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Association Between Arsenic Exposure and Global Post-translational Histone Modifications Among Adults in Bangladesh

Yana Chervona¹, Megan N. Hall³, Adriana Arita¹, Fen Wu¹, Hong Sun¹, Hsiang-Chi Tseng¹, Eunus Ali², Mohammad Nasir Uddin², Xinhua Liu⁴, Maria Antonietta Zoroddu⁶, Mary V. Gamble^{5,§,†}, and Max Costa^{1,§,†}

¹New York University School of Medicine, Department of Environmental Medicine, New York, NY

²Columbia University Arsenic Project in Bangladesh

³Departments of Epidemiology, Mailman School of Public Health, Columbia University, New York, NY

⁴Biostatistics, Mailman School of Public Health, Columbia University, New York, NY

⁵Department of Environmental Health Sciences Mailman School of Public Health, Columbia University, New York, NY

⁶Department of Chemistry, University of Sassari, Sassari, Italy.

Abstract

Background—Exposure to arsenic (As) is associated with an increased risk of several cancers, as well as, cardiovascular disease, and childhood neuro-developmental deficits. Arsenic compounds are weakly mutagenic, alter gene expression and post-translational histone modifications (PTHMs) *in vitro*.

Methods—Water and urinary As concentrations, as well as, global levels of histone 3 lysine 9 dimethylation and acetylation (H3K9me2 and H3K9ac), histone 3 lysine 27 trimethylation and acetylation (H3K27me3 and H3K27ac), histone 3 lysine 18 acetylation (H3K18ac) and histone 3 lysine 4 trimethylation (H3K4me3) were measured in peripheral blood mononuclear cells (PBMCs) from a subset of participants (N=40) of a folate clinical trial in Bangladesh (FACT study).

Results—Total urinary As (uAs) was positively correlated with H3K9me2 ($r=0.36$, $p=0.02$) and inversely with H3K9ac ($r=-0.47$, $p=0.002$). The associations between As and other PTHMs differed in a gender-dependent manner. Water As (wAs) was positively correlated with H3K4me3 ($r=0.45$, $p=0.05$) and H3K27me3 ($r=0.50$, $p=0.03$) among females and negatively correlated among males (H3K4me3: $r=-0.44$, $p=0.05$; H3K27me3: $r=-0.34$, $p=0.14$). Conversely, wAs was inversely associated with H3K27ac among females ($r=-0.44$, $p=0.05$) and positively associated among males ($r=0.29$, $p=0.21$). A similar pattern was observed for H3K18ac (females: $r=-0.22$, $p=0.36$; males: $r=0.27$, $p=0.24$).

Conclusion—Exposure to As is associated with alterations of global PTHMs; gender-specific patterns of association were observed between As exposure and several histone marks.

§ Corresponding Authors Tel. (845)-731-3515 Fax: (845)-731-2118 Max.Costa@nyumc.org Tel. (212) 305-7949 Fax: (212) 305-3857 mvg7@columbia.edu.

†These authors contributed equally to this study.

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Impact—These findings contribute to the growing body of evidence linking As exposure to epigenetic dysregulation, which may play a role in the pathogenesis of As toxicity.

Keywords

Arsenic; epigenetics; histone modifications; Bangladesh; gender difference

Introduction

It is estimated that approximately 150 million people in at least 70 countries are exposed to naturally occurring arsenic (As) through contaminated drinking water (1). In Asia, an estimated 60 million people are chronically exposed, and roughly half reside in Bangladesh where exposure levels often exceed the permissible U.S. EPA levels (2, 3). In the United States, excess lifetime risk for lung and bladder cancers were shown to be elevated in the Western and New England regions of the US, where there is As contamination of drinking water; excess fatality rates were 152 per million and 117 per million for domestic and public supplies in the Western region, and 125 per million for domestic supplies in New England (4).

