Epigenetics in Pediatrics

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Educational Gap

Epigenetics mechanisms are involved in most diseases and represent the biological effect of some environmental exposures. As knowledge in the field increases, clinicians could see more epigenetically targeted treatments and preventive measures for pediatric conditions. Thus, it is imperative to have an understanding of these mechanisms and their association with disease.

Objectives  After completing this article, readers should be able to:

1. Recognize the different types of epigenetic mechanisms.
2. Understand the role of epigenetics in disease development.

Abstract

Epigenetic mechanisms are external modifications of DNA that cause changes in gene function and are involved in many diseases. Specific examples of pediatric diseases with a known or suspected epigenetic component include Beckwith-Wiedemann syndrome, childhood leukemia, allergies, asthma, fetal alcohol spectrum disorders, childhood obesity, and type 2 diabetes mellitus. Currently, epigenetically active treatments are being used to treat childhood leukemia. Potential epigenetically active treatments and preventive regimens are under study for other diseases. Pediatricians need to be aware of the epigenetic basis of disease to help inform clinical decision making in the future.

INTRODUCTION

A mother gives birth to monozygotic twins. One twin is large at birth and has an enlarged tongue and hypoglycemia. The other twin appears normal. What could be causing these profound clinical differences between 2 infants who are genetically identical?

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ABBREVIATIONS

AML  acute myeloid leukemia
BWS  Beckwith-Wiedemann syndrome
FAS  fetal alcohol syndrome
FASD  fetal alcohol spectrum disorder
miRNA  microRNA
mRNA  messenger RNA
ncRNA  noncoding RNA
SAM  S-adenosylmethionine
EPIGENETICS

Epigenetics (“atop” or “surrounding” genetics) refers to changes in gene function that do not alter its underlying structure of DNA but result in genes being switched on or off in a reversible way. Environmental exposures that occur throughout a person’s lifetime (from fetal life through old age) can not only cause somatic mutations within DNA but also affect how genes function and whether they are turned on and off without changing the DNA sequence. Although the field of epigenetics may seem a matter for biochemists and basic scientists, there are many clinically relevant aspects that require an understanding by all physicians, especially pediatricians. Epigenetic principles are currently used for diagnosis and treatment of some childhood diseases and will likely be used in the near future for predicting and preventing disease.

The field of epigenetics includes any mitotically or meiotically heritable change that does not change the actual DNA sequence. (1) Epigenetic mechanisms regulate gene expression, making it possible for genes to function differently in various tissues. This system also allows a more flexible way to respond to each individual’s environment. Because of this adaptability, epigenetic mechanisms have been suspected or identified in most diseases, including cancer, diabetes, obesity, asthma, and cardiovascular disease. Exposures throughout life, including toxins, diet, and stress, can influence epigenetic processes. Adverse maternal exposures during pregnancy are thought to be especially important for later disease development because establishment of epigenetic markers occurs during fetal development. This review describes currently known epigenetic mechanisms and explores how these are related or may be related to pediatric disease, treatment, and prevention.

EPIGENETIC MECHANISMS

There are 3 currently identified types of epigenetic mechanisms: DNA methylation, histone modification, and small, noncoding RNAs (ncRNA). These mechanisms work together to regulate gene expression patterns. DNA methylation and histone modification influence transcription, whereas ncRNAs may affect transcription (producing RNA copy of gene sequence) or interfere with translation (producing amino acid sequence from messenger RNA [mRNA] sequence). (2)

DNA Methylation

DNA methylation results in silencing the involved gene. During DNA methylation, a methyl group is added to a cytosine nucleotide (Figure 1). In humans, methylation occurs at a cytosine that is adjacent to a guanine nucleotide, called a CpG site. The methylation of the cytosine nucleotide is performed using a methyl group from S-adenosylmethionine (SAM). SAM is derived from methionine through the one-carbon metabolism pathway (Figure 2). Dietary factors, including folate, riboflavin, vitamin B12, betaine, and choline, are involved in generating methionine for SAM through this pathway. Thus, diet, especially during the prenatal period, is important in DNA methylation and its association with disease.

