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Gene-environment interaction and maternal arsenic methylation efficiency during pregnancy

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ABSTRACT

Background: Single nucleotide polymorphisms (SNPs) may influence arsenic methylation efficiency, affecting arsenic metabolism. Whether gene-environment interactions affect arsenic metabolism during pregnancy remains unclear, which may have implications for pregnancy outcomes.

Objective: We aimed to investigate main effects as well as potential SNP-arsenic interactions on arsenic methylation efficiency in pregnant women.

Method: We recruited 1613 pregnant women in Bangladesh, and collected two urine samples from each participant, one at 4–16 weeks, and the second at 21–37 weeks of pregnancy. We determined the proportions of each arsenic metabolite [inorganic As (iAs)%, monomethylarsonic acid (MMA)%, and dimethylarsinic acid (DMA)%] from the total urinary arsenic level of each sample. A panel of 63 candidate SNPs was selected for genotyping based on their reported associations with arsenic metabolism (including in *As3MT*, *N6AMT1*, and *GSTO2* genes). We used linear regression models to assess the association between each SNP and DMA% with an additive allelic assumption, as well as SNP-arsenic interaction on DMA%. These analyses were performed separately for two urine collection time-points to capture differences in susceptibility to arsenic toxicity.

Result: Intron variants for *As3MT* were associated with DMA%. rs9527 ($\beta = -2.98\%$, $P_{FDR} = 0.008$) and rs1046778 ($\beta = 1.64\%$, $P_{FDR} = 0.008$) were associated with this measure in the early gestational period; rs3740393 ($\beta = 2.54\%$, $P_{FDR} = 0.002$) and rs1046778 ($\beta = 1.97\%$, $P_{FDR} = 0.003$) in the mid-to-late gestational period. Further, *As3MT*, *GSTO2*, and *N6AMT1* polymorphisms showed different effect sizes on DMA% conditional on arsenic exposure levels. However, SNP-arsenic interactions were not statistically significant after adjusting for false discovery rate (FDR). rs1048546 in *N6AMT1* had the highest significance level in the SNP-arsenic interaction test during mid-to-late gestation ($\beta = -1.8\%$ vs. 1.4%, $P_{GxE\_FDR} = 0.075$). Finally, *As3MT* and *As3MT/CNNM2* haplotypes were associated with DMA% at both time points.

Conclusion: We found that not all genetic associations reported in arsenic methylation efficiency replicate in pregnant women. Arsenic exposure level has a limited effect in modifying the association between genetic variation and arsenic methylation efficiency.

Abbreviations: iAs, inorganic arsenic; MMA, monomethylarsonic acid; DMA, dimethylarsinic acid; As3MT, arsenic(III) methyltransferase; SNP, single nucleotide polymorphisms; GSTO2, glutathione S-transferase omega 2; N6AMT1, N-6 adenine-specific DNA methyltransferase 1; CNNM2, cyclin and CBS domain divalent metal cation transport mediator 2; FDR, false discovery rate

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1. Introduction

Arsenic is a naturally occurring metalloid that is a toxicant and human carcinogen (ATSDR, 2007; Welch et al., 2000; WHO, 2007). Arsenic distributes sparsely in surface water and groundwater systems; about 200 million people are exposed to arsenic, from drinking water over the World Health Organization (WHO) safety standard of 10 μg/L (Petrusevski et al., 2007). Chronic arsenic exposure affects multiple organs and systems, which leads to skin diseases, cancers, diabetes, cardiovascular diseases, negative reproductive outcomes and developmental problems (ATSDR, 2007; Ratnaike, 2003). Because arsenic crosses the placenta, exposure poses particular risks to both pregnant women and developing fetuses (Punshon et al., 2015). Epidemiological studies report that prenatal arsenic exposure is associated with maternal symptoms including anemia, nausea, vomiting, and abnormal cramping (Hopenhayn et al., 2006; Kile et al., 2014), and negative birth outcomes including reduced gestational weeks, low birth weight, spontaneous abortion, stillbirth, neonatal mortality, and infant mortality (Ahmad et al., 2001; Bloom et al., 2014; Cherry et al., 2008; Chou et al., 2014; Kile et al., 2016; Quansah et al., 2015; Rahman et al., 2010; Rahman et al., 2009; Yang et al., 2003).

