ontogeny, and chromosome organization, as EISs are very ancient systems. Jablonka and Lamb (1995) argue that EISs developed in primitive unicellular organisms in response to the changing environment, and these systems played a significant role in the evolution and transition to multicellular organisms. "First, they enabled the emergence of a new unit of structure and function, the phenotypically distinct cell lineage. Second, they allowed the formation of the stable interdependences between epigenetically distinct cell lineages, which results in the evolution of integrated organisms from loose groups of cells" (p.205). They suggest that evolution to multicellularity occurred in the following steps:

first, large size was selected for, therefore groups of cells benefited. In response to the environment, differential phenotypes arose between the loosely grouped cells, and these phenotypes were preserved by EISs. Properties within groups were more similar than between groups, and therefore the groups of cells themselves acted as units of selection. Selection between groups resulted in the development of particular group properties, which then evolved into a division of labor. Cells became more self-sufficient, relying less on the surrounding environment and more on their interactions with other cell lineages. This ultimately resulted in the formation of multicellular individuals. Following the transition to multicellular organisms, EISs developed further and contributed to evolutionary changes through direct and indirect mechanisms. Directly, mechanisms such as selection, migration, drift, epimutation pressure, and epiallele-induction in populations affect the frequencies of epialleles, leading to evolutionary change. Indirectly, evolution has new opportunities once alternative inheritance systems exist. For example, "Organisms with EISs can evolve complex multicellular organizations, but possession of EISs endangers them because readily induced epigenetic states can destroy the harmonious development of descendants" (Jablonka and Lamb, 1995, p. 276). Without EISs, organisms with many types of differentiated cells would not have evolved, as cell memory was critical for these developments.

There are three types of epigenetic inheritance systems in cell memory: steady state systems, structural inheritance systems, and chromatin-marking systems (Jablonka and Lamb, 1995). Steady-state systems involve metabolic patterns that are self-perpetuating. An example of this is the positive feedback loop that regulates a gene's transcription activity. In structural inheritance systems, previously existing structures in the cell are used as a template to create new structures. Chromatin-marking systems are the most well known type of EIS, and they involve the organization of the DNA in chromosomes. Information is passed through generations

in chromatin marks, which take the form of additional chemical groups or binding proteins that are attached to the DNA. For example, the methylation pattern of a gene affects the activity and function. Typically low levels of methylation represent a potential for activity, while high levels of methylation result in inactivity. Changes in methylation patterns do not affect the coding sequence itself, but these patterns can be inherited by future generations (Jablonka and Lamb, 1998A).

Studies have shown that embryos will not develop normally without the proper epigenetic information. The most abundant research has been done on genomic imprinting, which has contributed to our understanding of EISs' importance in development. Chromosomes, regions of chromosomes, and genes are transmitted from parent to offspring and expressed based on the sex of the parent from which they were inherited. Genomic imprinting evidence is relevant to evolutionary biology because it shows that epigenetic marks present in parents are transmitted to offspring, and the majority of epigenetic marks would be unnoticeable outside of experimental manipulation. Even if a zygote inherits the proper genetic material, if the epigenetic marks are incorrect the zygote will develop abnormally (Jablonka and Lamb, 1998A).

While Jablonka and Lamb have been the major contributors to the discussion on epigenetics and evolution, there are many others that have added to the field. McVean (1998) establishes two methods in which epigenetic inheritance systems could play a role in evolution: they may be adaptations themselves which have enabled further change, leading to greater variation among genomes, or epigenetic change produces variants that act alongside genes as units of selection. Bossdorf et al. (2008) proposes a similar argument, addressing how epigenetic processes can lead to microevolution among natural populations. First, epigenetic processes may be an additional system for natural selection to act on. Second, if epigenetic processes can

be influenced by the environment and ecological interactions, then they may represent a new, rapid pathway for evolutionary change.

Shea et al. (2011) categorizes heritable epigenetic mechanisms into two classes (selection-based and detection-based) with differing evolutionary implications. In selection-based effects, natural selection on variants occurs and the information is passed between generations. In detection-based selection, organisms detect information in the environment and pass it on to their offspring. Both of these sources of information allow the organism to adapt and be adaptations, but these different routes produce different evolutionary outcomes. Selection-based effects occur in nature, particularly in unicellular organisms, and these epigenetic effects can persist for hundreds of generations. An example of how natural selection can act on these effects is a study done on the bacteria *Escherichia coli*. Adam et al. (2008) observed that when the bacteria were grown on an antibiotic medium, antibiotic resistant epigenetic variations were selected for. The epigenetic variations that were selected for were passed on to offspring, and the presence of these adapted epialleles allowed the offspring to develop resistance. These results showed a rapid evolution of adaptation. On the other hand, in detection-based effects, offspring produce specific phenotypes based on their parental history. For example, the herb *Campanulastrum americanum* grows as either an annual or biennial based on the environment the mother grew in, either a woodland understory or light gap (Galloway, 2005). Experiments, such as this one, suggest that an epigenetic variant matches with the maternal environment. This information is passed on to offspring, who respond with appropriate phenotypes that are already adapted to the environment.

Even when the original environmental stimulus that altered the phenotypes is no longer present, epigenetic inheritance systems act as memory systems that allow the different phenotypes with identical genotypes to be passed on to future generations. Mate preference is

interesting to analyze, as evolution is built around reproductive success. Crews et al. (2007) studied mate preference among rats exposed to endocrine-disrupting chemicals (EDC) in the environment to determine if these contaminants have epigenetic effects on the germ line. Male and female rats whose ancestors were exposed to the antiandrogenic fungicide vinclozolin demonstrated different behaviors: female rats three generations following the exposure preferred male rats whose ancestors did not have a history of exposure, while the male rats with the disease phenotype from their ancestors did not discriminate and prefer females based on this characteristic. Therefore, this effect was sex-specific. In other words, EDCs have not only transgenerational but transpopulational effects, as males are often the dispersing sex in mammals. The EDC exposure did not alter the DNA of the organisms itself, but rather the epigenome was mutated by methylation patterns. The researchers concluded that the consequences of EDCs are epigenetically inherited through multiple generations, and play a major role in sexual selection. This asymmetry in mating preference could have major evolutionary implications. When environmentally induced epialleles maintain through generations regardless of subsequent environmental change, they accumulate and are more likely to result in stable phenotypic variation. The relationship of epialleles to fitness is critical, as this will determine if the epigenetic variants are maintained by selection, providing direct evidence for the role of epigenetic inheritance in evolution.