Chronic exposure to As is known to cause skin, lung, and bladder cancers, and has been implicated in the development of liver and prostate cancers in several epidemiological studies (5-9). Furthermore, As exposure has also been associated with cardiovascular disease, neurologic and childhood neuro-developmental deficits, and hypertension (10-14). However, although As exposure is associated with a diverse set of health outcomes, the mechanisms underlying the carcinogenic and non-carcinogenic effects of As are poorly understood. An emerging body of evidence suggests that As exposure may alter epigenetic marks, including DNA methylation and post-translational histone modifications (PTHMs) (15, 16).

Post-translational modifications of lysine (K) residues on histone (H) tails play a fundamental role in the regulation of chromatin structure, gene and non-coding RNA transcription and nuclear architecture (17, 18). Enrichment in acetylation (ac) of histone tails in promoters is typically associated with transcriptional activation, while the functional consequences of methylation (me) depend on the number of methyl groups, the residue itself, and its location within the histone tail. For example, H3K4me₂, H3K4me₃, and H3K9ac are correlated with open chromatin and active gene expression, while H3K27me₂, H3K27me₃, H3K9me₂, and H3K9me₃ in gene promoters are associated with inactive chromatin and gene repression (19). In addition, some marks, such as H3K4me₁ and H3K27ac are found in the enhancer elements of genes and can influence gene expression even at large distances from the gene (20). Moreover, alteration of epigenetic mechanisms, such as DNA methylation and histone modifications, has been reported in numerous diseases, including cancer, neurological, and cardiovascular diseases (21-23).

A number of previous studies have demonstrated that As exposure induces a myriad of global PTHMs *in vitro*, including reduction of acetylation in histone H3 and H4, loss of H4K16ac, increase in H3K14ac, increase in H3K9ac, increase in H3K4me₂ and H3K4me₃, loss of H3K27me₃, loss or gain of H3K9me₂, as well as increase in H3Ser10 and H2AX phosphorylation (24-34). Recent work has also demonstrated that human peripheral blood mononuclear cells (PBMCs) or white blood cells can be utilized to measure the association between metal exposure and post-translational histone modifications. Furthermore, previous work by our group demonstrated that As exposure was associated with alterations in leukocyte DNA methylation and that global hypomethylation of leukocyte DNA appeared to increase the risk for As-induced premalignant skin lesions (24, 25, 35, 36). Importantly,

PBMC (or bone marrow progenitor cells) are a specific target for As toxicity. As an effective therapeutic drug for APL, As^{III} distributes to PBMC progenitor cells and influences their cellular function (37-39).

We conducted a pilot study to examine the influence of chronic As exposure on global levels of six post-translational histone marks, including: H3K9me2 and H3K27me3, H3K4me3, H3K9ac, H3K18ac and H3K27ac, using PBMC from an ongoing trial of folic acid and creatine supplementation (FACT) in Bangladesh. We found that environmental exposure to As is associated with alterations of all of the marks examined, and observed that four of the six marks, including: H3K4me3, H3K27me3, H3K18ac and H3K27ac, were altered in a gender-specific manner.

Methods

Study Site

The study site is a 25-km² region within Araihaazar, approximately 30 km east of Dhaka, Bangladesh. The wide range of well-water As concentrations (0.1–960 µg/L) from over 10,000 wells within this region presents a unique opportunity to study potential effects of As exposure. Informed consent was obtained by Bangladeshi field staff physicians. The study was approved by the institutional review boards of Columbia University Medical Center and the Bangladesh Medical Research Council and is registered with ClinicalTrials.gov.

Eligibility Criteria and Study Design

The HEALS cohort study is part of Columbia University's Superfund Research Program that was launched in 2000, and currently includes roughly 30,000 participants. The present study utilizes samples from a subset of 600 HEALS participants who were recruited for enrollment into FACT study. This clinical trial, aimed primarily at lowering blood As concentrations through nutritional supplementation, enrolled a total of 600 study participants, and fieldwork was completed in June 2011. At the time of enrollment, all FACT study participants had water As between 50 – 500 µg/L and were provided with a water filtration system that removed As. Women who were currently pregnant and/or planned to become pregnant, participants taking nutritional supplements, or having known renal or gastrointestinal disease were excluded from the study. To evaluate the effects of As exposure on histone modifications, PBMCs from 40 FACT study participants (20 from each gender) known to have a wide range of water As exposure (50-500 µg/L As) were analyzed.

