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RESEARCH PAPER

Associations between post translational histone modifications, myelomeningocele risk, environmental arsenic exposure, and folate deficiency among participants in a case control study in Bangladesh

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ABSTRACT

Arsenic exposure may contribute to disease risk in humans through alterations in the epigenome. Previous studies reported that arsenic exposure is associated with changes in plasma histone concentrations. Posttranslational histone modifications have been found to differ between the brain tissue of human embryos with neural tube defects and that of controls. Our objectives were to investigate the relationships between plasma histone 3 levels, history of having an infant with myelomeningocele, biomarkers of arsenic exposure, and maternal folate deficiency. These studies took place in Bangladesh, a country with high environmental arsenic exposure through contaminated drinking water. We performed ELISA assays to investigate plasma concentration of total histone 3 (H3) and the histone modification H3K27me3. The plasma samples were collected from 85 adult women as part of a case-control study of arsenic and myelomeningocele risk in Bangladesh. We found significant associations between plasma %H3K27me3 levels and risk of myelomeningocele ($P<0.05$). Mothers with higher %H3K27me3 in their plasma had lower risk of having an infant with myelomeningocele (odds ratio: 0.91, 95% confidence interval: 0.84, 0.98). We also found that arsenic exposure, as estimated by arsenic concentration in toenails, was associated with lower total H3 concentrations in plasma, but only among women with folate deficiency ($\beta = -9.99$, standard error = 3.91, $P=0.02$). Our results suggest that %H3K27me3 in maternal plasma differs between mothers of infants with myelomeningocele and mothers of infants without myelomeningocele, and may be a marker for myelomeningocele risk. Women with folate deficiency may be more susceptible to the epigenetic effects of environmental arsenic exposure.

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Introduction

Neural tube defects are debilitating birth defects characterized by high rates of mortality and lifelong disabilities in surviving children. Neural tube defects occur when the developing neural plate fails to elevate and fuse in the first 3 to 4 weeks of gestation, causing death or permanent damage to the spinal cord.[1] It is increasingly recognized that the majority of human neural tube defects have a multifactorial and complex etiology, and that environmental factors, such as maternal diet and exposure to chemicals, may affect the risk of these disorders.[2]

Epigenetic mechanisms are suspected to contribute to neural tube defects, and mutations in a growing number of epigenetic regulators have been shown to result in neural tube defects in animal models.[3,4] In humans, the role of epigenetics in neural tube defects is supported by the success of folic acid supplementation programs in the prevention of many cases. One of many potential mechanisms that may explain folic acid beneficial effects is that folate plays an important role in epigenetic regulation through its effect on DNA methylation. In addition to DNA methylation, posttranslational histone modifications have been investigated as epigenetic mechanisms leading to neural tube defects.

Histone modifications play a fundamental role in the regulation of chromatin structure, gene and noncoding RNA transcription, and nuclear architecture.[5,6] Enrichment in the acetylation of histone tails in promoters is typically associated with transcriptional activation; however, the functional consequences of methylation depend on the number of methyl groups, the residue itself, and its location within the histone tail.[7] Supporting this hypothesis is the observation that the anticonvulsant medication valproic acid, a well-recognized teratogen associated with neural tube defects, is also an inhibitor of enzymes involved in the acetylation of histones, suggesting that histone modification may play a role in the expression of genes important for normal neural tube development.[3,8,9]

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It has been reported that exposure to arsenic is associated with changes in epigenetic regulation, and recent reviews have summarized the evidence supporting the hypothesis that arsenic alters methylation of gene promoters; histone acetylation, methylation, and phosphorylation; and microRNA expression.[10-14] Arsenic induces neural tube defects in animal models, and our recent studies in humans suggest that arsenic exposure influences the risk of neural tube defects.[15] Whether arsenic affects neural tube defect risk through changes in the epigenome is unknown.

Our study aimed, therefore, to investigate whether environmental arsenic exposure was associated with histone levels as well as with levels of a particular posttranslational histone modification. We also investigated whether these epigenetic markers in mothers were associated with folate state in mothers or higher risk of neural tube defects in offspring. Our samples were drawn from women in Bangladesh, a country experiencing high arsenic exposure related to contaminated drinking water and also high rates of folate deficiency.

