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# Long-term health consequences of prenatal arsenic exposure: Impacts to the genome and the epigenome

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## Abstract

Arsenic continues to poison the water of millions of individuals around the globe. In spite of the potentially devastating effects of arsenic on worldwide human health, the impacts of such exposure on vulnerable populations including pregnant women and their infants are understudied. Data from human populations exposed early in life highlight the increased mortality risks related to both cancer and non-cancer endpoints. The molecular underpinnings for these effects are largely unknown. Here we highlight the current studies linking prenatal arsenic exposure and health effects, particularly those that examined associations between arsenic exposure and altered genomic and epigenetic signaling. Current needs in the field to increase our understanding of the molecular basis for adult disease are mentioned.

### Keywords

arsenic; prenatal exposure; genome; epigenome

## Introduction

Inorganic arsenic (iAs) continues to poison individuals around the globe with current estimates exceeding 100 million people (Uddin and Huda 2011). While the World Health Organization (WHO) established a recommended limit of 10 μg/L (WHO 2006) for arsenic in drinking water, levels that exceed this value have been detected in drinking water sources in areas throughout the world, including but not limited to Bangladesh, India, Mexico, the United States, and Vietnam (ATSDR 2007). New locations with drinking water contaminated with iAs continue to be identified as highlighted by our recent identification of elevated iAs in private wells in North Carolina (Ayotte, Montgomery et al. 2003, Camacho, Gutiérrez et al. 2011, Sanders, Messier et al. 2012). These elevated levels are of concern as iAs is a known carcinogen with target sites including the liver, lung, prostate, skin, and urinary bladder (NTP 2011). Exposure to iAs has also been linked to a variety of non-cancer health effects as well, including adverse effects on memory and intellectual function,

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diabetes, heart disease, liver hypertrophy, and respiratory system disease (Kapaj, Peterson et al. 2006).

## Prenatal arsenic exposure and health effects

Prenatal and early-life exposure to iAs and subsequent health effects represents a global health issue yet is one that is understudied. iAs has toxic effects on the developing fetus in which even fairly modest levels have been associated with detrimental birth outcomes. Such effects of exposure include decreased birth weight, decreased head and chest circumferences (Rahman, Vahter et al. 2009) as well as increased risk of infection in infants (Rahman, Vahter et al. 2011, Farzan, Korrick et al. 2013). Retrospective cohort analyses have highlighted that in addition to the immediate health effects, early-life exposure to iAs is linked to adult diseases including both cancer and non-cancer endpoints (Dauphiné, Ferreccio et al. 2011, Smith, Marshall et al. 2012, Naujokas, Anderson et al. 2013).

## Susceptibility factors influencing response to iAs

In addition to life stage at the time of exposure, the efficiency with which individuals metabolize iAs is a known risk factor for disease. iAs is metabolized in humans to produce monomethylated and dimethylated arsenicals (MMAs and DMAs, respectively). The six major urinary arsenicals associated with iAs exposure include arsenite (iAsIII), arsenate (iAsV), monomethylarsonous acid (MMAIII), monomethylarsonic acid (MMAV), dimethylarsinous acid (DMAIII) and dimethylarsinic acid (DMAV) (Thomas, Styblo et al. 2001, Vahter 2002). In general, total urinary arsenic in iAs-exposed individuals is composed of 10–20% total (i.e. trivalent + pentavalent) iAs, 10–20% total MMAs, and 60–80% total DMAs (Vahter 1999, Vahter 2002). The detection of organoarsenicals such as arsenobetaine, arsenocholine, and arsenosugars in urine are a result of their presence in seafood and are not products of iAs metabolism (Choi, Choi et al. 2010).

High urinary proportions of MMAs and high ratios of MMAs/DMAs, which are thought to be indicators of an inefficient methylation of iAs, have been associated with the development of several adverse effects in humans including urinary bladder cancer, non-melanoma skin cancers, carotid atherosclerosis, and chromosomal aberrations (reviewed in (Tseng 2007)). A single enzyme, arsenic (+3 oxidation state) methyltransferase (AS3MT), has been shown to catalyze both the oxidative methylation and reduction reactions (Thomas, Waters et al. 2004). It has recently been shown that the genotype for *AS3MT* is associated with levels of urinary proportions of MMAs and DMAs. It was also demonstrated that *AS3MT* genotype is associated with risk for disease (e.g. skin lesions)(Pierce, Kibriya et al. 2012). These data highlight the role of underlying genetics in iAs metabolism and the susceptibility to iAs-induced disease. In consideration of prenatal exposure, it is known that pregnancy can alter the metabolism efficiency of iAs (Hopenhayn, Huang et al. 2003, Gardner, Engström et al. 2012). However, the impact of altered metabolism on pregnant women's or children's health is not well established.

## Molecular signaling events associated with prenatal exposure: Genomic signaling events

Because of the serious health impacts resulting from iAs exposure during critical times of development, understanding the biological mechanisms that underlie these effects is of utmost importance. Prenatal exposure to arsenic has been related to alterations in gene expression profiles in both rodents (Liu, Xie et al. 2004, Liu, Xie et al. 2006) and humans (Fry, Navasumrit et al. 2007). Groundbreaking work from the Waalkes laboratory demonstrated that mice exposed transplacentally to iAs have altered expression levels of oncogenes, tumor suppressor genes, and stress-related genes in their livers as adults (Liu, Xie et al. 2004, Liu, Xie et al. 2006). In humans, gene expression changes associated with inflammatory signaling pathways are altered in cord blood samples from newborns exposed to varying levels of iAs (Fry, Navasumrit et al. 2007). These altered transcriptional changes were predicted to be regulated by transcription factors including nuclear factor kappa beta (NF-κB) and metal regulatory transcription factor 1 (MTF1). The iAs-associated transcriptional changes were linked to other pro-inflammatory pathways such as those mediated by interleukin 1 (IL-1). Pro-inflammatory signaling has been shown to be altered in peripheral blood leukocytes (PBLs) of adults with iAs-induced skin lesions compared to adults without lesions (Argos, Kibriya et al. 2006). These data suggest that the modulation of transcriptional regulators and their subsequent activation of their targets is a key factor in the cellular response to arsenic. In future research it would be of interest to determine whether genotype for these iAs-responsive transcription factors influence iAs-associated disease and to establish which molecular events are directly related to the transcriptional activation in humans.