Sample collection and handling

Blood samples were obtained by venipuncture, and collected into heparin-containing vacutainer tubes, which were placed in IsoRack/IsoPack cool packs (Brinkmann Instruments, Westbury, NY). Within 4 hrs, samples were transported in hand-carried coolers to the local laboratory, situated in our field clinic in Araihaazar. Samples were centrifuged at 3,000 × *g* for 10 min at 4°C, and the plasma fraction was stored at -80°C. PBS was added to the remaining cells followed by a ficollhpaque gradient extraction of PBMCs using standard techniques. PBMCs were stored at -80°C. Blood and plasma samples were hand-carried frozen on dry ice to Columbia University.

Water Arsenic

A survey of all wells in the study region assessed water As concentrations of tube wells at each participant's home between January and May 2000 (Van Geen et al. 2002). Samples were analyzed at Columbia University's Lamont Doherty Earth Observatory by graphite furnace atomic absorption (GFAA), which has a detection limit of 5 µg/L (*AAAnalyst 600*, PerkinElmer, Shelton, CT). Those samples found to have nondetectable As concentrations

by GFAA were subsequently analyzed by inductively coupled mass spectrometry (ICP-MS), with a detection limit of 0.1 µg/L (Cheng et al. 2004).

Urinary Arsenic

Urine samples were collected in 50-mL acid-washed polypropylene tubes. These were kept in portable coolers, and within 4 hrs were frozen at -20°C in Arai-hazar, and subsequently hand carried on dry ice to New York. Total urinary As (uAs) concentrations were measured by GFAA spectrometry using an AAnalyst 600 graphite furnace system (PerkinElmer, Shelton, CT) in the Columbia University Trace Metals Core Lab, as described previously (Nixon et al. 1991). This laboratory participates in a quality control program run by the Institut de Sante Publique du Quebec, Canada. Intra-class correlation coefficients (ICCs) between the Columbia laboratory's values and samples calibrated at the Quebec laboratory were 0.99. Urinary creatinine was analyzed using a method based on the Jaffe reaction and was used to correct for hydration status (40)

Histone Extraction

Histones were extracted from the PBMCs as previously described (24, 41). Briefly, cells were washed with ice-cold PBS and lysed in ice-cold radioimmunoprecipitation assay (RIPA) buffer (50 mM Tris-HCl, pH 7.4, 1% NP-40, 0.25% Na-deoxycholate, 150 mM NaCl, 1 mM EDTA, 1 mM PMSF, supplemented with a protease inhibitor mixture (Roche Applied Sciences, Indianapolis, IN) for 10 min. The pellet was collected by centrifugation at $10,000 \times g$ for 10 min. The pellet was washed once in 10 mM Tris-Cl and 37 mM EDTA (pH 7.4), and resuspended in 200 µl 0.4 N H_2SO_4 . After an overnight incubation at 4°C , the supernatant was collected by centrifugation at $14,000 \times g$ for 15 min, and mixed with 1.8 ml cold acetone and kept at -20°C overnight. Histones were collected by centrifugation at $14,000 \times g$ for 15 min. After one wash with acetone, the histones were air dried and suspended in sterile deionized water. Total protein concentration in each sample was measured using the Bradford Assay according to the manufacturer's instructions (Bio-Rad Laboratories, Hercules, CA).