The development of arsenic-related negative health effects is not only associated with the dosage of the exposure, but also with the individual arsenic metabolic capacities (Ahsan et al., 2007; Lindberg et al., 2008). In humans, inorganic arsenic (iAs) undergoes metabolic conversion from pentavalent to trivalent forms with subsequent methylation reactions (Drobna et al., n.d.; Vahter, 2002). Through catalysis by arsenate reductase (glutaredoxin, encoded by *GLRX*), arsenate (As^{V}) is rapidly reduced to arsenite (As^{III}) in blood, with reducing agent glutathione (GSH) (Schlawicke Engstrom et al., 2009). As^{III} is methylated to monomethylarsonic acid (MMA^{V}) in the liver with the methyl-donor S-adenosylmethionine (SAM), catalyzed by arsenite methyltransferase (encoded by *As3MT*) (Hayakawa et al., 2005; Marafante et al., 1985; Styblo et al., 1999). MMA^{V} is reduced to monomethylarsonous acid (MMA^{III}) and methylated to dimethylarsinic acid (DMA^{V}), which can convert to dimethylarsinous acid (DMA^{III}). All of these arsenic species can be found in urine, but MMA^{III} and DMA^{III} are highly reactive and rapidly converted to their pentavalent forms; these forms are the less toxic products of arsenic (Mandal et al., 2001; Styblo et al., 2000).

The enzymes involved in these steps determine the efficiency of methylating inorganic arsenic, thereby influencing arsenic metabolism. Genetic variation in genes encoding these enzymes affects the proportions of arsenic metabolites within the total arsenic level in urine (Ahsan et al., 2007; Steinmaus et al., 2006). Indeed, SNPs in *As3MT* are associated with arsenic methylation efficiency (Agusa et al., 2009; Antonelli et al., 2014; Engström et al., 2011; Hernández et al., 2008), as well as with arsenic-related negative health effects, including diabetes risk in people exposed to high level of arsenic in Mexico (Drobná et al., 2013) and basal cell carcinoma in a European population (Engström et al., 2015). A study of the Biomarkers of Exposure to ARsenic (BEAR) cohort also found *As3MT* polymorphisms to be associated with both arsenic methylation efficiency at the time of delivery and birth weight, depending upon infant sex (Drobna et al., 2016). These findings suggest that SNPs underlie a causal pathway in health outcomes are mediated through arsenic methylation efficiency. Under this hypothesis, individuals with better detoxification capacity have a lower risk of negative health effects.

Nonetheless, prior studies have several limitations. First, arsenic methylation efficiency is an important risk factor for negative health outcome and it increases during pregnancy(Gardner et al., 2012; Gardner et al., 2011; Hopenhayn et al., 2003). Thus, measuring urinary arsenic before a negative outcome is necessary to avoid misclassification of exposure, especially for pregnant women. Secondly, this change during pregnancy may result from altered gene expression during critical developmental periods (Kile et al., 2012). Thus, although associations between SNPs and arsenic methylation efficiency are established in non-pregnant populations, they should be validated for links with pregnancy outcomes. Second, arsenic exposure levels, genetic variation, and pregnancy all have an independent influence on arsenic methylation efficiency (Gardner et al., 2012). However, the interaction between arsenic exposure and genetic variation is poorly understood. Few studies reported a gene-arsenic interaction effect on cardiovascular risk factors and arsenic-induced skin lesions (Argos et al., 2018; Farzan et al., 2015; Pierce et al., 2013; Wu et al., 2014). It is possible that arsenic exposure interacts with genetic variants affecting arsenic methylation efficiency to alter health outcomes. However, no study has reported a SNP-arsenic interaction effect on arsenic methylation efficiency.