Posttranslational histone modifications have been found in a variety of bodily fluids, including plasma, and are hypothesized to play a role in cell-to-cell communication and disease pathogenesis, most notably diseases related to inflammation[16] and coagulation.[17] Recent studies demonstrate that exposure to environmental chemicals are associated with these epigenetic markers in plasma; for example, a recent study has shown that specific posttranslational histone modifications are associated with markers of environmental exposure to particulate matter.[18] Plasma is an easily accessible tissue, and identification of epigenetic markers in plasma that are related to both environmental exposures and disease risk may aid in estimating an individual risk, as well as in surveillance efforts. To our knowledge, no previous study has used plasma samples to investigate environmental arsenic exposure and its associations with histones and posttranslational histone modifications.

Methods

Study population

Between April and November 2013, we conducted a case-control study in communities served by Dhaka Community Hospital (DCH) in Bangladesh. Details of the case ascertainment and control selection strategies have been reported previously.[15,19] Briefly, eligible cases were children under the age of 1 y with myelomeningocele, a common and severe form of neural tube defect, in which the membranes and the spinal cord protrude at birth. In cases of myelomeningocele, the spinal cord and meninges (the tissues covering the spinal cord) protrude from the back. An experienced pediatrician (Dr. Ibne Hasan) confirmed cases of myelomeningocele. Controls were matched (1:1) by sex and age from pregnancy registries from DCH-affiliated health centers using the following method: potential controls were separated into groups corresponding to sex and birth quarter, and placement on the list of potential controls was assigned by random digit assignment. Once a case was enrolled, potential controls were approached in order of assignment on this list. Fifty-seven cases and 55 controls, along with their mothers, were enrolled. Participation was 98% among potential cases and 83% among potential controls. Informed consent was obtained from all participants. The Human Research Committees at Boston Children's Hospital and DCH approved this study.

Questionnaires and medical history

Trained interviewers asked parents regarding their medical histories and environmental exposures, including the use of medications during pregnancy, family history, water consumption, other potential environmental and occupational exposures, as well as reproductive history. Periconceptional folic acid supplementation use was defined as reporting any intake of a folic acid-containing supplement within the 2 months before the awareness of pregnancy.

Arsenic exposure

Drinking Water

Drinking water samples were obtained from the tube well each mother identified as her primary source of drinking water at the time she became aware of her pregnancy. Water samples were collected in 50 ml polypropylene tubes (BD Falcon, BD Bioscience, Bedford, MA, USA), preserved with reagent grade nitric acid (Merck, Germany) to a pH < 2, and stored at room temperature. Arsenic concentration in water was analyzed using inductively coupled plasma mass spectrometry (ICP-MS) according to US Environmental Protection Agency method 200.8 (Spectrum Analytical, Inc., Agawam, MA, USA). For quality control, instrument performance was validated by a spiked laboratory control sample (ICP, Analytical Mixture 12 Solution A, High Purity Standards, Charleston, SC, USA) with percent recoveries ranging from 98 to 107%. Samples below the 0.15 μg/L limit of detection (LOD) were reassigned a value of half the LOD for statistical analyses. Families found to have drinking water inorganic arsenic concentrations $\geq$ 50 μg/L (Bangladesh standard) were advised to seek alternative sources of drinking water.

Toenails

Toenail samples were collected from mothers and were placed in individual sealed envelopes, digested, and analyzed at the Harvard T.H. Chan School of Public Health (HSPH) metals laboratory using ICP-MS, following methods described by Chen et al.[20] A method blank and a human certified reference material GBW070601 (Institute for Geophysical and Geochemical Exploration, Langfang, China) were included with each batch of toenails during the digestion process. Toenail arsenic concentrations were blank-corrected and further corrected for systemic errors by normalizing the sample concentration against the measured concentration of the batch-specific reference material, and this corrected value was used in all statistical analyses. National Institute of Standards and Technology (NIST) 1640d was analyzed for arsenic concentration after every 10 samples. The average recovery of the NIST standard was 105%. The average LOD for the samples was 0.14 μg/g. Samples below the 0.14 μg/g LOD were reassigned a value of half the LOD for statistical analyses.