Wymore (2011) also discusses the effects of endocrine-disrupting chemicals (EDCs) on gene expression. The particular EDC studied in fish populations, EE2 (which mimics natural estrogen), affects the development and sexual differentiation, in addition to altering the gene expression that controls behavior. Genetically male fish were severely impacted, as many developed feminized reproductive ducts and were physiologically female. This change in sex created a more feminized sex ratio, which ultimately decreased reproductive success of the

population as a whole. In another study, female fish that were exposed to EE2 were unable to make appropriate decisions about mating. In situations like this, populations can decline and ultimately die off (Wymore, 2011). Studies such as these demonstrate how environmental factors can regulate gene expression and mating behaviors, ultimately affecting the overall population's fitness and survival.

Another interesting population to study when looking into epigenetics is plants, as they are exposed to many environmental conditions, such as temperature, light, salt, water, and pathogens during the life span (Bond and Finnegan, 2007). In response to the environment, plants adapt by altering their gene expression. Plant studies supply the greatest evidence of heritable epigenetic marks displaying phenotypic variation. There are two types of studies in plants that investigate the potential significance of epigenetic phenomena to evolution: genomic surveys of epigenetic marks and expression patterns, and studies on specific phenotypes (example: flowering time) (Rapp and Wendel, 2005). Methylated epialleles can affect floral shape, vegetative and seed pigmentation, pathogen resistance, and development, and these effects are demonstrated in studies of the plant species *Arabidopsis thaliana*, *Zea mays*, and *Linaria vulgaris* (Kalisz and Purugganan, 2004). Traditional models of microevolution would predict organisms with identical genotypes to respond to selection in the same way, but because epiallele phenotypes differ so would the expected outcomes. If the results of the discussed plant models are suggestive of the effects of epialleles in natural populations, then epialleles are expected to play a major role in fitness. It is predicted that epiallelic phenotypes could influence the following: mating system or pollination syndromes, disease patterns (pathogen resistance), physiology, herbivory and seed predation, developmental and phenological traits, and ultimately trait evolution (Kalisz and Purugganan, 2004). The stability

and frequency of epialleles in natural populations need to be evaluated in order to determine how important epialleles truly are in nature.

While the field of epigenetics is in its infancy, research has demonstrated that the existence of this additional inheritance system should make us rethink our understanding of evolution, and perhaps develop a new evolutionary model.

Future directions for the epigenetic perspective of evolution

The research that has been completed in the field of epigenetics thus far is just scratching the surface, and much is left to be done. However, interest in this topic is growing, as a recent meta-analysis showed that in the NIH PubMed database, there were roughly 12,000 papers published between 2010-2013 containing the words “epigenetic”, “epigenetics”, or “epigenome” (Burggren and Crews, 2014). While research exploring epigenetic inheritance is currently being done, there is still a great deal that we do not understand. Much of what we know is based off specific variants in small populations under laboratory conditions. Thus, future research needs to be extended to natural populations. For example, in natural plant populations it is necessary to assess how variable methylation patterns are within populations, the extent to which methylation patterns affect phenotypes, and how stable methylation patterns are across generations. If these aspects are studied and understood they could serve as the starting point for a revised evolutionary model. By identifying epigenetic modifications that have the greatest influence on behavioral phenotype, and consequent fitness, we would have a better understanding of evolutionary processes. Therefore future research should aim at finding these significant epigenetic modifications, as they will be the best predictors of differences in phenotype, therefore serving as the targets in natural selection (Ledon-Retting et al., 2012).

Evolution is based on reproductive success. Therefore, mate selection is an extremely important area of research that should be explored in the context of epigenetics. As previously discussed, studies have been done on sexual selection among populations with altered epigenomes. Results from these studies suggest that changes induced by the environment can have a major impact on the sustainability of a population, and therefore the evolution of that species. All of the previously discussed studies suggest that the evolution of many characteristics is mediated by epigenetic mechanisms, which proposes that mutation is not the only driving force behind evolutionary change. The topic of epigenetics and evolution has received criticism, but that is simply because it presents novel ideas that offer a much more complex system of inheritance. Future studies need to address this complexity, and assess how important epigenetic variation is in its contribution to fitness differences in comparison to genetic variation contributions.

Scrutinizing the epigenetics revolution

In a way the epigenetic revolution has already been criticized, as I identified aspects of the current theory that do not represent a fundamental shift in our thinking, but there are additional problems with epigenetic research.

While developments in the field of epigenetics are extremely exciting, is the optimism that this field endorses a little blinding, as it presents a potential new way to detect, prevent, and treat disease? As with any new proposal, epigenetics has received some criticism, and it is necessary to discuss both sides of the matter. Tsankova et al. (2007) encourages caution when looking at epigenetic mechanisms, and Nestler (2013) claims that “much more work is therefore needed before we will know the extent to which epigenetic mechanisms represent a third factor-beyond nature and nurture- in controlling an individual’s traits in health and disease.”

Yes, we can acknowledge that epigenetic inheritance systems exist, but what is the magnitude of their contributions?

Maurizio Meloni and Giuseppe Testa published an article titled “Scrutinizing the Epigenetic Revolution” in 2014, where they criticize the current excitement epigenetics is stirring. They begin by discussing the ambiguity of epigenetic terminology, and how the field is “succeeding by blurring” (p. 432). Historically, researchers used the term ‘epigenetic transmission’ to refer to any phenomena that could not be explained by genetics. Therefore the phrase “epigenetic transmission of acquired characteristics” could be used without any supporting molecular mechanism evidence (Carey, 2012). This ambiguity of terminology is a major problem, as researchers could use the same term but be referring to different phenomena.

Meloni and Testa (2014) label it as a “passive revolution”, a phrase created by the political theorist Antonio Gramsci in the 1930s (Gramsci and Forgacs, 1988). “According to his definition a revolution is passive when, far from being a radical break, it unfolds as a long-term process in which progressive and backward-looking forces coexist and overlap. It is passive because it does not have the strength for changing ‘the essential’ and ends up thereby processing in a sort of limping way. And yet, despite an uncertain route in which vocal gestures end up often void or usher into bombastic but sterile statements, its impacts can nonetheless prove revolutionary” (Meloni and Testa, 2014). This passive revolution is exactly how the genome-environment aspect of epigenetics can be viewed: this characteristic is both progressive and backward looking, as it is new information and evidence about an old idea.