## Epigenetic signaling events

In addition to the control that transcription factors play in the response to prenatal iAs exposure, it is hypothesized that various epigenetic mechanisms may regulate transcriptional changes. There is increased understanding of the role of the epigenome in maintaining cellular homeostasis. Currently, the most studied components of the epigenome are cytosine DNA methylation at CpG sites, covalent post-translational histone modifications, and microRNAs (miRNAs) (Baccarelli and Bollati 2009). These epigenetic components play crucial roles in the regulation of gene expression, collectively acting at both transcriptional and post-transcriptional levels (Haluskova 2010).

The best studied of these three epigenetic modifications is DNA methylation, specifically 5-meC. The establishment of fetal DNA methylation patterns is essential for proper development and can influence the health status of individuals in adulthood (Kim, Friso et al. 2009). The impact of iAs on the DNA methylome therefore is a tantalizing potential mechanism that may link exposure to long-term health effects. Some of the earliest evidence linking prenatal exposure with altered patterns of DNA methylation was in the mouse model where newborn males exposed to a carcinogenic dose of iAsIII had global reduction of DNA methylation in GC-rich regions and altered expression of multiple genes in the liver (Xie, Liu et al. 2007).

Links between iAs exposure, DNA methylation and dietary compounds have been explored. The Gamble laboratory demonstrated that folate availability plays an important role in the DNA methylation patterns of iAs-exposed populations. In exposed populations in Bangladesh, there was a positive association of global hypermethylation of PBL DNA with iAs concentrations in urine and plasma, but only when plasma blood folate concentrations were sufficient (Pilsner, Liu et al. 2007, Pilsner, Liu et al. 2009). In the latter study, this effect was observed only among individuals without iAs-associated skin lesions. Our laboratory has examined genome-wide, gene-specific DNA methylation in iAs-exposed individuals. In a Mexican cohort, we demonstrated that the promoters of 183 genes were differentially methylated in individuals with arsenic-associated skin lesions compared to unexposed individuals with unlesioned skin (Smeester, Rager et al. 2011). Most of these genes were hypermethylated in individuals with skin lesions (99%), and several had known links to arsenic-associated diseases such as diabetes mellitus, cardiovascular disease, and various cancers. Our laboratory has shown that there are distinct patterns of gene-specific DNA methylation patterns associated with inter-individual differences in iAs metabolism (Bailey, Wu et al. 2013). In particular, distinct groups of genes with DNA methylation patterns associated with urinary levels of iAs, MMAs, and DMAs were identified. There was some overlap in terms of common genes between the three arsenical groups as well.

Prenatal arsenic exposure has also been associated with global changes in DNA in PBLs of a cohort in Bangladesh methylation (Kile, Baccarelli et al. 2012), as well as gene-specific changes in PBLs from a cohort in Thailand (Intarasunanont, Navasumrit et al. 2012) where tumor protein 53 (*TP53*) demonstrated increased promoter methylation related to prenatal iAs exposure. Highlighting low level iAs-associated changes to the epigenome, estrogen receptor 1 (*ESR1*) and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (*PPARGC1A*) showed altered DNA methylation in a US cohort (Koestler, Avissar-Whiting et al. 2013). Moving forward it will be critical to establish whether changes in DNA methylation have functional consequence at a transcriptional and/or translational level and the relevance of PBL DNA methylation at it relates to changes in iAs-induced disease.

## Proteomic signaling events

A current gap in the understanding of molecular effects of prenatal arsenic exposure is the impact on protein response at a large scale (e.g. proteomic signaling). To date, a single study has examined protein alterations associated with prenatal iAs exposure. Ahmed et al. (2011) analyzed 18 cord blood proteins for associations with maternal urinary arsenic in Bangladeshi newborns. Using total maternal urinary arsenic (U-tAs) measured at gestational week (GW) 30 as a measure of iAs exposure, two proteins, namely interleukin 8 (IL8) and tumor necrosis factor (TNF), were found to have expression levels that formed U-shaped curves across U-tAs quartiles (Ahmed, Mahabbat-e Khoda et al. 2011). Supporting the transcriptional response observed previously (Fry, Navasumrit et al. 2007), the shifts in protein expression levels provide further evidence for a pro-inflammatory response resulting from prenatal exposure. The molecular events that underlie the activation of the identified proteins could be transcriptionally-mediated via changes in epigenetic modifications such as histone post-translational modifications, altered miRNA expression levels, and/or shifts in DNA methylation. These direct relationships remain unexplored.

## Conclusions

The molecular basis for the health effects associated with prenatal arsenic exposure is not well understood. It is likely a combination of complex factors that includes level and extent of iAs exposure, maternal and fetal genotype that impact iAs metabolism, altered enzymatic activity that influences epigenetic patterning, and both epigenetic and non-epigenetic transcriptional regulatory mechanisms that ultimately culminate in altered proteomic response. It is plausible that some of these molecular events will be stable over time, thus contributing to altered disease risk later in life.

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