Histone Modification Analysis

The levels of each histone modification were determined using the sandwich enzyme-linked immunoabsorbent assay (ELISA), as previously described (24). Briefly, polystyrene 96-well microplates (Fisher Scientific, Pittsburg, PA) were coated with Histone H3 antibody (Abcam, Cambridge, MA) and incubated overnight at 4°C . Plates were then blocked for 2 hrs at room temperature with 5% milk in PBST (1× PBS, 0.05% TWEEN-20), washed with PBST, and the desired amount of standards [H3K9me2 and H3K4me3] recombinant proteins (Active Motif, Carlsbad, CA) or mixed calf histone proteins (Sigma, Saint Louis, MO)] were added to each well, followed by the addition of histones diluted in water. The plates were then incubated at room temperature for 1.5 hours with agitation on an orbital shaker. After incubation, the wells were washed, and primary antibody, such as H3 (Sigma, St. Louis, MO, USA), or H3K9me2, or H3K9ac, or H3K18ac, or H3K27ac (Abcam, Cambridge, MA, USA), or H3K4me3 (Millipore, Billerica, MA, USA) was added to each well separately and incubated at room temperature for 1 hr with agitation. After another wash, secondary rabbit anti-goat IgG-HRP or mouse anti-goat IgG-HRP antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) was added to each well and incubated at room temperature for 1 hr without agitation. Wells were then washed and TMB (3,3', 5,5''-tetramethylbenzidine; Fisher Scientific, Pittsburg, PA, USA) solution was added to each well and incubated at room temperature for 30 min in the dark. The reaction was stopped by adding 2 M H_2SO_4 to each well. All analyses were performed in triplicate. The optical density was read at 450 nm using the SoftMax Pro software (version 5.2) and the SpectraMax 190 microplate reader (both from Molecular Devices, Sunnyvale, CA, USA).

The percentages of each histone modification were derived from standard curves specific to each histone mark. The respective within- and between-assay coefficients of variation for each modification were: H3K9me2: 7.2 and 7.1%, H3K9ac: 5.1 and 10.9%, H3K4me3: 5.2 and 9.4%, H3K18ac: 3.9 and 13%, H3K27me3: 6.5 and 7.3%, and H3K27ac: 6.3 and 7.7%. To calculate the within-assay coefficient of variation, all samples were run in triplicate on the same plate on the same day. To calculate the between-assay coefficient of variation, multiple samples were run in triplicate for each modification on different days.

Statistical Analysis

We calculated descriptive statistics for the total sample and by water As category (high vs. low) for As exposure variables (water As and urinary As) and covariates (age, sex, television ownership, cigarette smoking, use of betel nut, body mass index (BMI), total homocysteine, s-adenosyl methionine, and s-adenosyl homocysteine).

Bivariate associations between As exposure and the histone marks (H3K18ac, H3K27ac, H3K27me3, H3K4me3, H3K9ac, and H3K9me2) were evaluated using water As as both a continuous and a categorical (high vs. low) variable and using uAs as a continuous variable. Scatterplots and Spearman's correlation coefficients were used for uAs and when water As was treated as a continuous variables. When water As was treated as a binary variable, Wilcoxon rank-sum tests were utilized to determine whether there was a statistically significant difference in the histone marks by high vs. low water As category. Given previous observations that males and females in Bangladesh differ significantly in susceptibility to the effects of As exposure, Spearman's correlations between water or uAs exposure and histone marks stratified by gender were used. In addition, median concentrations of the histone marks by water As category and gender were calculated; a Kruskal-Wallis test was used to determine whether there was a statistically significant difference among the four groups and the Wilcoxon rank-sum test was used to compare specific groups. Correlations between the histone marks were evaluated using Spearman's correlation coefficients.

To evaluate potential confounding variables, associations between 1) covariates and water or uAs exposure and 2) covariates and histone marks were examined. When both variables being examined were continuous, Spearman's correlation coefficients were used. To examine associations between a categorical variable and a continuous variable t-tests or the non-parametric Wilcoxon rank sum test was used when appropriate. Multiple linear regression models were then used to further examine the associations between water or uAs exposure and the histone marks, after adjustment for age and land ownership, i.e. covariates observed to be associated with both the exposure and outcome variables. Age was modeled as a continuous variable and land ownership was modeled as a binary (yes/no) variable. Variables with skewed distributions were natural log transformed before inclusion in linear regression models in order to 1) create approximately normal distributions for dependent variables, 2) improve the linearity of the relationship between independent and dependent variables, or 3) to reduce the impact of extreme values of an independent variable. Urinary As, age, H3K18ac, H3K27ac, H3K27me3, H3K4me3, and H3K9ac were log transformed. All analyses were performed using SAS (version 9.2; SAS Institute Inc., Cary, NC); all statistical tests were two sided with a significance level of 0.05.