Heijmans and Mill (2012) also identify several major biological, technical, and methodological problems in epigenetic research. First, researchers do not know exactly what they are looking for. The densest area of research has been on methylation, but as previously

mentioned there are many other aspects of the epigenome that are equally important, and therefore deserve more attention. Additionally, even the methylome (comprised of all methyl marks on DNA) is not well understood. Heijmans and Mill (2012) suggest that there are other areas of the methylome that could be more important to gene regulation than the areas we have studied. Therefore we do not really know what we are looking for and where, making this a major limitation of epigenetic research. Next, we do not have the proper technology currently to discover precise findings. While major leaps have been made in genome technology, we are not there with epigenome technology. When researchers are able to cover a large amount of the genome, the precision of sites is compromised, and vice versa. When methods are used that incorporate both coverage and precision, researchers run into the problem of comparing data across studies. Lastly, researchers are lacking appropriate sample sizes when assessing epigenetic epidemiology (Heijmans and Mill, 2012).

The overall message of Heijmans and Mill's (2012) critique of epigenetic research is that we are getting ahead of ourselves: the expectations are too high. A similar situation occurred when genetic epidemiology started making an appearance, and claims were made that once the genome was fully mapped, the cause and treatment of major diseases would be better understood. While genetic studies have been successful, the outcomes have not matched the hype that was originally created. Therefore Heijmans and Mill urge caution. Despite their critique, they do end their review on a positive note, claiming that with time and proper research we could someday reach the goals of epigenetic research.

Criticism of Evolutionary Aspect

While it cannot be denied that epigenetic inheritance systems exist and are important, some critics do not see its relevance to evolution. There is a great deal that is still not

understood about epigenetics, which results in many more questions than answers. When Jablonka and Lamb (1989) first started discussing the role of epigenetic inheritance systems in evolution, they acknowledged expected criticism because they were suggesting that heredity and evolution were much more complicated and confusing than initially thought. It is important to note that throughout all of Jablonka and Lamb's works, they do no attempt to downplay the role of genetic material in evolution; instead they are proposing an additional inheritance system. This was something they were criticized for, as people interpreted their work as discrediting the importance of genetic inheritance. A basic argument states that since evolution can be explained without EISs, why are we attempting to fit them in (Jablonka and Lamb, 1998B)? McVean (1998) is one of the supporters of this claim, and argues that the evolution of ontogeny, genome organization, and reproductive isolation can be explained by genetic mechanisms without EISs. While he does acknowledge the importance of EISs in the maintenance of tissues following an organism's development, he maintains that they do not play a role in ontogeny.

One of the simplest criticisms is the "Lamarckian" aspect of the argument, as most scientists discarded this proposed theory a long time ago. For example, Moore (1998) stated in a commentary on Jablonka and Lamb's (1998A) piece that "their Darwinian avowals notwithstanding, an irritating undercurrent of "Lamarckism" percolates through many of their references to possible environmental influences on mutation and inheritance" (p. 229). In addition to the criticism of Lamarckian references used throughout epigenetic research, people also have a problem with the language used. As previously discussed, there are many different definitions of the term epigenetics, and it becomes confusing when researchers use the same term to refer to different phenomena. Bird (1998) argues against the epigenetic dimension to evolution by criticizing the language used by Jablonka and Lamb (1998A), specifically that they

discuss epigenetics only as subcellular events. Bird (1998) states that if inherited traits that do not alter the DNA are being discussed, then cultures and human languages should also be considered epialleles.

Outside of genomic imprinting, there are three major arguments against the role of epigenetic inheritance systems (EISs) in evolution: epigenetic inheritance across generations (transgenerational) is rare and pathological; if transgenerational inheritance does occur, it is not stable and therefore not significant to evolution; epigenetic inheritance is only important to unicellular asexual organisms or multicellular organisms that use fragmentation to reproduce (Jablonka and Lamb, 1998A). Even genomic imprinting has received its own criticism as studies show how information from the previous generation is often erased in the germ line of the next, and therefore these changes cannot be labeled as permanent. This suggests that there is a selection against carrying information from the previous generation (Jablonka and Lamb, 1995).

There is sufficient evidence that epigenetic variation persists through mitotic divisions, but in order for epigenetics to affect inheritance and be of evolutionary significance, epigenetic states must persist through meiosis. Richards (2006) argues against the meiotic transmission of epigenetic states, stating that in a developmental context, epigenetic states are erased. For example, during early mammalian development, methylation marks are removed. This resetting process supports the idea that epigenetic states are limited to one generation and cannot be passed on to offspring. Transgenerational stability of DNA methylation has been shown in unicellular organisms (Adam et al., 2008), plants (Johannes et al., 2009), and mammals (Morgan et al., 1999; Crews et al., 2007), but a major argument is that epigenetic variants are only stable for a few generations, and do not persist in the long-term. An example of this is that in the experimentally induced DNA methylation of the plant *Arabidopsis thaliana*, between generations two and five half of the methylation sites reverted to their wild-type forms (Shea et al., 2011).

Another argument against the stability of transgenerational inheritance is made by Burggren and Crews (2014), who discuss the reversibility of epigenetic changes, and believe that epigenetic changes cannot be compared to mutation or natural selection because these alterations are not permanent.

On the other hand, phenotypes can rapidly change in response to environment conditions, a characteristic that the mechanisms mutation and natural selection do not possess. For example, zebrafish that are exposed to hypoxic water pass on a certain tolerance of these harmful conditions to their offspring through epigenetic modification. What is unknown is the cost of this epigenetic modification, which could outweigh the irrelevant benefits when zebrafish reproduce in water that has normal oxygen levels. The inheritance of epialleles could be a type of adaptive evolution, created in response to the ever-changing environment, moving much faster than the mechanisms of natural selection and mutation. Lastly, Kirschner and Gerhart (1998) argue that evidence supporting the role of epigenetic inheritance in evolutionary mechanisms is lacking, particularly that there is no evidence for a mechanism that selects for epigenetic stability. Kirschner and Gerhart (1998) continue by saying that even if such a mechanism exists, there is no evidence that it would play a role in evolution.