The most critical period for the establishment of DNA methylation is early gestation. Methylation of most genes is reset after fertilization through active demethylation of the paternal genome and passive demethylation of the maternal genome. (3) Remethylation of the genome takes place in a tissue-specific manner after implantation, leading to differential expression of certain genes in various tissues and cells. (4) DNA methylation is transmitted mitotically to daughter cells in a highly persistent manner. In fact, twin studies have found that methylation is heritable and more concordant in monozygotic twins compared with dizygotic twins. (4)(5) Although normally maintained, this process is reversible because methylation abnormalities are seen in cancer cells and DNA methylation tends to change with age. (6)(7)(8) For example, Fraga et al (9) found less concordance in global measures of methylation in older compared with younger monozygotic twins.

A special type of epigenetic effect associated with DNA methylation is genomic imprinting. Everyone inherits 2 copies of genes, one from each parent, usually resulting in both copies of the gene being expressed. Imprinting refers to the process of inactivating (marking) one copy of the gene so that only a single copy of a gene is expressed normally. Although generally involving DNA methylation, other epigenetic mechanisms make imprinted genes less prone to changes over time. (4)

Imprinting disorders are diseases caused by inappropriate functioning of the marks controlling gene expression at imprinted sites. In these diseases, the typical expression (one copy expressed) is altered so that either both copies are expressed or neither copy is expressed. There are several ways in which the expression at imprinted sites could be altered, including uniparental disomy in which both copies are from the same parent, genetic mutations, or an epigenetic change.

Histone Modifications

Genomic DNA is packaged into chromatin, creating a highly compact complex that consists of DNA and proteins. The structure of chromatin can be affected by a variety of factors, resulting in differences in gene expression. Chromatin
can be in either the inactive form heterochromatin, which results in transcriptional repression, or the active form euchromatin, allowing transcription and gene expression. Nucleosomes are the basic building blocks of chromatin and consist of approximately 146 base pairs of DNA wrapped around histone proteins. The N-terminal regions of these histones protrude from the nucleosome and can interact with other proteins. Sites on the N-terminal region are subject to posttranslational modification, including acetylation, methylation, ubiquitination, and phosphorylation (10) (Fig 1). These modifications result in differing gene expression.

**Noncoding RNAs**

Although protein-coding genes are well studied in genetics and genomics, these areas only account for a small fraction...
of the genome (approximately 2%–3%). Non–protein-coding regulatory regions are also highly relevant in gene expression regulation and disease processes. ncRNA is a broad category that can be broken into several different classes of which microRNA (miRNA) is the most commonly studied. These various classes of ncRNA interact with chromatin, mRNA, and other elements of transcriptional and translational processes.

miRNAs are small (only approximately 22 nucleotides in length) and act by interfering with the translation of mRNA into proteins, resulting in reduced gene function (11) (Fig 1). More than 60% of protein-coding genes are thought to be regulated by miRNAs, making them extremely prevalent in disease processes and normal cellular functioning.

EPIGENETICS IN PEDIATRIC DISEASE

Because epigenetic mechanisms are reset during early in utero development, the prenatal period is perhaps the most important in linking epigenetics to disease. Epigenetics provides at least part of the mechanism for how diseases processes occur prenatally, which is especially evident in obesity, diabetes mellitus, and metabolic syndrome. However, some research also indicates that postnatal, early childhood, and even adult exposures may play a significant role as well. To better understand how epigenetics is involved in disease, the following examples provide details on differing levels of evidence of epigenetic involvement in childhood disease.

Beckwith-Wiedemann Syndrome

Imprinting disorders are perhaps one of the most concrete examples of epigenetic influence on disease. Beckwith-Wiedemann syndrome (BWS) is an imprinting disorder with symptoms and signs that include macrosomia, macroglossia, visceromegaly of abdominal organs, umbilical defect, and hemihyperplasia, although clinical presentation is highly variable. (12) In BWS, monozygotic twins may be discordant for disease at birth even though their DNA sequence is exactly the same. (13)