Results

A total of 40 participants were selected for this pilot study; 50% were male, and the average age was 39. The characteristics of the participants are shown in Table 1 for the entire group by water As (wAs) exposure category (low vs. high). We selected 20 study participants with relatively low As exposure (low water As group) and 20 with relatively high As exposure

(high water As group). The median water As concentration for the low exposure group was 55 $\mu\text{g/L}$ (range: 50-81 $\mu\text{g/L}$). For the high exposure group, the median water As concentration was 216 $\mu\text{g/L}$ and the range was wider (150-500 $\mu\text{g/L}$). Compared to those with high water As exposure, participants in the low water As group were more likely to own land (55% vs. 35%) or a television (65% vs. 55%), and were also more likely to have ever smoked (30% vs. 20%) or used betel nut (30% vs. 25%). Participants from the low water As group also had lower total homocysteine (Hcys) concentrations (8.3 vs. 9.2 $\mu\text{mol/L}$).

Spearman's correlation coefficients between As exposure (water and uAs) and the histone marks for the entire group and by gender are shown in Table 2. Both water and uAs were inversely correlated with H3K9Ac in the entire group [water As: $r = -0.40$ ($p=0.01$); uAs: $r = -0.47$ ($p=0.002$)]. Water and uAs were both positively correlated with H3K9me2, although the association with water As did not reach statistical significance [water As: $r = 0.27$ ($p=0.10$); uAs: $r = 0.36$ ($p=0.02$)]. The associations between As exposure and H3K9Ac or H3K9me2 did not differ significantly by gender (Table 2).

There were no statistically significant correlations between water or uAs exposure and H3K18Ac, H3K27ac, H3K27me3, or H3K4me3 in the entire group. However, the associations between As exposure and these four histone marks differed by gender (Table 2 and figure 1). Water As was significantly positively correlated with H3K4me3 ($r=0.45$, $p=0.05$) and H3K27me3 ($r=0.50$, $p=0.03$) among females and negatively correlated with H3K4me3 ($r = -0.44$, $p=0.05$) and H3K27me3 ($r = -0.34$, $p=0.14$) among males. The p -value for the test of difference by gender was 0.005 for H3K4me3 and 0.009 for H3K27me3. Similar associations were observed using uAs as the exposure.

Water As was inversely associated with the global levels of H3K27ac among females ($r = -0.44$) and positively associated among males ($r=0.29$). Correlations between water As and H3K18ac by gender also exhibited a similar pattern (females, $r = -0.22$; males $r = 0.27$). None of these correlations within gender reach statistical significance. The test of difference by gender was significant for H3K27ac ($p=0.02$) but not for H3K18ac ($p=0.14$). Findings for H3K27ac and H3K18ac were similar when uAs was used as the exposure variable (Table 2); however, when using uAs as the exposure the p -value for the test of difference by gender was 0.03 for H3K18ac.

Table 3 shows the Spearman's correlations between histone marks. H3K18ac was positively correlated with H3K27ac ($r=0.44$, $p=0.004$) and negatively correlated with H3K27me3 ($r=-0.47$, $p=0.002$). H3K27ac, in turn, was positively correlated with H3K9me2 ($r=0.35$, $p=0.03$). H3K9ac was also inversely, but not significantly, correlated with H3K9me2 ($r=-0.27$, $p=0.10$).

Overall, results from the linear regression models were similar to our main analyses. In addition, the results of unadjusted models and those adjusted for age and land ownership were essentially the same (data not shown). The only notable difference between the linear regression results and those in Table 2 is that the inverse association between water arsenic and H3K27me3 in males presented in Table 2 was not observed in the regression models. There was also no statistically significant difference in the covariate-adjusted associations between wAs and H3K27me3 by sex.