Critics point out the lack of epigenetic mechanisms in multicellular organisms, as most of the evidence lies with unicellular organisms. There are two rebuttals to this argument: developmental and selective. A developmental argument against this is that organisms such as plants and fungi have greater windows for epigenetic changes to be passed on because the separation of germ line and soma occurs much later than in animals, where this separation occurs early in development. The selective argument contests that epigenetic mechanisms are not as important in animals because they have other systems that allow them to appropriately respond to their environment, such as a nervous system and motility (Shea et al., 2011). Moore

(1998) is one of the critics that acknowledges the relevance of epigenetic arguments to simple, unicellular organisms, but expresses his concern with the lack of evidence in mammals.

While one cannot deny the existence of epigenetic mechanisms, many have a hard time believing that these modifications of gene expression have lasting evolutionary effects, which is understandable, considering a great deal is still unknown about the field.

Implications of the Current Research: Looking Forward

“Epigenetics has clearly provided a banner under which a new scientific movement has advanced” (Haig, 2012, p.15).

We have identified two separate important aspects of epigenetics: that it provides the molecular mechanisms through which nature and nurture interact, and that epigenetic changes can be transmitted through generations. The first point, that epigenetics explains the gap we have had in our knowledge of genome-environment interactions, can be viewed as an extension of the old paradigm. Researchers and health care providers have all been on the same page that the environment can influence phenotypes, and therefore we cannot label this aspect of epigenetics as a paradigm shift. As Kuhn asserted, a paradigm shift encompasses a change at the basic theoretical level. While the aspect of epigenetics that explains gene-environment interactions is not a fundamental shift in our thinking, it does have major implications for our current view of health and disease. The role of the environment should be a major focus, as identification of harmful environmental factors could aid in the prevention and treatment of disease.

On the other hand, the second aspect of epigenetics, its transgenerational nature, does constitute a paradigm shift. The idea that molecular changes occur that do not alter the DNA sequence itself can be passed through multiple generations of offspring is a novel one, and demonstrates a clear change in our thinking. Epigenetics raises awareness of the importance of life histories, and should motivate people to take more responsibility as their environment and life choices not only impact themselves, but can also affect their future offspring. The health of

current and future mothers *and* fathers is critical to the well being of their offspring, and people need to be made aware of this.

The plasticity of the epigenome is also an important aspect of the field, and research demonstrates that early life environments are not the only critical windows in development, and in fact the epigenome can continue to be altered throughout the life course. We can think of our cells as having a creative quality: networks of genes, cells, tissues, and other major components of living systems take in signals from the surrounding environment, and respond in an appropriate, often adaptive way. These abilities allow us to identify the adapted organism as having a “wise body” (Strohman, 1993). “Body wisdom” was first coined by Walter B Cannon in 1932 (Cannon, 1932) as an important aspect of life, and “It is recaptured as a modern concept of organism based on past, conserved genetic adaptation *coupled with current epigenetic regulation*” (Strohman, 1993, p. 139).

The way disease can be treated is currently changing as researchers have established epigenetic etiologies of disease, and this treatment is known as epigenetic therapy. There are many agents that can alter methylation and histone modification patterns, and these drugs are currently being tested in clinical trials (Egger et al., 2004). The reversibility of epigenetic modifications is promising for therapy as these agents can potentially reverse detrimental epigenetic patterns. For example, if a set of genes is silenced because of an increase DNA methylation, an agent that inhibits DNA methylation can be used to reactivate the genes, ultimately reversing the pathological state (Egger et al., 2004). The recent discovery of epigenetic etiologies of disease creates optimism, as epigenetic changes can serve as targets for therapy, and prevention strategies can be developed once we have a greater understanding of the epigenome.

Though many therapeutic agents are currently being studied and offer a potential way to reverse epigenetic changes, knowing what we do now, the focus of health care can shift more toward prevention and away from therapy. Knowing the role that environmental factors, such as stress and nutrition, play in the development of not only your own health but your future offspring's health, there should be more of a focus on responsibility. There are different levels of change that can occur: changes you can make on a personal level, such as diet and lifestyle; changes that can be made at the societal level, such as living and working environments, and healthcare; changes in social structure, such as class and gender (Hedlund, 2012).

The 19th century can be labeled as the era of evolution and genetics because of Darwin and Mendel's discoveries, the 20th century is the era of DNA because of the findings of Watson and Crick, and current research and discussion is suggesting that the 21st century is the era of epigenetics. While much is left to be done, the information that has been discovered thus far is already changing how we view human health and disease, and the implications of the field are far reaching.

References

- Abdolmaleky HM, Cheng KH, Faraone SV, Wilcox M, Glatt SJ, Gao F, et al. Hypomethylation of MB-COMT promoter is a major risk factor for schizophrenia and bipolar disorder. *Human Molecular Genetics*. 2006;15(21):3132-3145.
- Abdolmaleky HM, Cheng KH, Russo A, Smith CL, Faraone SV, Wilcox M, et al. Hypermethylation of the reelin (*RELN*) promoter in the brain of schizophrenic patients: a preliminary report. *American Journal of Medical Genetics. Part B: Neuropsychiatric Genetics*. 2005;134B(1):60-66.
- Abdolmaleky HM, Shafa R, Tsuang MT, Thiagalingam S. Psychiatric Epigenetics: A Key to the Molecular Basis of and Therapy for Psychiatric Disorders. *Psychiatric Times*. 2013;10-12,14-15.
- Abel T, Zukin RS. Epigenetic targets of HDAC inhibition in neurodegenerative and psychiatric disorders. *Current Opinion in Pharmacology*. 2008;8:57-64.
- Adam M, Murali B, Glenn N, Potter SS. Epigenetic inheritance based evolution of antibiotic resistance in bacteria. *BMC Evolutionary Biology*. 2008;8:52.
- Allis CD, Jenuwein T, Reinberg D. *Epigenetics*. Cold Spring Harbor, NY: Cold Spring Harbor Lab; 2007.
- Ashley CT, Sutcliffe JS, Kunst CB, Leiner HA, Eichler EE, Nelson DL, et al. Human and murine FMR-1: alternative splicing and translational initiation downstream of the CGG-repeat. *Nature Genetics*. 1993;4(3):244-251.
- American Psychiatric Association. *Diagnostic and statistical manual of mental disorders: DSM-IV*. 4th ed. Washington, DC: American Psychiatric Association; 1994.
- American Psychiatric Association: *Diagnostic and statistical manual of mental disorders: DSM V*. 5th ed. Washington, DC: American Psychiatric Association; 2013.
- Balazs R, Vernon J, Hardy J. Epigenetic mechanisms in Alzheimer's disease: progress but much to do. *Neurobiology of Aging*. 2011;32:1181-1187.
- Barker DJ, Osmond C. Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. *The Lancet*. 1986;1:1077-1081.
- Barker DJP. The origins of the developmental origins theory. *Journal of Internal Medicine*. 2007;261:412-417.
- Bird A. Comments on "Epigenetic inheritance in evolution". *Journal of Evolutionary Biology*. 1998;11:185-188.
- Bird A. Perceptions of epigenetics. *Nature*. 2007;447:396-398.