There are several different molecular causes of BWS, leading to variability in presentation; therefore, there are no set clinical diagnostic criteria for this disease. Instead, diagnosis is based on clinical suspicion and a confirmatory molecular test. In approximately 20% of cases of BWS, a person has inherited both copies of a specific gene from his or her father, leading to some genes having increased expression and others having lower expression than expected. However, the most common cause of BWS is a change in DNA methylation, in particular genes leading to altered expression (52%–57%). (12) Other causes include genetic mutations in 10% and unknown causes in 13% to 15%. (12)

Early diagnosis of BWS is important because affected children are at higher risk for embryonal tumors, especially Wilms tumor and hepatoblastoma. (12) The specific location of loss of imprinting greatly affects the risk of tumor development. Depending on the genes affected, tumor risk can range from approximately 3% to 43%. (14) Thus, knowing the specific epigenetic alteration can help inform clinical monitoring for cancer in these patients.

Childhood Acute Myeloid Leukemia

Some of the earliest work in epigenetics has been in cancer because epigenetic changes can be clearly documented in cancer cells. Childhood cancer is likely no exception, but because cancer in children is rare, epigenetics processes are less well known. The most common form of childhood
cancer is leukemia, accounting for approximately 30% of all cancer diagnoses. (15) Two subtypes account for most cases: acute lymphoblastic leukemia and acute myeloid leukemia (AML). AML accounts for only approximately 18% of all childhood leukemia diagnoses. (16) Although found less commonly in children, some genes involved in DNA methylation regulation are known to be mutated in AML. In addition, many environmental risk factors associated with changes in DNA methylation have been suggested, including advanced maternal age, increased birth weight, and maternal use of alcohol during pregnancy. (17) These risk factors, along with findings of epigenetic changes in diseased cells, indicate a role for epigenetics in the development of childhood AML. (18)

Epigenetic treatments are in use or being tested in adult and childhood AML. DNA-demethylating agents are currently being used to treat adult AML and myelodysplastic syndromes. (19) In children, testing of demethylating agents to treat relapsed or refractory AML is in the initial stages. (20) Another possible epigenetic treatment involves agents that change histone modifications. These drugs are being tested in clinical trials in adults with AML. (20) In the case of cancer epigenetics, treatments may be easier to develop because they can be specifically targeted to the cancer cells rather than a more global application. However, problems still arise because of the lack of specificity of epigenetic treatments, leading to adverse effects. For example, demethylating agents are carcinogenic in animal studies, potentially leading to the development of secondary cancers mediated by a global reduction in DNA methylation that leads to increased expression of growth-promoting genes. (14)

Asthma

The development of asthma is thought to have a large environmental component and has been linked to exposures such as air pollution, cigarette smoke, and fuel exhaust. (21) Two sets of complementary studies are being performed to examine how epigenetic changes relate to exposures and disease. One set links the exposure to epigenetic changes, and the other links these changes to disease risk.

The epidemiologic evidence of an association between maternal smoking and asthma is strong. (22) Given this consistent association, it makes sense to explore further how epigenetics could link maternal smoking to childhood asthma. The initial step in this process is to explore epigenetic changes based on maternal smoking. In multiple tissue types, global DNA methylation has been altered after exposure to maternal smoking. (23) Gene-specific differences have also been documented. Joubert et al (24) found that maternal smoking was associated with methylation changes in 3 genes in cord blood from a large cohort and a small replication sample. Supporting evidence is found in a study in adults. One of the same genes identified by Joubert et al was found to be differentially methylated in the peripheral blood of those with long-term exposure to tobacco smoke. (25) Only one study so far has been able to link differential DNA methylation in cord blood based on exposure to a particular constituent of tobacco smoke to early childhood asthma. (23)

Air pollution can induce epigenetic changes and is also a well-established risk factor for the development of asthma. However, air pollution is really a group of different exposures and so is much more difficult to study. Although not as strong as the evidence for maternal smoking, childhood exposure to air pollution is still important in the development of asthma. (26)(27) A number of studies have also found an association between air pollution and epigenetic changes. For example, a study in California found that children living in cities with higher levels of pollution had changes in DNA methylation at a specific site related to T-cell function. (27) Other studies have found genespecific DNA methylation, histone changes, and miRNA changes after exposure to different types of air pollution. (26) The next step in research would be to link these epigenetic changes with childhood asthma. Currently, research is sparse and somewhat inconsistent. Although some differences in DNA methylation have been found in airway epithelial cells in children with asthma and allergies, additional studies have found no differences compared with control children. (21) Additional research has found that certain genes known to be associated with asthma and allergies are subject to epigenetic regulation, suggesting a link with an unknown exposure. (22) More research is needed to assess fully the association between epigenetic changes due to air pollution exposure and the development of childhood asthma.

