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Review Article

# Investigating Epigenetic Effects of Prenatal Exposure to Toxic Metals in Newborns: Challenges and Benefits

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## Key Words

Arsenic · Lead · Prenatal exposure · DNA methylation · Epigenetics

## Abstract

Increasing evidence suggests that epigenetic alterations can have a great impact on human health, and that epigenetic mechanisms (DNA methylation, histone modifications, and microRNAs) may be particularly relevant in responding to environmental toxicant exposure early in life. The epigenome plays a vital role in embryonic development, tissue differentiation, and development of disease by controlling gene expression. In this review, we discuss what is currently known about epigenetic alterations in response to prenatal exposure to inorganic arsenic (iAs) and lead (Pb), focusing specifically on their effects on DNA methylation. We then describe how epigenetic alterations are studied in newborns as potential biomarkers of in utero environmental toxicant exposure, and the benefits and challenges of this approach. In summary, the studies highlighted herein indicate how epigenetic mechanisms have an impact on early-life exposure to iAs and Pb and the research that is being done to move towards an understanding of the relationships between toxicant-induced epigenetic alterations and disease development. Although much remains unknown, several groups are working to understand the correlative and causal effects of early-life toxic metal exposure on epigenetic changes and to find out how these may result in later disease development.

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## Introduction

Environmental contaminants including toxic metals are widespread and often disproportionately affect certain populations within the USA. Toxic metal exposures have been associated with a number of diseases, including cardiovascular, neurological, and autoimmune diseases as well as cancer [1]. The molecular mechanisms underlying toxic metal-induced diseases are complex and in many cases can be linked to oxidative stress and altered expression of genes in key cellular pathways [2]. Recent reports have demonstrated epigenetic alterations with toxic metal exposure [3–5].

Current research efforts have focused on a growing understanding of the importance of the epigenome and the role it plays in cellular homeostasis. Studies are also beginning to shed light on the notion that epigenetic alterations resulting from early-life exposures may play a major role in mediating the links between disease and the environment. This review focuses on toxic metal-induced dysregulation of DNA methylation, with particular attention given to imprinted genes. Herein, we highlight the effects of exposure to inorganic arsenic (iAs) and lead (Pb) on DNA methylation. We discuss how epigenetic alterations are being studied in newborns as biomarkers of in utero environmental toxicant exposure and the benefits and challenges of this approach. Lastly, we provide a brief summary of unresolved questions about how iAs and Pb exposures alter epigenetic profiles in early life and the larger implications of these changes.

## Developmental Origins of Disease and Epigenetics

The developmental origins of disease hypothesis postulates that altered maternal nutrition and/or endocrine status during prenatal development result in persistent changes in development, physiology, and metabolism, predisposing the developing embryo or fetus to cardiovascular, metabolic, and endocrine-related diseases in adult life [6]. This is thought to reflect the heightened vulnerability to environmental influences during specific developmental periods. Human and animal studies support that prenatal growth is substantially influenced by the in utero environment.

Fetal programming is a phenomenon whereby a stimulus or insult during critical periods can have persistent effects on the structure, physiology, and metabolism of that individual later in life [6]. Environmental exposures during early development can have lasting effects, and epigenetic modifications play a vital role in the response to in utero environmental factors such as toxic exposures and nutrition. These exposures result in changes in regulatory and growth-related gene expression that are important components of fetal programming.

Epigenetics refers to modifications that occur ‘above the genome’ that provide somatically heritable, stable regulatory information outside the DNA sequence [7]. DNA methylation involves the addition of a methyl group to the 5-carbon position of the cytosine ring [5-methylcytosine (5-mC)] via DNA methyltransferase enzymes. Cytosines followed by guanines are the targets of DNA methylation, and most CpG dinucleotides are methylated throughout the genome. The exception to this is CpG islands, which are stretches of sequences that are densely populated with unmethylated CpGs. These CpG islands, and the sequence immediately adjacent to CpG islands (CpG shores [8]), can exhibit altered methylation resulting from environmental influences, and this can contribute to disease. Some CpG islands are methylated differentially on the two chromosomal copies inherited from each parent; these are associated with genomically imprinted genes as discussed below. DNA methylation plays an important role in the regulation of transcription by either attracting or inhibiting the binding of transcriptional modulators. Changes in the DNA methylation patterns are the most common

alterations in cancers [9]. Complete reprogramming of DNA methylation occurs during gametogenesis and just after fertilization (when cell- and tissue-specific DNA methylation patterns are established), and these patterns can be modified during puberty and during the aging process [9]. DNA methylation patterns are likely most susceptible to environmental influences during the processes of methylation reprogramming and during maintenance methylation as cells prepare for division. Prior to dividing, the methylation profiles need to be faithfully replicated on the nascent DNA of the daughter cell. DNA synthesis rates are high during prenatal development and this may represent one of the most critical windows of vulnerability to environmental influences on the epigenome.