- Bond DM, Finnegan EJ. Passing the message on: inheritance of epigenetic traits. *Trends in Plant Science*. 2007;12(5):211-216.
- Boniolo G, Testa G. The Identity of Living Beings, Epigenetics, and the Modesty of Philosophy. *Erkenntnis*. 2011;76:279-298.
- Bossdorf O, Richards CL, Pigliucci M. Epigenetics for ecologists. *Ecology Letters*. 2008;11:106-115.
- Burggren WW. Epigenetics as a source of variation in comparative animal physiology-or-Lamarck is lookin' pretty good these days. *The Journal of Experimental Biology*. 2014;217:682-689.
- Burggren WW, Crews D. Epigenetics in Comparative Biology: Why We Should Pay Attention. *Integrative and Comparative Biology*. 2014;54(1):7-20.
- Butterfield H. *The Origins of Modern Science, 1300-1800*. London: The Macmillan Company; 1949. p 1-7.
- Bygren LO. Intergenerational health responses to adverse and enriched environments. *Annual Review of Public Health*. 2013;34:49-60.
- Cannon WB. *The Wisdom of the Body*. New York: W.W. Norton; 1932.
- Carey B. Genes as Mirrors of Life Experiences. *The New York Times*. 2010 Nov 8.
- Carey N. *The Epigenetics Revolution: How Modern Biology is Rewriting Our Understanding of Genetics, Disease, and Inheritance*. New York: Columbia University Press; 2012.
- Chao W, D'Amore PA. IGF2: Epigenetic regulation and role in development and disease. *Cytokine & Growth Factor Reviews*. 2008;19(2):111-120.
- Crews D. Epigenetics and its implications for behavioral neuroendocrinology. *Frontiers in Neuroendocrinology*. 2008;29:344-357.
- Crews D. Epigenetics, brain, behavior, and the environment. *Hormones*. 2010;9(1):41-50.
- Crews D, Gore AC. Transgenerational Epigenetics: Current Controversies and Debates. In: Tollefsbol T, editor. *Transgenerational Epigenetics: evidence and debate*. London: Academic Press; 2014. p 371-390.
- Crews D, Gore AC, Hsu TS, Dangleben NL, Spinetta M, Schallert T, et al. Transgenerational epigenetic imprints on mate preference. *Proceedings of the National Academy of Sciences*. 2007;104(14): 5942-5946.
- Crick F. Memory and molecular turnover. *Nature*. 1984;312(5990):101.
- Danaei G, Fincane MM, Lu Y, Singh GM, Cowan MJ, Paciorek CJ, et al. National, regional, and

- global trends in fasting plasma glucose and diabetes prevalence since 1980: systematic analysis of health examination surveys and epidemiological studies with 370 country years and 2.7 million participants. *The Lancet*. 2011;378:31-40.
- Darnaudeau M, Maccari S. Epigenetic programming of the stress response in male and female rats by prenatal restraint stress. *Brain Research Reviews*. 2008;57(2):571-585.
- Darwin C. *On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life*. London, UK: W. Clowes and Sons; 1859.
- Dietz DM, LaPlant Q, Watts EL, Hodes GE, Russo SJ, Feng J, et al. Paternal Transmission of Stress-Induced Pathologies. *Biological Psychiatry*. 2011;70:408-414.
- Dobzhansky T. *Nothing in Biology Makes Sense except in the Light of Evolution*. *The American Biology Teacher*. 1973;35(3):129-129.
- Dolinoy DC, Weidman JR, Jirtle RL. Epigenetic gene regulation: Linking early developmental environmental to adult disease. *Reproductive Toxicology*. 2007;23:297-307.
- Dover GJ. The Barker Hypothesis: how pediatricians will diagnose and prevent common adult-onset diseases. *Transactions of the American Clinical and Climatological Association*. 2009;120:199-207.
- Dupras C, Ravitsky V, Williams-Jones B. Epigenetics and the environment in bioethics. *Bioethics*. 2014;28(7):327-334.
- Eckhardt F, Beck S, Gut IG, Berlin K. Future potential of the Human Epigenome Project. *Expert Review of Molecular Diagnostics*. 2004;4(5):609-618.
- Egger G, Liang G, Aparicio A, Jones PA. Epigenetics in human disease and prospects for epigenetic therapy. *Nature*. 2004;429:457-463.
- Ferrante RJ, Kubilus JK, Lee J, Ryu H, Beesen A, Zucker B, et al. Histone deacetylase inhibition by sodium butyrate chemotherapy ameliorates the neurodegenerative phenotype in Huntington's disease mice. *Journal of Neuroscience*. 2003;23(28):9418-9427.
- Fraga MF, Ballestar E, Oaz MF, Ropero S, Setien F, Ballestar ML, et al. Epigenetic differences arise during the lifetime of monozygotic twins. *Proceedings of the National Academy of Sciences*. 2005;102(30):10604-10609.
- Francis RC. *Epigenetics: The Ultimate Mystery of Inheritance*. New York, London: W.W. Norton Company; 2011.
- Francis D, Diorio J, Liu D, Meaney MJ. Nongenomic transmission across generations of maternal behavior and stress responses in the rat. *Science*. 1999;286:1155-1158.
- Franklin TB, Russig H, Weiss IC, Graff J, Linder N, Michalon A, et al. Epigenetic