Another more clinically relevant area is the use of epigenetic treatments in childhood asthma. Several trials have been conducted using choline and vitamin B6. As previously mentioned, dietary factors such as choline and vitamin B6 are involved in the generation of methionine for DNA methylation through one-carbon metabolism and thus affect DNA methylation and histone methylation. Studies exploring choline dietary supplementation suggested improvement in symptoms in adult patients but were based on relatively small numbers. (28) Other dietary trials used vitamin B12 as a possible treatment. Although one study in adults and one in children found improvement in symptoms, another study did not. (28) Additional studies are needed in children to confirm an effect of choline or vitamin B6, as well as other epigenetic
dietary factors, to determine their role in the treatment of childhood asthma.

**Fetal Alcohol Syndrome**

Maternal alcohol use during pregnancy is one of the largest contributors to intellectual disability in the world and can result in a child with a fetal alcohol spectrum disorder (FASD). FASDs include fetal alcohol syndrome (FAS), partial FAS, alcohol-related neurodevelopmental disorder, and alcohol-related birth defects. Clinical features of FASDs can include minor facial anomalies (short palpebral fissures, smooth philtrum and thin vermilion border of the upper lip), prenatal and/or postnatal growth deficiency, and central nervous system structural and functional abnormalities. (29)

One possible pathway for a maternal alcohol use effect in epigenetics is through its role in the pathways that convert dietary components to methionine for DNA methylation (Fig 2). Alcohol interferes with folate metabolism and reduces overall methylation levels in mice exposed to alcohol in utero. (30)(31) Other studies using mouse models have found specific sites with altered DNA methylation, as well as alterations in histones and ncRNAs, after in utero exposure to alcohol. (32) Human studies are currently lacking in this area.

Another area of research in FAS involves treatments to help minimize some of the harmful effects of prenatal alcohol exposure. Animal studies have documented that choline ameliorates some of the associated problems in central nervous system function. (33)(34)(35)(36)(37) Trials are under way in human populations, both during pregnancy and in children who have been exposed to alcohol in utero, to determine whether choline can be beneficial in children with FAS. (38) Studies in mice indicate altered epigenetics in the fetal brain due to a low-choline maternal diet. (39) Jiang et al (40) found that choline supplementation in pregnant women could alter the DNA methylation pattern in cortisol-regulating genes in the placenta and cord blood. Thus, it will be informative to determine the role of choline supplementation during pregnancy and in childhood as a possible treatment for FAS.

**Obesity, Diabetes, and Metabolic Syndrome**

After a significant increase in the 1980s and 1990s, the number of children considered obese may have stabilized in recent years. However, the prevalence of obesity is still almost 17% among children aged 2 to 19 years in the United States. (41) This high number leads to several comorbid conditions during childhood, including type 2 diabetes and metabolic syndrome. Some of this increase in adverse childhood outcomes can be linked to maternal exposures during pregnancy, and research is beginning to link maternal exposure to epigenetic mechanisms.

In adults, there has been increasing evidence of an association between maternal gestational exposures and adult development of obesity, diabetes, and coronary heart disease. Studies have suggested that the mechanism for this association is through modified epigenetics. One example is work performed on the long-term outcome of children exposed prenatally to famine in the Netherlands during the Dutch Hunger Winter in 1944–1945. Studies of this population of children documented that those exposed to famine during early gestation had differential methylation in the IGF2 gene compared with same-sex siblings who were not exposed. (42) This association was not seen for those exposed to famine during late gestation. Those exposed to famine during early gestation were also at increased risk of coronary heart disease and obesity in adulthood. (43)

Although few studies have been performed in children, there is some evidence that the imprinted sites around the genes H19 and IGF2 play a role in childhood obesity and other metabolic dysfunctions. (44) This is the same region involved in the overgrowth and obesity associated with BWS. A study found that DNA methylation levels at H19 and IGF2 in placental tissue and cord blood correlated with birth weight. (45) Maternal diabetes during pregnancy is also a potential risk factor for metabolic dysfunctions; one study that used a genome-wide methylation array found differential methylation patterns in children born to diabetic mothers vs control children. (46) Although more work needs to be done, evidence is emerging that maternal exposures during pregnancy have an effect on obesity, diabetes, and metabolic syndrome in childhood.