## Toxic Metal Exposure and Epigenetics

### *Arsenic*

Arsenic and lead are ranked number one and two on the Agency for Toxic Substances and Disease Registry (ATSDR) 2011 Substance Priority List [10]. This list ranks substances based on their toxicities and potential for human exposure at locations on the National Priorities List (NPL). Arsenic occurs naturally in the environment as an element of the earth's crust. When combined with other elements such as oxygen, chlorine and sulfur, As becomes an inorganic compound (iAs). Higher-than-average iAs is found in certain occupational settings, hazardous waste sites, and areas with high levels of naturally occurring iAs (soil, rocks, and water). Drinking water contaminated with iAs is the major source of iAs exposure worldwide. The World Health Organization (WHO) recommends that iAs in drinking water must not exceed 10 parts per billion, but it is estimated that more than 100 million people worldwide are exposed to levels of iAs in drinking water that are considered harmful to human health [11]. Although iAs exposure has been extensively studied and linked to numerous chronic conditions including cardiovascular disease, diabetes mellitus, neurological effects and cancers of the skin, lung, liver, and urinary bladder [12], the precise molecular mechanisms connecting exposure to disease are not well understood.

Evidence suggests that there are long-term health consequences of prenatal iAs exposure and that this may occur through epigenetic mechanisms. iAs exposure and effects on DNA methylation have been studied in vitro, in vivo, and within human populations, as reviewed in [13], yet the biological consequences of the observed changes have not been established. In addition, a causal relationship between iAs exposure, DNA methylation changes, and oncogenesis has not been established. The long-term health consequences associated with prenatal iAs exposure support that iAs may exert its effects through epigenetic mechanisms, as it is not a mutagen. The effects of chronic iAs exposure and development of disease have been more extensively described by Bailey and Fry [13].

### *Lead*

Pb is a naturally occurring metal and a ubiquitous nondegradable toxic pollutant of the environment through natural and anthropogenic sources. Pb has both acute and chronic effects on human health. The most common sources of Pb exposure are inhalation of Pb-contaminated dust, ingestion of Pb-tainted food and/or water, and direct contact with Pb-polluted soil. As a result of the government-mandated removal of Pb from paints and gasoline in the USA, it is less of a contamination hazard; yet it remains a threat to human health as it can still be found in many products including batteries, ceramics, toys, and plumbing pipes.

Within the USA, there are populations at a higher risk of Pb exposure based on the age of their housing and their occupation. Children belong to the highest risk group because they remain most vulnerable to Pb for several years following birth during brain and neurological

development. In addition, they are more sensitive to Pb poisoning because they have thinner skin that more easily absorbs Pb. Young children frequently put items in their mouth, which increases exposure if the items contain Pb or are contaminated with Pb from other sources [14]. The ability of Pb to freely cross the placental barrier and to be mobilized from maternal bone stores during pregnancy place the developing infant at risk of Pb exposure, and even low-level exposure may be harmful [15]. Epidemiological studies provide compelling evidence that blood Pb levels above the current Centers for Disease Control action level (5 µg/dl) have detrimental effects on the developing brain. Pb exposure-related health outcomes have been most often studied in the field of neurological development and disease. Even at very low levels, prenatal Pb exposure results in poorer cognitive performance in boys [16]. The mechanisms by which prenatal Pb exposure compromises human development and leads to late-onset disease are not fully understood but may involve DNA methylation alterations. There are few studies to date on the epigenetic effects of prenatal Pb exposure in humans. In 2009, Pilsner et al. [17] published the first human study showing that cumulative measures of Pb in maternal bone were associated with changes in DNA methylation levels in the umbilical cord blood (UCB) leukocytes of the offspring.

Toxic metals are widespread environmental contaminants, and there has been an increased interest in understanding the molecular factors involved in the etiology of metal-induced diseases in recent years [18]. There are several studies showing epigenetic alterations following environmental toxicant exposure, implicating epigenetic mechanisms as a potential link between exposure and later-life disease.

### DNA Methylation and Genomic Imprinting

Approximately 90 genes in humans have been identified thus far that are subject to a unique form of gene regulation referred to as genomic imprinting, whereby only one of the two inherited parental alleles is functional. The other allele is permanently silenced in a parental origin-dependent manner by epigenetic mechanisms, including DNA methylation that is differentially established in sperm and egg. Methylation patterns in these regulatory regions of imprinted genes may be particularly susceptible to environmental effects [19].

Appropriate expression of imprinted genes is critical for normal growth and development. These genes are often organized in clusters within imprinted domains and are coordinately regulated by imprinting control regions. Therefore, the disruption of epigenetic regulatory mechanisms at these regions can alter the imprinting and/or expression of multiple imprinted genes [20]. The DNA methylation patterns associated with imprinted genes are mitotically heritable [21]. In very early development, the epigenetic state of the cell, and perhaps at imprinted genes, is labile and can be influenced by environmental factors. Accumulating evidence supports the notion that early life is a critical window of vulnerability during which there may be an increased susceptibility to epigenetic dysregulation at imprinted regulatory regions.