Plasma folate analysis

Whole blood was collected from mothers via venipuncture into EDTA tubes. Samples were centrifuged at 2,000 rpm for 12 min. Plasma was collected in 5 ml cryovials and stored at −20°C. Plasma samples were shipped to HSPH on dry ice. Folate analyses were performed at the Vitamin Metabolism Laboratory at the United States Department of Agriculture - Human Nutrition Research Center at Tufts University (Jean Mayer laboratory). We measured total folate concentration of the plasma samples by microbial assay with the use of *Lactobacillus casei*.[21] We serially diluted 5 μL of each plasma sample and plated the samples in triplicate onto a 96-microtiter well plate with 150 μL of *L. casei* growth medium.[21] We incubated the plates overnight in a 37°C humid incubator and measured the absorbance, which indicated microbial growth, with the use of a 96-well plate reader (PowerWave HT; BioTek Instruments, Inc., Winooski, VT, USA) at 595 nm. To test if any arsenic in plasma affected the microbial assay, we spiked 3 random samples with 5 ng/ml folic acid, and detected no inhibitory components in the plasma. The coefficients of variation (CVs) for the assay using one plasma sample with high folate concentration and one sample with low folate concentration were 6.78% and 4.73%, respectively.

Plasma histone concentration

The concentrations of total histone 3 (H3) and H3K27me3 in plasma were measured using sandwich ELISA.[22] Polystyrene microplates (96-well; Fisher Scientific, Pittsburgh, PA, USA) were coated with 100 μL of H3 antibody (Abcam ab16061, Cambridge, MA, USA) at a concentration of 1:20,000 diluted in phosphate-buffered saline (PBS) and incubated overnight at 4°C. Plates were washed in PBS with TWEEN-20 (PBST) (1X PBS, 0.05% TWEEN-20) and blocked with 3% milk in PBST for 1.5 h at room temperature with agitation on an orbital shaker at 450 rpm. The standard curve for the histones [total H3 (Active Motif 31207) and H3K27me3 (Active Motif 31216)] were made by diluting appropriate amount of recombinant protein (Active Motif, Carlsbad, CA, USA) in MQ water. Two quality control plasma samples were prepared by pooling 10 μL of plasma from the first 50 samples and the next 50 samples, respectively.[23]

Each plasma sample (5 μL) was diluted in 95 μL of Milli-Q water before analysis. After coating incubation, plates were washed with PBST. Case and control samples (100 μL each) were added in triplicate to the plate and incubated at room temperature with agitation for 1.5 h. Following incubation, the plates were washed with PBST. We diluted antibodies to total H3 at 1:40,000 (Sigma H0164, St. Louis, MO, USA), and H3K27me3 at 1:4,000 (Active Motif 39155) in 1% PBST milk. We added 100 μL of diluted primary antibody to each well and incubated for 1 h at room temperature with agitation. Plates were then washed with TBST. Secondary goat anti-rabbit IgG-HRP antibody (100 μL; Santa Cruz Biotechnology sc-2004, Santa Cruz, CA, USA) at 1:2,000 in TBST was added to each well and incubated for 1 h without agitation. Following incubation with secondary antibody, wells were washed 4 times with TBST. We then added 3,3′ 5,5′– tetramethylbenzidine (TMB; 100 μL) (Fisher Scientific, Pittsburgh, PA, USA) to each well and incubated at room temperature. The reaction was stopped after 30 min by adding 100 μL of 2 M H_2SO4. The absorbance was read at 450 nm using the Infinite M200 PRO spectrophotometer (TECAN, Mannedorf, Switzerland).

For the quality control samples, the within-assay CVs ranged from 1.83 to 5.53% for total H3 and 1.63 to 8.00% for H3K27me3. For the study samples, the between-assay CVs were 11.37 and 22.55% for total H3 and 5.13 and 6.72% for H3K27me3. Twenty-seven plasma samples (24%) with CVs greater than 10% were excluded from the analysis.

Statistical analysis

Because water arsenic concentration, maternal toenail arsenic concentration, and maternal plasma folate concentration were skewed, these values were log-transformed for analysis to approximate a normal distribution. %H3K27me3 was calculated by dividing H3K27me3 concentration by the total H3 concentration and was used as the measure of this histone subtype in analyses to be consistent with prior studies.[23] Maternal plasma folate concentration was used as a continuous variable, and was also used as a dichotomous variable in tests of effect modification. When used as a dichotomous variable, low plasma folate concentration was defined as <4 ng/ml, consistent with the current World Health Organization (WHO) definition of folate deficiency.[24]