Other than *As3MT*, SNPs in *N6AMT1*, *GSTO*, and *GLRX*, as well as some genes that may not be closely involved in the arsenic methylation process, are associated with the concentrations or percentages of urinary arsenic metabolites (Antonelli et al., 2014; Beebe-Dimmer et al., 2012; Gao et al., 2015; Harari et al., 2013). For example, one-carbon metabolism (OCM) is a biochemical pathway that is able to provide methyl groups for arsenic methylation, and related nutritional status is reported associated with arsenic methylation efficiency in pregnant and non-pregnant cohorts (Bozack et al., 2018; Kurzius-Spencer et al., 2017; Laine et al., 2018). Methylenetetrahydrofolate reductase (encoded by *MTHFR*) is an important enzyme involved in OCM, and plays a role in the supplement of SAM in arsenic metabolism (Chung et al., 2010; Wlodarczyk et al., 2012). An association between *MTHFR* SNPs and arsenic metabolism was identified in both an animal model and an epidemiologic study (Steinmaus et al., 2007; Wlodarczyk et al., 2012). However, the effects of variants of MTHFR and other selected genes are not described in pregnant women.

Bangladesh has a severe public health problem of arsenic exposure in drinking water as well as a high prevalence of low birth weight (Naujokas et al., 2013; WHO, 2012). To evaluate genetic susceptibility to arsenic toxicity in pregnant women and developing fetuses, we evaluated the association between genetic variants and arsenic methylation efficiency in this critical period. In a group of pregnant women in Bangladesh exposed to varying levels of arsenic, we aimed to identify genetic association(s) and gene-environment interaction effects on arsenic methylation efficiency. We investigated these effects during two time-points, at early and mid-to-late gestational periods of pregnancy.

2. Methods

2.1. Study population

The Bangladesh prospective reproductive cohort study (Project Jeebon) is a community-based observational study of adult pregnant women to investigate arsenic-related reproductive health problems and birth outcomes. We recruited 1613 participants from 2008 through 2011 in two study centers of Dhaka Community Hospital (DCH), located in Pabna Upazila and Sirajdikhan Upazila of Bangladesh. All participants were in the first trimester of pregnancy at the recruitment visit (visit 1, 4–16 weeks of gestation). The participants attended DCH's prenatal health program until birth and delivered in DCH's hospital or clinic or at home with DCH-trained midwives. The second study visit (visit 2, 21–37 weeks of gestation) occurred at mid-to-late gestation. All participants received a prenatal vitamin with folate (400 μg), and 99.9% of the participants reported they took one tablet per day during pregnancy. More details of recruitment criteria and protocols of this cohort study have been published elsewhere (Kile et al., 2014).

All protocols were approved by the Institutional Review Boards (IRB) at both the Harvard School of Public Health and the Dhaka Community Hospital Trust. We obtained informed consent from each participant before the study.

2.2. Water arsenic exposure

Water arsenic samples were collected at visit 1 for arsenic concentration quantification. Briefly, uniformly-trained DCH research assistants collected a 50-mL water sample from each participant's primary water source, to assess the concentration of total arsenic in drinking water (DW-As). Water samples were acidified for stabilization and shipped to the laboratory at room temperature. Arsenic concentration was analyzed by inductively coupled plasma-mass spectrometer (ICP-MS) using U.S. EPA method 200.8 (Environmental Laboratory Services, North Syracuse, New York) (Longbottom et al., 1994). The detection limit was 1 μg/L for the instrument. Observations under the detection limit (N = 329, 20.5%) were assigned the value of 0.5 μg/L in statistical analysis. Details are published elsewhere (Kile et al., 2014).