Discussion

There is considerable interest among the scientific community in the field of epigenetics, with particular attention being paid to the study of post-translational histone modifications and their functional roles in gene expression dysregulation, and carcinogenesis. Cancer cells

exhibit both aberrant DNA methylation patterns and changes in the global levels of specific histone modifications, but the specific pattern of epigenetic modifications that precedes the onset of neoplasia is not well characterized (22). Given that As is a highly prevalent environmental contaminant world-wide and that some arsenicals (i.e. As₂O₃) are given to patients to treat disease, understanding the potential effects of As on the epigenome is of great importance.

This pilot study provides unique insights into the epigenetic effects of chronic As exposure in a generally healthy population in Bangladesh. Histone post-translational modifications can change chromatin structure via an intricate set of interactions and influence both global and gene-specific DNA methylation, thereby regulating genomic stability and gene expression (19, 42, 43). The observed changes in the global levels of histone marks, such as an increase in H3K9me₂, a mark of transcriptional repression, and a decrease in H3K9ac, a mark that is usually associated with relaxed and active chromatin, suggests that chronic As exposure may be associated with global transcriptional repression. An abundance of chromatin silencing marks may favor the silencing of tumor suppressor genes which can predispose to cancer development. Moreover, H3K9 methylation and hypo-acetylation play an important role in the establishment of DNA methylation (44).

Earlier work by our group has shown that chronic As exposure in Bangladesh was positively associated with genomic DNA methylation in a dose dependent manner among folate-sufficient individuals (15). Recently, Christiani *et al.* reported that exposure to higher levels of arsenic in Bangladesh was positively associated with DNA methylation in LINE-1 repeated elements, in both maternal and fetal leukocytes (45). Although contradictory to the findings of some studies, the observed correlation between DNA hypermethylation and As exposure may potentially be explained by the findings reported here. H3K9me₂ is a critical mark for cytosine methylation of DNA (46). A persistent increase of H3K9me₂ following chronic As exposure could potentially trigger *de novo* DNA methylation, which would more permanently suppress gene expression and contribute to arsenic-induced long term silencing of tumor suppressor genes. Both *in vitro* and *in vivo* studies have demonstrated that As exposure could lead to increased DNA methylation in the promoter region of tumor suppressor genes such as p53 and p16 (47, 48). With respect to histone acetylation, a number of studies have linked both global and gene specific DNA hypermethylation, with the loss of histone acetylation (30, 49, 50). Jensen *et al.*, demonstrated that As induced malignant transformation was accompanied by DNA hypermethylation and loss of histone 3 acetylation at gene promoters (30). Taken together, the As-associated increase in H3K9me₂ and decrease in H3K9ac may provide some insight into the underlying mechanisms and physiologic implications of increased genomic DNA methylation observed in the earlier work.

The fact that As exposure appears to influence histone marks such as H3K27me₃, H3K4me₃, H3K18ac and H3K27ac, in a gender specific manner represents an interesting and significant finding. The gender differences occurred in both methylation and acetylation marks, however the direction of the association with As by gender differed by the type of mark (i.e. H3K18ac and H3K27ac increased in males and decreased in females, while H3K27me₃ and H3K4me₃ increased in females and decreased in males). The effect modification by gender was unexpected. While it is possible that some of the statistically significant differences that were observed may have occurred by chance due to the small sample size, there is some evidence of estrogen-sensitive histone marks. For example, Bredfeldt *et al.* have demonstrated that estrogen receptor signaling regulates a HMT enhancer, ultimately reducing levels of H3K27me₃ in hormone-responsive cells (51). In addition, Hamilton *et al.* illustrated that As is an endocrine disruptor and influences ER-mediated gene expression (52). Interestingly, in the preliminary analyses of these same n=40

FACT participants, *S*-adenosylhomocysteine (SAH), a strong inhibitor of most methylation reactions, including DNA and As methylation, also influenced histone marks in a gender-specific manner (53, 54). In the dataset, SAH was associated with significant decreases in two methylation marks: H3K9me2 in females and H3K27me3 among males (data not shown). Unfortunately due to the small sample size and the fact that folate data is not yet available for these subjects, the analysis of this finding is limited, but will be investigated further in future work.