We assessed the associations between histone concentrations and case status of offspring using unconditional logistic regression models. We did not use conditional models due to the uneven numbers of cases and controls, but instead forced the matching variables, including age and sex into all models, as suggested by Rothman and Greenland.[25] Other variables (maternal age, paternal age, receiving an ultrasound during pregnancy, medication use, maternal plasma folate concentration, and folate deficiency) were evaluated as potential confounders. Variables that were significant at the $P<0.05$ level were chosen as potential confounders, and kept in models if they changed the estimate by 10%. Separate models were constructed for each exposure (total H3 concentration or %H3K27me3) and outcome (case status of offspring). Logistic regression models were constructed for each analysis as follows: the first model was adjusted for infant age and sex while additional models were also adjusted for maternal plasma folate concentration (Model 2); plasma folate concentration and maternal toenail arsenic (Model 3); and plasma folate concentration, and maternal toenail and water arsenic concentrations (Model 4). We conducted linear regression analyses to assess the association between arsenic exposure with total H3 concentration and %H3K27me3.

In our linear regression models, we adjusted for case status of offspring to minimize the potential bias associated with the unequal sampling probabilities of mothers of cases and mothers of controls. As with the previous analyses, we evaluated potential confounding by maternal age, paternal age, receiving an ultrasound during pregnancy, medication use, maternal plasma folate concentration, and folate deficiency. Variables that were significant at the $P < 0.05$ level were chosen as potential confounders, and kept in models if they changed the estimate by 10%. We were not able to weight observations by sampling

Table 1. Characteristics of study population.

Characteristics	Controls (n = 40)	Cases (n = 45)	*P*-value
Maternal Characteristics			
Age at Delivery (years)	22.3 (4.4)	24.5 (5.4)	0.046
Ultrasound During Pregnancy (%)	87.5	88.9	0.84
Reported Folic Acid Use During Pregnancy (%)	60.0	44.4	0.15
Folate Deficiency (%)*	32.5	33.3	0.94
Plasma Folate (ng/ml)	3.5 (2.6)	4.2 (4.9)	0.39
Infant Characteristics			
Sex, Male (%)	60.0	57.8	0.84
Age (Months)	8.4 (5.2)	6.4 (5.7)	0.10

Data are shown as means (standard deviations) for continuous variables or proportions for categorical variables.
*Defined as maternal plasma folate concentration < 4 ng/ml.

Table 3. Associations between Plasma Histone Levels and Case Status of Offspring.

Variables	Models*	OR (95%CI)#
Total H3 Concentration	Model 1	1.00 (0.99, 1.01)
	Model 2	0.997 (0.99, 1.01)
	Model 3	0.994 (0.98, 1.01)
	Model 4	0.994 (0.98, 1.01)
%H3K27me3	Model 1	0.91 (0.84, 0.98)
	Model 2	0.91 (0.84, 0.98)
	Model 3	0.89 (0.82, 0.98)
	Model 4	0.89 (0.82, 0.98)

*Model 1 was adjusted for infant sex and infant age. Model 2 was adjusted for infant sex, infant age, and ln (maternal plasma folate concentration). Model 3 was adjusted for infant sex, infant age, ln (maternal plasma folate concentration), and ln (maternal toenail arsenic). Model 4 was adjusted for infant sex, infant age, ln (maternal plasma folate concentration), ln (maternal toenail arsenic), and ln (maternal water arsenic).
#OR: odd ratio; 95%CI: 95% Confidence Interval

probabilities, as the prevalence of myelomeningocele in Bangladesh is unknown. For all analyses, statistical significance was considered at the 2-tailed $P<0.05$ level. All statistical analyses were conducted using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA).

Results

A total of 85 plasma samples (45 from mothers of cases, 40 from mothers of controls) passed quality control criteria for histone concentration, and were included in the current analytical data set. Table 1 shows the characteristics of the study population based on case-control status of offspring. Mothers of infants with myelomeningocele were slightly older than mothers of controls ($P = 0.046$). We did not observe differences in maternal plasma folate concentration, use of ultrasound during pregnancy, or reported folic acid supplementation based on status of offspring, though differences in reported folic acid use between mothers of cases and mothers of controls were observed in previous reports using the data from the full study population.[15] Comparisons between the included (n = 40) and excluded (n = 15) mothers of controls, and between the included (n = 45) and excluded (n = 12) mothers of infants with myelomeningocele, showed no significant differences between the included and excluded groups with respect to maternal age, infant age, and infant sex. However, the included mothers of both controls and cases were more likely to have reported use of folic acid supplements during pregnancy (data not shown).