2.3. Arsenic methylation efficiency

We collected two spot urine samples for each participant at visit 1 and visit 2, respectively. We quantified inorganic and methylated arsenic species from urine, including As^{III}, As^{V}, MMA, and DMA. Urine samples were stored at −80 °C and shipped to Dr. Yumei Hsueh's laboratory in the Department of Public Health, School of Medicine at Taipei Medical University, Taiwan. Urine samples were thawed at room temperature, sonicated for dispersion, and filtered through a Sep-Pak C18 column (Mallinckrodt Baker Inc., NJ, USA). Each sample was fractionated by high-performance liquid chromatography (HPLC; Waters 501, Waters Associates, Milford, MA, USA) with Phenomenex columns (Nucleosil, Torrance, CA, USA). Concentrations of each arsenic metabolite in urine (iAs^{III}, iAs^{V}, MMA, and DMA) were analyzed by hydride generator atomic absorption spectrometry (HG-AAS, Perkin Element). We used SRM 2670 from the National Institute of Standard and Technology (NIST, Gaithersburg, MD, USA) as a reference standard. The recovery rates for iAs^{III}, iAs^{V}, MMA, and DMA ranged from 93.8% to 102.2%. The detection limits were 0.02 μg/L, 0.06 μg/L, 0.07 μg/L, and 0.10 μg/L, respectively (Hsueh et al., 1997; Hsueh et al., 2002). Numbers of observations under the detection limits were 810, 476, 316, and 1 for visit 1; and 841, 374, 172, and 1 for visit 2, respectively. The results below the limit of detection were kept as the original value in the statistical analysis.

We analyzed the concentration of urinary creatinine by colorimetric assay (Roche Modular P800 instrument, Roche Inc., Mannheim, Germany) to control for dilution of the urine.

2.4. Genotyping

Maternal blood samples for each participant were collected at visit 1 for genotyping. Trained healthcare workers drew 6 mL of blood into a 7-mL vacutainer and refrigerated until transferred to −80 °C freezers. We extracted DNA in the Molecular Epidemiology laboratory at the Harvard T.H. Chan School of Public Health, Boston, MA, USA.

We selected a panel of 63 candidate SNPs (Table S1), based on reported evidence of their associations with arsenic methylation efficiency. These SNPs are located in genes encoding enzymes or proteins directly or indirectly related to the arsenic methylation pathway including arsenic methyltransferase and glutathione s-transferase families, as well as methylenetetrahydrofolate reductase in the OCM pathway. We used TaqMan genotyping with the ABI Prism 7900 HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA). Two proxy SNPs were used to substitute SNPs that cannot be genotyped by this method (Table S1). We randomly selected 5% of samples as validation duplicates in genotyping quality control (QC) procedures.

Our QC criteria include minor allele frequencies (MAF) > 0.02, sample call rate > 0.95, SNP call rate > 0.95, and Hardy-Weinberg equilibrium (HWE) *p*-value > 0.000001. The QC procedure is shown in Fig. 1.

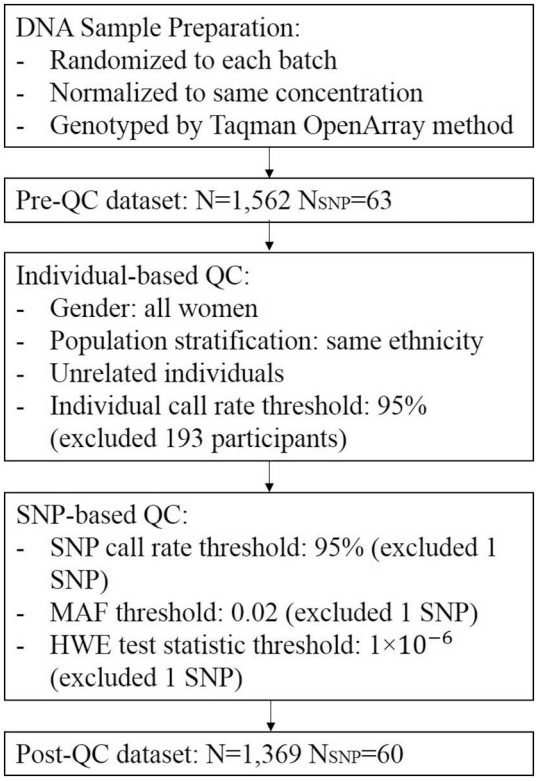

Fig. 1. The SNP-based and individual-based genotyping data quality control (QC) criteria and exclusion situations.

2.5. Statistical analysis

Demographic information was summarized conditional on high and low DW-As groups (DW-As > 2 μg/L or DW-As ≤ 2 μg/L). We drew triangle plots to show the ratio of arsenic species in urine using R 2.14.2 (Hamilton, 2016; Team, 2000).