Another interesting finding was the correlations between the histone marks. H3K18ac was positively correlated with H3K27ac ($r=0.44$, $p=0.004$) and negatively correlated with H3K27me3 ($r=-0.47$, $p=0.002$). H3K27ac, in turn, was positively correlated with H3K9me2 ($r=0.35$, $p=0.03$). As would be expected given the opposite direction of gene expression afforded by the presence of these two opposing marks, H3K9ac was also inversely, but not significantly, correlated with H3K9me2 ($r=-0.27$, $p=0.10$). Contrary to an earlier hypothesis, however, a correlation between H3K4me3 and H3K9me2, which would be consistent with their reciprocal regulation (i.e. H3K4me3 is an activating mark and H3K9me2 is a repressive mark), was not observed. The inability to detect a correlation between these two marks might be due to the small sample size of this pilot study.

With respect to our findings, some potential mechanisms that may underlie the observed alterations in histone post-translational modifications include: an increase in histone methyltransferases (HMT), such as G9a, which is responsible for the methylation of H3K9 and whose mRNA and protein expression increased following As exposure; induction of two H3K4 methyl “erasers,” lysine-specific demethylase (LSD) 1 and 2, which were also required for global DNA methylation; and a very potent As-induced inhibition of pyruvate dehydrogenase, an enzyme that catalyzes the final step in acetyl CoA biosynthesis (32, 55-57). Acetyl CoA is a requisite substrate for histone acetylation; inhibition of acetyl CoA biosynthesis would decrease histone acetylation by depletion of substrate leading to chromatin condensation and increased DNA methylation.

In conclusion, H3K9ac and H3K9me2 were found to be associated with As exposure similarly in males and females, while H3K4me3, H3K18ac, H3K27ac and H3K27me3 were associated with As exposure in a gender-specific manner. Future studies will be directed at examining the proposed mechanisms underlying the observed alterations in histone post-translational modifications and further investigating the gender effects. In addition, exploring the genomic location of the altered histone marks will be of importance in understanding whether they impact mostly gene expression by producing changes in gene promoters or whether they affect other genomic regulatory components such as non-coding RNA. Finally, additional work is warranted to evaluate the potential impact of these histone modifications on various As-induced health outcomes.

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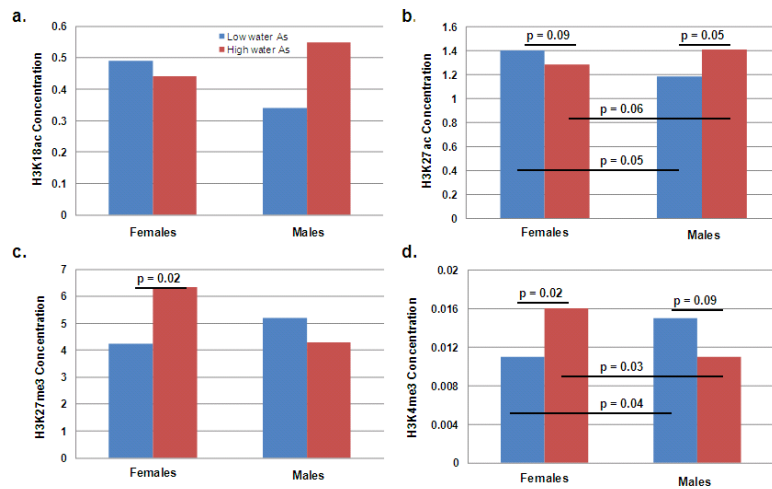


Figure 1.