Table 2 displays the arsenic concentrations in water and toenails observed in our study. Twenty-seven water samples (31.8%) and 2 toenail samples (2.4%) had arsenic concentrations below the level of detection (LOD). While most of the study population were exposed to water arsenic concentrations below the current US and WHO standard of 10 μg/L, over 25% had levels higher than this standard, and some had exposure to water that had arsenic concentrations greater than 50 times that standard. Toenail arsenic and water arsenic concentrations were highly correlated ($r = 0.78$, $P<0.0001$).

We did not find a significant association between plasma folate concentration and either total H3 concentration or %H3K27me3, nor did we find any significant association between covariates (potential confounders) and histone concentrations in univariate models (all $P>0.05$, data not shown).

We found a significant association between %H3K27me3 and case status such that women with higher levels of %H3K27me3 had lower odds of having an infant with myelomeningocele [odds ratio (OR): 0.91, 95% confidence interval (CI): 0.84, 0.98]. This association did not change after adjustment for maternal plasma folate concentration, maternal toenail arsenic concentration, and maternal water arsenic concentration (Table 3).

We found that among women with folate deficiency (< 4 ng/ml), toenail arsenic concentration was inversely associated with total H3 levels [β (standard error) = −9.99 (3.91), $P = 0.02$] (Table 4). No significant association was observed between toenail arsenic concentration and %H3K27me3. This

Table 2. Distribution of Maternal Arsenic Exposure Levels and Plasma Histone Levels.

Variable	Mean	Standard Deviation	Minimum	25th Percentile	Median	75th Percentile	Maximum
Maternal Arsenic Exposure							
Drinking Water Arsenic (μg/L)	35.7	85.8	< LOD*	< LOD*	1.73	36.3	506
Maternal Toenail Arsenic (μg/g)	2.19	3.96	< LOD**	0.38	0.69	2.29	27.7
Plasma Histones							
Total H3 (ng/μL)	160	43.2	108	129	158	177	455
H3K27me3 (ng/μL)	31.8	12.0	10.7	24.1	29.2	37.4	71.7
%H3K27me3#	20.2	6.55	5.92	15.5	19.8	23.7	44.3

*Average LOD for water arsenic samples was 0.15 μg/L.
**Average LOD for toenail arsenic samples was 0.14 μg/g.
#Calculated by the percentage of the plasma H3K27me3 level divided by the total histone 3 concentration.
LOD, limit of detection.

Table 4. Associations between Maternal Arsenic with Plasma Histone Levels Stratified by Maternal Folate Status

	Total H3 Concentration				%H3K27me3			
	Plasma Folate ≥ 4 ng/ml		Plasma Folate < 4 ng/ml		Plasma Folate ≥ 4 ng/ml		Plasma Folate < 4 ng/ml	
Variables	β (SE)	*P*-value	β (SE)	*P*-value	β (SE)	*P*-value	β (SE)	*P*-value
Ln (Maternal Toenail Arsenic)	−5.24 (5.84)	0.37	−9.99 (3.91)	0.02	−0.51 (0.71)	0.48	−0.27 (1.26)	0.83
Ln (Maternal Water Arsenic)	−3.36 (2.22)	0.14	−2.09 (2.00)	0.31	−0.004 (0.28)	0.99	−0.29 (0.58)	0.62

All models were adjusted for infant sex, infant age, and case status of offspring.

pattern was also seen when water arsenic concentration was used as the measure of environmental arsenic exposure (Table 4).

Discussion

Using samples from a recently completed case control study in Bangladesh, we observed that plasma levels of the epigenetic histone modification H3K27me3 in mothers were significantly associated with myelomeningocele risk in offspring. H3K27me3 was selected because it has been associated with neural tube defects *in vitro*,[26] and in the amniotic fluid of neural tube-affected pregnancies.[27] We further found that arsenic exposure was associated with plasma total histone concentrations, but only among women who had concurrent folate deficiency.