### Investigating Past Environmental Exposures in Newborns

Based on the developmental origins of disease hypothesis, a likely means by which early-life environmental exposures cause late-onset disease is through epigenetic mechanisms, particularly during reprogramming of the epigenome that takes place during embryonic development. The epigenome is highly vulnerable to environmental insults during this time [22]. Understanding epigenetic events in early life and how environmental exposures influence fetal outcomes is important for elucidating molecular mechanisms and how the

epigenome can be modified or manipulated to prevent later undesirable outcomes. The study of epigenetics and perinatal health is becoming increasingly important as the body of evidence supporting the idea that diverse environmental exposures can alter epigenetic programming and the transgenerational risk of disease is growing. Perinatal exposures to As, tobacco smoke, air pollutants, and endocrine-disrupting chemicals have all been shown to alter epigenetic profiles [23].

#### *Evidence of Exposure in the Prenatal Environment*

To date, there are several studies that seek to better understand how environmental exposures in utero affect the epigenetic profile of the offspring. Individuals exposed to severe caloric restriction in utero have a higher incidence of several chronic adult diseases including type 2 diabetes, coronary heart disease, neurological disorders, obesity, and certain cancers [24]. One of the most profound findings of the Dutch famine study that has followed individuals exposed to severe caloric restriction in utero is that the changes in methylation were still detectable six decades after the caloric restriction occurred. This demonstrates that the effects of exposure are persistent, supporting the idea that historical exposure information is stably archived by the genome [25]. Studies investigating maternal nutrition have found that folic acid supplementation before or during pregnancy is associated with altered DNA methylation at two differentially methylated regions regulating imprinted insulin-like growth factor 2, with males showing more prominent effects than females [26]. Additional work by our group has shown that maternal influences including smoking status, BMI, depressed mood, and antibiotic use during pregnancy can result in altered DNA methylation profiles at imprinted gene regulatory regions in newborns. High prenatal iAs exposure and DNA methylation at LINE-1 repetitive elements are positively associated in both maternal and fetal leukocytes [27]. In addition, we have previously published on prenatal iAs exposure and effects on the epigenome in UCB [28]. Prenatal Pb exposure has been studied in the Early Life Exposures in Mexico to Environmental Toxicants (ELEMENTS) cohort, in which maternal patella Pb is associated with UCB LINE-1 methylation, while maternal tibia Pb is negatively associated with UCB genomic DNA methylation of Alu repeats [17].

### **Benefits and Challenges in Measuring Exposures in Newborns**

#### *Benefits*

A better understanding of the epigenetic targets and mechanisms involved is required if we hope to prevent adult onset disease directly related to suboptimal conditions in the intra-uterine environment. Studying epigenetic alterations in newborns from samples collected at the time of parturition provides the benefit of identifying changes that resulted from what was experienced in utero. From these types of analyses, it may also be possible to detect changes that were heritably transmitted through the gametes of the prior generation. A benefit of measuring iAs and Pb exposure in cord blood is that these cells may be exposed to higher maternal blood levels of toxic metals. If calcium intake is not sufficient during pregnancy, maternal bone stores of toxic metals are often released into the blood stream due to the bone resorption process, thus exposing the infant to increased levels of toxic metals. Studying epigenetic profiles in newborns can provide information about the collective effects of the in utero environment on the infant's epigenome.

#### *Challenges*

There are considerable challenges associated with studying the effects of prenatal iAs and Pb exposure and epigenetic mechanisms in newborns. Noncancer cohort studies investi-

gating epigenetics in preclinical populations with specific environmental exposures have the unique challenge of relying on surrogate tissues (buccal swabs, cord blood, placentas, etc.), which may have variable epigenetic correlations with target tissues. Measuring iAs and Pb exposure in surrogate tissues presents challenges in terms of explaining the biological relevance of these effects. In vitro studies have relied on immortalized cell lines for studying epigenetic mechanisms of iAs and Pb exposure, which may not be representative of in vivo effects. An additional challenge faced in environmental exposure studies is the understanding of the relationship between DNA methylation, histone modifications, and miRNAs at specific gene targets and how alterations in these mechanisms together contribute to disease outcome. All three epigenetic mechanisms are rarely studied concurrently, and functional endpoints have seldom been assessed in relation to the epigenetic modifications. Lastly, we do not expect to see large differences in methylation from in utero exposures as such differences would likely not be compatible with viability [29]. Thus, analysis requires technologies that are capable of detecting small differences in DNA methylation.

### Conclusions and Future Directions

The studies highlighted in this review indicate that the epigenome is impacted by early-life exposure to iAs and Pb. A causal relationship between toxicant-induced epigenetic alterations and disease development has not been established. More work is needed to understand the causal effects of early-life toxic metal exposure on epigenetic changes and how these changes result in the onset/progression of various disease states.

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