Median concentrations of histone marks by sex and water As category^a for (a) H3K18ac, (b) H3K27ac, (c) H3K27me3, and (d) H3K4me3^b

^a Low water As range = 50-81 $\mu\text{g/L}$, high water As range=150-500 $\mu\text{g/L}$, ^bBased on a Kruskal-Wallis test, there is a statistically significant overall difference among the four groups for H3K27Ac ($p=0.049$), H3K27me3 ($p=0.048$), and H3K4me3 ($p=0.02$). Using Wilcoxon rank-sum tests, we also tested for 1) differences in histone marks by high/low water As within gender and 2) differences in histone marks by gender within water As category (high/low) – p -values < 0.10 are indicated.

Table 1General characteristics for the total study sample and by water As group^a

	Entire Group (n=40)	Low water As group ^b (n=20)	High water As group ^c (n=20)
Age (yrs)	39 (13.5)	42.0 (12.5)	34.0 (12.5)
BMI (kg/m ²)	19.5 (3.3)	20.5 (3.4)	18.5 (2.7)
Water As (µg/L)	115.5 (161)	55.0 (4.9)	216.0 (71.0)
Urinary As (µg/L)	91.5 (115.0)	69.0 (62.5)	165.0 (158.0)
Urinary creatinine (mg/dL)	39.0 (35.8)	42.7 (37.8)	35.6 (34.8)
Urinary As / gm Creatinine	247.8 (376.3)	133.4 (75.6)	509.7 (221.6)
Total homocysteine (µmol/L)	8.9 (5.5)	8.3 (6.9)	9.2 (5.4)
s-adenosyl methionine (µmol/L)	2.14 (0.95)	2.5 (1.2)	2.06 (0.64)
s-adenosyl homocysteine (µmol/L)	0.25 (0.17)	0.22 (0.15)	0.28 (0.17)
Female (%)	50	50	50
Land ownership (%)	45	55	35
Television ownership (%)	60	65	55
Current cigarette smoking (%)	25	30	20
Current betel nut use (%)	27.5	30	25

^aMedian (interquartile range) unless otherwise noted^bwater As 50-81 µg/L^cwater As 150-500 µg/L

Table 2
Spearman's correlation coefficients (p-value) between As exposure and histone marks by gender

	Water As						Urinary As ^a					
	Total		Females		Males		Total		Females		Males	
		p-value ^b										
H3K18ac	0.08 (0.62)	-0.22 (0.36)	0.27 (0.24)	0.14	0.11 (0.50)	-0.21 (0.37)	0.49 (0.03)	0.03				
H3K27ac	0.07 (0.65)	-0.44 (0.05)	0.29 (0.21)	0.02	-0.009 (0.96)	-0.48 (0.03)	0.36 (0.12)	0.009				
H3K27me3	0.06 (0.72)	0.50 (0.03)	-0.34 (0.14)	0.009	0.13 (0.44)	0.45 (0.04)	-0.42 (0.06)	0.007				
H3K4me3	-0.04 (0.81)	0.45 (0.05)	-0.44 (0.05)	0.005	0.19 (0.23)	0.39 (0.09)	-0.18 (0.44)	0.08				
H3K9ac	-0.40 (0.01)	-0.44 (0.05)	-0.40 (0.08)	0.89	-0.47 (0.002)	-0.63 (0.003)	-0.25 (0.28)	0.16				
H3K9me2	0.27 (0.10)	0.17 (0.49)	0.27 (0.25)	0.76	0.36 (0.02)	0.12 (0.61)	0.52 (0.02)	0.18				

^a urinary As adjusted for uCr using the residual method

^b p-value for the test of difference by gender

Table 3

Spearman's correlation coefficients (p-value) between histone marks (n=40)

	H3K18ac	H3K27ac	H3K27me3	H3K4me3	H3K9ac	H3K9me2
H3K18ac	1	0.44 (0.004)	-0.47 (0.002)	0.14 (0.39)	0.06 (0.73)	0.23 (0.16)
H3K27ac		1	0.04 (0.79)	-0.14 (0.38)	0.15 (0.36)	0.35 (0.03)
H3K27me3			1	0.04 (0.79)	-0.04 (0.81)	-0.05 (0.75)
H3K4me3				1	-0.16 (0.31)	0.18 (0.27)
H3K9ac					1	-0.27 (0.10)
H3K9me2						1