**CONCLUSION**

The promise of epigenetics is in the ability to harness specific markers of disease status and devise treatments to prevent or treat disease, which is especially important in pediatrics because early interventions can greatly alter a child’s life. Some epigenetic treatments are already used in cancer, and some trials of other epigenetic treatments are currently under way, even if the specific epigenetic mechanisms are not well understood.

Many interesting associations have been found between epigenetic alterations and exposures or disease, but, for the most part, the pathways and processes have not been fully elucidated. However, epigenetic mechanisms may be linked to a number of different pediatric and adult diseases, and the ability to use this information clinically holds great potential.
Summary

- On the basis of evidence and consensus, the 3 types of epigenetic mechanisms are DNA methylation, histone modification, and ncRNA interaction. (2)
- On the basis of consistent data from observational studies, Beckwith-Wiedemann syndrome is caused by an epigenetic change in 52% to 57% of cases. (12)
- On the basis of clinical studies, epigenetic changes are present in childhood acute myeloid leukemia (AML). (18) and on the basis of epidemiologic data, some risk factors for AML are also linked to epigenetic changes. (17)
- On the basis of strong evidence from animal studies, DNA methylation is altered in fetal alcohol syndrome (30)(31)(32) and may be at least partly corrected by choline supplementation. (33)(34)(35)(36)(37)
- On the basis of consistent observational data, maternal smoking is a risk factor for childhood asthma. (22) On the basis of limited observation studies, a link between maternal smoking and epigenetic changes has been identified, and a single study has linked maternal smoking to childhood asthma. (23)(24)
- On the basis of limited observational studies, methylation levels of growth-related genes have been associated with birth weight, and in limited observational studies, these regions have been linked to childhood obesity. (44)(45)

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PIR Quiz

1. You are describing the importance of early prenatal care and appropriate nutrition to a group of first-year medical students participating in an Introduction to Clinical Medicine course. While discussing the interaction between genetics and environment, you note that several dietary factors, including folate and vitamin B₁₂, are important in facilitating:
   A. Apoptosis.
   B. Copy number variation.
   C. DNA methylation.
   D. Histone modification.
   E. Noncoding RNA gene silencing.

2. Numerous environmental risk factors, including maternal age, increased birth weight, and maternal use of alcohol during pregnancy, have been associated with epigenetic changes. These changes:
   A. Are heritable.
   B. Are permanent.
   C. Change DNA structure.
   D. Occur in all tissues to the same degree.
   E. Occur only during fetal development.

3. A 6-month-old infant is seen in your clinic with physical examination findings that include macroglossia, macrosomia, umbilical hernia, visceromegaly, ear lobe creases, and cryptorchidism. The most common cause of this syndromic condition is:
   A. A change in DNA methylation.
   B. A genetic mutation.
   C. Teratogen exposure.
   D. Uniparental disomy.
   E. Unknown.

4. A pregnant 19-year-old college student who does not drink sees no reason to stop smoking during pregnancy. She wants to know how smoking can hurt the fetus. You explain that there is strong evidence that the offspring of women who smoke during pregnancy will be at higher risk for developing:
   A. Asthma.
   B. Depression.
   C. Diabetes mellitus.
   D. Learning disability.
   E. Obesity.

5. You are counseling an 11-year-old about his weight. His body mass index is 30, and his weight curve continues to move above the 98th percentile for age. The child responds by telling you, “It’s not my fault. Everyone in my family is heavy. It’s genetic!” To some degree, he may be correct. Of the following, which maternal epigenetic factor has been associated with obesity in offspring?
   A. Alcohol exposure.
   B. Gestational diabetes.
   C. Hyperthermia.
   D. Poor third trimester nutrition.
   E. Smoking.
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