- transmission of the impact of early stress across generations. *Biological Psychiatry*. 2010;68:408-415.
- Galloway LF. Maternal effects provide phenotypic adaptation to local environmental conditions. *New Phytologist*. 2005;166:93-99.
- Gaisler-Salomon I. Inheriting Stress. *The New York Times*. 2014 March 7; Sunday Review.
- Gecz J, Gedon AK, Sutherland GR, Mulley JC. Identification of the gene FMR2, associated with FRAXE mental retardation. *Nature Genetics*. 1996;13(1):105-108.
- Gilbert SF, Epel D. *Ecological Developmental Biology: Integrating Epigenetics, Medicine, and Evolution*. Sunderland, MA: Sinauer; 2008.
- Goldstein JL. Paradigm shifts in science: insights from the arts. *Nature Medicine*. 2012;18(10):iii-vii.
- Gräff J, Mansuy I. Epigenetic codes in cognition and behavior. *Behavioral Brain Research*. 2008;192:70-87.
- Gramsci A, Forgacs D. *An Antonio Gramsci Reader: Selected Writings, 1916-1935*. New York: Schocken; 1988.
- Grandma's curse. *The Economist*. 2012 Nov 3; Science and Technology.
- Gurdinguio R, Sanchez-Mut JV, Esteller M. Epigenetic mechanisms in neurological diseases: genes, syndromes, and therapies. *The Lancet Neurology*. 2009;8:1056-1072.
- Guthman J, Mansfield B. The implications of environmental epigenetics: A new direction for geographic inquiry on health, space, and nature-society relations. *Progress in Human Geography*. 2012;37(4):486-504.
- Guy J, Gan J, Selfridge J, Cobb S, Bird A. Reversal of neurological deficits in a mouse model of Rett Syndrome. *Science*. 2007;315:1143-1147.
- Haig D. The epidemiology of epigenetics. *International Journal of Epidemiology*. 2012;41(1):13-16.
- Hales CN, Barker DJ, Clark PM, Cox LJ, Fall C, Osmond C, et al. Fetal and infant growth and impaired glucose tolerance at age 64. *British Medical Journal*. 1991;303:1019-1022.
- Hedlund M. Epigenetic Responsibility. *Medicine Studies*. 2012;3(3):171-183.
- Heijmans BT, Mill J. Commentary: The seven plagues of epigenetic epidemiology. *International Journal of Epidemiology*. 2012;41:74-78.
- Heijmans BT, Tobi EW, Stein AD, Putter H, Blauw GJ, Susser ES, et al. Persistent epigenetic

- differences associated with prenatal exposure to famine in humans. *Proceedings of the National Academy of Sciences USA*. 2008;105(44):17046-17049.
- Henry C, Kabbaju M, Simon H, Le Moal M, Maccari S. Prenatal stress increases the hypothalamo-pituitary-adrenal axis response in young and adult rats. *Journal of Neuroendocrinology*. 1994;6:341-345.
- Hockly E, Richon VM, Woodman B, Smith DL, Zhou X, Rosa E, et al. Suberoylanilide hydroxamic acid, a histone deacetylase inhibitor, ameliorates motor deficits in a mouse model of Huntington's disease. *Proceedings of the National Academy of Sciences USA*. 2003;100(4):2041-2046.
- Holliday R, Pugh JE. DNA Modification Mechanisms and Gene Activity during Development. *Science*. 1975;187(4173):226-232.
- Holmes A, le Guisquet AM, Vogel E, Millstein RA, Leman S, Belzung C. Early life genetic, epigenetic and environmental factors shaping emotionality in rodents. *Neuroscience and Biobehavioral Reviews*. 2005;29:1335-1346.
- Hultman CM, Sparen P, Takei N, Murray RM, Cnattingus S. Prenatal and perinatal risk factors for schizophrenia, affective psychosis of early onset: case-control study. *British Medical Journal*. 1999;318:412-426.
- Jablonka E, Lamb MJ. The inheritance of acquired epigenetic variations. *Journal of Theoretical Biology*. 1989;139:69-83.
- Jablonka E, Lamb MJ. *Epigenetic Inheritance and Evolution: The Lamarckian Dimension*. New York: Oxford University Press; 1995.
- Jablonka E, Lamb MJ. Epigenetic inheritance in evolution. *Journal of Evolutionary Biology*. 1998A;11:159-183.
- Jablonka E, Lamb MJ. Genic neo-Darwinism – is it the whole story? *Journal of Evolutionary Biology*. 1998B;11:243-260.
- Jablonka E, Lamb MJ. *Evolution in Four Dimensions*. Cambridge (MA): The MIT Press; 2005.
- Jiménez-Chillarón JC, Díaz R, Ramón-Krauel M, Ribó S. Transgenerational Epigenetic Inheritance of Type 2 Diabetes. In: Tollefsbol T, editor. *Transgenerational Epigenetics: evidence and debate*. London: Academic Press; 2014. p 281-301.
- Jirtle RL. Epigenetics: How Genes and Environment Interact. National Institutes of Health (U.S). 2012 April. Available from: <http://videocast.nih.gov/summary.asp?Live=10520&bhcp=1>.
- Jirtle RL, Skinner MK. Environmental epigenomics and disease susceptibility. *Nature Reviews: Genetics*. 2007;8:253-262.
- Johannes F, Porcher E, Teixeira FK, Saliba-Colombani V, Simon M, Agier N, et al. Assessing