Epigenetic mechanisms have an important role in gene regulation during fetal development and are suspected to contribute to neural tube defects. Mutations in genes that affect histone modification, in particular acetylation, result in neural tube defects in mice.[28] In humans, histone modification patterns differed between brains from fetuses with spina bifida and those from fetuses that were electively terminated.[29] The hypothesis that histone modifications in the mother may influence neural tube defect risk in children is supported by the observation that the antiepileptic drug valproate, which is an inhibitor of enzymes involved in deacetylation of histones,[8] is a well-known risk factor for neural tube defects in humans.[30,31]

Our study found that increasing levels of H3K27me3 in maternal plasma was associated with lower risk of myelomeningocele (OR: 0.91, 95%CI: 0.84, 0.98). Ours is the first study in humans to link maternal plasma levels of %H3K27me3 to myelomeningocele in offspring, and to suggest that epigenetic modifications in mothers as well as embryos may play a role in these disorders. Studies in experimental models support an important role of H3K27 in neural tube development. In cell culture, histone methylation at K27 is associated with repressed expression of several developmental genes,[32] and animal studies in $Sp^{-/-}$ mice show that increased H3K27 methylation is a marker of increased risk of lumbar neural tube defects.[33] In experiments performed in zebrafish, a family of H3K27 demethylases was found to be important in anterior-posterior development.[34] In humans, amniotic fluid stem cells cultured from myelomeningocele-affected pregnancies have demonstrated high levels of H3K27me3,[27] and the H3K27me3 mark in human embryonic stem cells is associated with regulation of dorsal patterning in the developing neural tube.[26] Our study suggests that %H3K27me3 in mothers may contribute to expression of genes important in neural tube closure in embryos. Future studies that incorporate gene expression data from embryos may better elucidate mechanisms by which this and other histone modifications affect myelomeningocele risk.

Our study took place in a setting of high environmental arsenic exposure, enabling a robust investigation of the associations between arsenic exposure and histone modifications. The majority of studies in arsenic and histone post-translational modifications have been conducted *in vitro*;[2,35-47] however, as recently reviewed by Howe and Gamble,[11] there is a growing body of literature from human populations that supports a link between arsenic exposure and post translational histone modifications.[23,47-50] For instance, a recent study by Pournara et al. (2016)[47] documented the inverse relationship between arsenic exposure via drinking water and decreases in global H3K9me3 in CD4+ cells, and H3K9me3 has been linked to metabolic disorder,[51] neurologic disorders,[52] and cancer.[53,54] Among the healthy population in Bangladesh, associations between higher drinking water and urinary arsenic exposures with alteration in various histone modifications were reported in a sex-dependent manner, suggesting the potential effects of arsenic exposure on epigenetics.[49] Furthermore, histone modification on H3K18ac and H3K36me3, which are particularly associated with higher arsenic concentrations in their biomarkers of urine and hair, were notably pronounced in the oxidative stress response gene promoters.[50] These findings corroborate histone modification as a potential mediator in the association between arsenic exposure and transcriptional regulation of oxidative stress response genes.[50] Most relevant to this study are recent investigations in Bangladesh that have shown that arsenic exposure among Bangladeshi adults was associated with %H3K36me2, a particular histone modification selected because of its association with cancer.[23] In that study, urinary arsenic was positively associated with %H3K36me2 in peripheral blood mononuclear cells in men but negatively associated with %H3K36me2 in women, suggesting a sex-specific effect of arsenic on this epigenetic marker.[23] The authors of these studies in Bangladesh did not report whether they evaluated potential folate deficiency to modify the effect of arsenic exposure on epigenetic markers.

We found that women's arsenic exposure as measured by toenail arsenic concentration was significantly associated with plasma H3 concentration, but this association was observed only among women with folate deficiency, suggesting that folate deficiency is a state in which the epigenetic effects of arsenic may be more prominent. It has been well-established that arsenic metabolism is dependent on folate, which facilitates methylation of arsenic into species that are more easily excreted.[55] Our studies provide further evidence that the role of

folate is not limited to carrying one-carbon units in metabolic pathways but may have an epigenetic role in disease as well, a finding consistent with animal studies.[56] We did not find an association between arsenic exposure and plasma %H3K27me3, suggesting that a different modification contributes to the change in plasma H3 in the presence of arsenic.

Our study has many important limitations, most significantly in its small sample size. Additionally, our associations between histone modification levels and myelomeningocele risk are limited by the case-control design because plasma samples were collected at the time of study visit, which was after the infant was born, and previous studies have shown that some histone modification levels change with variation of environmental exposures.[57,58] However, measures of histone concentration, arsenic, and folate were concurrent, and so our observations of arsenic's relationships with histone concentrations differing by folate status are not limited by time of collection.

Conclusions

Our results suggest that %H3K27me3 in maternal plasma differs between mothers of infants with myelomeningocele and mothers of infants without myelomeningocele, and may be a marker for myelomeningocele risk. Women with folate deficiency may be more susceptible to the epigenetic effects of environmental arsenic exposure.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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