- the impact of transgenerational epigenetic variation on complex traits. *PLOS Genetics*. 2009;5.
- Kaati G. Case Studies on Epigenetic Inheritance. In: Haslberger AG, Gressler S, editors. *Epigenetics and Human Health*. KGaA, Weinheim: WILEY-VCH Verlag GmbH & Co; 2010. p 63-86.
- Kaati G, Bygren LO, Edvinsson S. Cardiovascular and diabetes mortality determined by nutrition during parents' and grandparents slow growth period. *European Journal of Human Genetics*. 2002;10:682-688.
- Kalisz S, Purugganan MD. Epialleles via DNA methylation: consequences for plant evolution. *TRENDS in Ecology and Evolution*. 2004;19(6):309-314.
- Kinnally EL, Feinberg C, Kim D, Ferguson K, Leibel R, Coplan JD. DNA methylation as a risk factor in the effects of early life stress. *Brain, Behavior, and Immunity*. 2011;25:1548-1553.
- Kirschner M, Gerhart J. Comment on "Epigenetic inheritance in evolution". *Journal of Evolutionary Biology*. 1998;11:213-217.
- Kitcher P. *Battling the undead: how (and how not) to resist genetic determinism. Thinking about Evolution: Historical, Philosophical, and Political Perspectives*. Cambridge, UK: Cambridge Univ. Press; 2001. p 396-414.
- Kuhn TS. *The Structure of Scientific Revolutions*. Chicago & London: The University of Chicago Press; 1962.
- Landecker H, Panofsky A. From social structure to gene regulation, and back: A critical introduction to environmental epigenetics for sociology. *Annual Review of Sociology*. 2013;39:333-357.
- Ledón-Rettig CC, Richards CL, Martin LB. Epigenetics for behavioral ecologists. *Behavioral Ecology*. 2012;311-324.
- Lester BM, Tronick E, Nestler E, Abel T, Kosofsky B, Kuzawa CW, et al. Behavioral Epigenetics. *Annals of the NY Academy of Sciences*. 2011;1266:14-33.
- Liu D, Diorio J, Tannenbaum B, Caldji C, Francis D, Freedman A, et al. Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. *Science*. 1997;277:1659-1662.
- Lucas A. Programming by early nutrition in man. *Ciba Foundation Symposia*. 1991;156:38-50.
- Mashoodh R, Champagne FA. Paternal Epigenetic Inheritance. In: Tollefsbol T, editor. *Transgenerational Epigenetics: evidence and debate*. London: Academic Press; 2014. p 221-235.

- Masterpasqua F. Psychology and Epigenetics. *Review of General Psychology*. 2009;13(3):194-201.
- Mastroeni D, McKee A, Grover A, Rogers J, Coleman PD. Epigenetic Differences in Cortical Neurons from a Pair of Monozygotic Twins Discordant for Alzheimer's Disease. *PLOS ONE*. 2009;4(8):1-6.
- McGowan PO, Szyf M. The epigenetics of social adversity in early life: Implications for mental health outcomes. *Neurobiology of Disease*. 2010;39:66-72.
- McVean G. Spandrels or spanners? The role of epigenetic inheritance in evolution. *Journal of Evolutionary Biology*. 1998;11:219-223.
- Meloni M, Testa G. Scrutinizing the epigenetics revolution. *BioSocieties*. 2014;9(4):431-456.
- Methylated Spirits. *The Economist*. 2009 Oct 15; Science and Technology.
- Migliore L, Coppedè F. The Impact of Genetic and Environmental Factors in Neurodegeneration: Emerging Role of Epigenetics. In: Haslberger AG, Gressler S, editors. *Epigenetics and Human Health*. KGaA, Weinheim: WILEY-VCH Verlag GmbH & Co; 2010. p 225-243.
- Miller CA, Sweatt JD. Covalent modification of DNA regulates memory formation. *Neuron*. 2007;53(6):857-869.
- Moore T. Comment on "Epigenetic inheritance in evolution". *Journal of Evolutionary Biology*. 1998;11:229-232.
- Morgan HD, Sutherland HE, Martin DIK, Whitelaw E. Epigenetic inheritance at the agouti locus in the mouse. *Nature Genetics*. 1999;23:314-318.
- Murrell A, Rakyan VK, Beck S. From genome to epigenome. *Human Molecular Genetics*. 2005;14(1):R3-R10.
- Neel JV. Diabetes Mellitus: A "Thrifty Genotype Rendered Detrimental by "Progress"? *American Journal of Human Genetics*. 1962;14, 353-362.
- Nestler E. Epigenetic inheritance: Fact or fiction? Report on Progress. The Dana Foundation. 2013 April. Available at: http://www.dana.org/news/features/detail_rop.aspx?id=42636.
- Oberlander TF, Weinberg J, Papsdorf M, Grunau R, Misri S, Devlin AM. Prenatal exposure to maternal depression, neonatal methylation of human glucocorticoid receptor gene (*NR3C1*) and infant cortisol stress responses. *Epigenetics*. 2008;3(2):97-106.
- Ordovás JM, Smith CE. Epigenetics and cardiovascular disease. *Nature Reviews Cardiology*. 2010;7:510-519.

- Pederson CB, Mortensen PB. Urbanization and traffic related exposures as risk factors for Schizophrenia. *BMC Psychiatry*. 2006;6(2).
- Pembrey ME, Bygren LO, Kaati G, Edvinsson S, Northstone K, Sjöström M, et al. and The ALSPAC Study Team. Sex-specific, male-line transgenerational responses in humans. *European Journal of Human Genetics*. 2006;14:159-166.
- Petronis A. Epigenetics and twins: three variations on the theme. *Trends in Genetics*. 2006;22:347-50.
- Petronis A. Epigenetics as a unifying principle in the aetiology of complex traits and diseases. *Nature*. 2010;465:721-727.
- Petronis A, Gottesman II, Kan P, Kennedy JL, Basile VS, Paterson AD, et al. Monozygotic Twins Exhibit Numerous Epigenetic Differences: Clues to Twin Discordance? *Schizophrenia Bulletin*. 2003;29(1):169-178.
- Pigliucci M. Do we need an extended evolutionary synthesis? *Evolution*. 2007;61(12):2743-2749.
- Poisoned Inheritance. *The Economist*. 2013 Dec 14; Science and Technology.
- Prickett AR, Oakey RJ. A survey of tissue-specific genomic imprinting in mammals. *Molecular Genetics Genomics*. 2012;287(8):621-630.
- Ptak C, Petronis A. Epigenetic approaches to psychiatric disorders. *Dialogues in Clinical Neurosciences*. 2010;12(1):25-35.
- Rachadoui N, Sarkar DK. Transgenerational Epigenetics and Brain Disorders. *International Review of Neurobiology*. 2014;115:51-73.
- Rapp RA, Wendel JF. Epigenetics and plant evolution. *New Phytologist*. 2005;168:81-91.
- Richards EJ. Inherited epigenetic variation-revisiting soft inheritance. *Nature*. 2006;6:395:401.
- Riggs AD. X inactivation, differentiation, and DNA methylation. *Cytogenetics and Cell Genetics*. 1975;14(1):9-25.
- Roseboom T, de Rooij S, Painter R. The Dutch famine and its long-term consequences for adult health. *Early Human Development*. 2006;82:485-491.
- Roseboom T, Painter R. Epidemiology of Transgenerational Epigenetics. In: Tollefsbol T, editor. *Transgenerational Epigenetics: evidence and debate*. London: Academic Press; 2014. p 59-66.
- Rothstein MA. Epigenetic Exceptionalism. *Journal of Law, Medicine & Ethics*. 2013;41(3):733-736.

- Rothstein MA, Cai Y, Marchant GE. The ghost in our genes: Legal and ethical implications of epigenetics. *Health Matrix Cleveland*. 2009;19(1):1-62.
- Rutten BPF, Mill J. Epigenetic Mediation of Environmental Influences in Major Psychotic Disorders. *Schizophrenia Bulletin*. 2009;35(6):1045-1056.
- Shachar-Dadon A, Schulkin J, Leshem M. Adversity Before Conception Will Affect Adult Progeny in Rats. *Developmental Psychology*. 2009;45(1):9-16.
- Shea N, Pen I, Uller T. Three epigenetic information channels and their different roles in evolution. *Journal of Evolutionary Biology*. 2011;24:1178-1187.
- Shulevitz J. Why Fathers Really Matter. *The New York Times*. 2012 Sept 8.
- Skinner MK. Role of Epigenetics in Developmental Biology and Transgenerational Inheritance. *Birth Defects Research (Part C)*. 2011;93:51-55.
- Singhal A, Fewtrell M, Cole TJ, Lucas A. Low nutrient intake and early growth for later insulin resistance in adolescents born preterm. *The Lancet*. 2003;361:1089-1097.
- Strohman RC. Ancient Genomes, Wise Bodies, Unhealthy People: Limits of a Genetic Paradigm in Biology and Medicine. *Perspectives in Biology and Medicine*. 1993;37(1):112-145.
- Sweatt JD. Experience-Dependent Epigenetic Modifications in the Central Nervous System. *Biological Psychiatry*. 2009;65:191-197.
- Sweatt JD. The emerging field of neuroepigenetics. *Neuron*. 2013;80(3):624-632.
- Tollefsbol TO. Transgenerational Epigenetics. In: Tollefsbol T, editor. *Transgenerational Epigenetics: evidence and debate*. London: Academic Press; 2014. p 1-8.
- Toyokawa S, Uddin M, Koenen KC, Galea S. How does the social environment 'get into the mind'? Epigenetics at the intersection of social and psychiatric epidemiology. *Social Science & Medicine*. 2012;74:67-74.
- Tsankova N, Renthal W, Kumar A, Nestler EJ. Epigenetic regulation in psychiatric disorders. *Nature Reviews Neuroscience*. 2007;8(5):355-367.
- Urduingio RG, Sanchez-Mut JV, Esteller M. Epigenetic mechanisms in neurological diseases: genes, syndromes, and therapies. *The Lancet Neurology*. 2009;8:1056-1072.
- Vineis P. The Research Program in Epigenetics: The Birth of a New Paradigm. In: Haslberger AG, Gressler S, editors. *Epigenetics and Human Health*. KGaA, Weinheim: WILEY-VCH Verlag GmbH & Co; 2010. p 3-6.
- Waddington CH. The epigenotype. *Endeavour*. 1942;1:18-20.

- Wadhwa PD, Buss C, Entringer S, Swanson JM. Developmental Origins of Health and Disease: Brief History of the Approach and Current Focus on Epigenetic Mechanisms. *Seminars in Reproductive Medicine*. 2009;27(5):358-368.
- Waterland RA, Jirtle RL. Transposable elements: Targets for Early Nutritional Effects on Epigenetic Gene Regulation. *Molecular Cell Biology*. 2003;23(15):5293-5300.
- Waterland RA, Michels KB. Epigenetic Epidemiology of the Developmental Origins Hypothesis. *Annual Review of Nutrition*. 2007;27:363-388.
- Watson JD, Crick FHC. Molecular Structure of Nucleic Acids. *Nature*. 1953;171:737-738.
- Wong AHC, Gottesman II, Petronis A. Phenotypic differences in genetically identical organisms: the epigenetic perspective. *Human Molecular Genetics*. 2005;14(1):R11-R18.
- World Health Organization. The right to health. 2013 Nov. Available from: <http://www.who.int/mediacentre/factsheets/fs323/en/>.
- World Health Organization. Cardiovascular diseases (CVDs). 2015 Jan. Available from: <http://www.who.int/mediacentre/factsheets/fs317/en/>.
- Wymore AS, Keeley ATH, Yturralde KM, Schroer MI, Propper CR, Whitham TG. Genes to ecosystems: exploring the frontiers of ecology with one of the smallest biological units. *New Phytologist*. 2011;191:19-36.
- Yehuda R, Engel SM, Brand SR, Seckl J, Marcus SM, Berkowitz GS. Transgenerational Effects of Posttraumatic Stress Disorder in Babies of Mothers Exposed to the World Trade Center Attacks during Pregnancy. *Journal of Clinical Endocrinology and Metabolism*. 2005;90(7):4115-4118.
- Yehuda R, Teicher MH, Seckl JR, Grossman RA, Morris A, Bierer LM. Parental Posttraumatic Stress Disorder as a Vulnerability Factor for Low Cortisol Trait in Offspring of Holocaust Survivors. *General Psychiatry*. 2007;64(9):1040-1048.
- Zaidan H, Leshem M, Gaisler-Salomon I. Prereproductive Stress to Female Rats Alters Corticotropin Releasing Factor Type 1 Expression in Ova and Behavior and Brain Corticotropin Releasing Factor Type 1 Expression in Offspring. *Biological Psychiatry*. 2013;74:680-687.
- Zullo A, Casamassimi A, Mancini FP, Napoli C. Cardiovascular Disease and Transgenerational Epigenetic Effects. In: Tollefsbol T, editor. *Transgenerational Epigenetics: evidence and debate*. London: Academic Press; 2014. p 321-341.
- Zuena AR, Mairesse J, Casolini P, Cinque C, Alema GS, Morley-Fletcher S, et al. Prenatal Restraint Stress Generates Two Distinct Behavioral and Neurochemical Profiles in Male and Female Rats. *PLoS ONE*. 2008;3(5): e2170.