The primary outcome of the analyses is arsenic methylation efficiency represented by the concentration of DMA over the sum of all arsenic species concentrations in urine (DMA%). The percentages of other species were calculated in the same way. The proportion of inorganic arsenic (iAs%) was the sum of the concentrations of As^{III} and As^{V} over the sum of all arsenic species concentrations in urine.

We performed linear regression models to assess the association between DMA% and each SNP controlling for DW-As (Model 1) using PLINK (Purcell et al., 2007). With an additive assumption of the allelic genetic model, the numbers of coded alleles were coded as 0, 1, and 2 for each SNP. The linkage disequilibrium pattern of the candidate SNPs may cause inflation of *p*-values of the analyses. Thus, we calculated the variance inflation factors using R; then we used Benjamini and Hochberg (2000) step-up false discovery rate (FDR) method to adjust for the inflation of *p*-values (Benjamini and Hochberg, 2000).

We applied two models to assess the SNP-arsenic interactions (Model 2 and 3). In Model 2, we performed linear regression to examine the association between DMA% and SNPs conditional on dichotomized low and high arsenic exposure groups using PLINK quantitative trait interaction analysis. The interaction effects were evaluated by comparing the regression coefficients between high and low exposure group using *Z*-score test.

In Model 3, we evaluated interactions by adding a SNP-arsenic exposure interaction term to the linear regression model. We used SAS software for this model because it allows DW-As to be a continuous variable (version 9.4; SAS Institute Inc., Cary, NC, USA). We adjusted for age, body mass index (BMI) at enrollment, household income (< 3000 takas or ≥3000 takas), and education level (< Secondary

Table 1
Demographic characteristics of the study participants of Project Jeebon*, by exposure level.

	Low As group	High As group	Study sample
	N		
Sample size			
Visit 1	673	690	1363
Visit 2	605	623	1228
Education level			
< Secondary education	335	315[a]	650
≥ Secondary education	328	358	686
Household income			
< 3000 takas	199	376[b]	575
≥ 3000 takas	464	297	761
	Mean (SD)		
Age, years	22.8 (4.1)	23.0 (4.3)[c]	22.9 (4.2)
BMI at enrollment, kg/m^2	20.9 (3.3)	20.0 (2.9)[d]	20.5 (3.2)
Gestational age, weeks[e]			
Visit 1	11.4 (3.1)	11.4 (2.9)	11.2 (3.1)
Visit 2	28.8 (1.8)	29.2 (2.0)	29.0 (1.9)
	Median (IQR)		
Adjusted total urinary arsenic, mg/g-creatinine			
Visit 1	48.7 (31.7–81.6)	137.6 (77.1–290.7)	79.9 (43.2–185.1)
Visit 2	60.5 (41.0–97.4)	151.5 (86.2–356.9)	92.2 (52.2–202.2)
Drinking water arsenic, μg/L			
Study sample	1.0 (0.5–1.5)	34.0 (10.0–109.0)	2.2 (1.0–34.0)
Overall sample	1.0 (0.5–1.5)	34.0 (10.0–109.0)	2.0 (0.9–33.0)

SD, standard deviation; IQR, interquartile range.

* The study sample includes 1363 participants with genotype data. The overall sample includes 1604 participants in this reproductive cohort. Low As Group: Drinking water arsenic ≤2 μg/L; high As Group: drinking water arsenic > 2 μg/L.

[a] Chi-sq test $p = 0.189$.

[b] Chi-sq test $p < 0.001$.

[c] Two sample t-test $p = 0.398$.

[d] Two sample t-test $p = 0.003$.

[e] Measured by ultrasound at each urine sample collection.

education or ≥Secondary education). All models were adjusted for false discovery rate (FDR).

The linkage disequilibrium (LD) between SNPs was evaluated by Haplotyper (Niu et al., 2002). We ended up with ten groups of alleles with $R^2 > 94\%$ aided by Haploview Software (Barrett et al., 2004). We analyzed the association between DMA% and specific haplotype sequences using PLINK, which performed haplotype-specific tests of each versus all others, and generated omnibus association statistics.

3. Results

Characteristics of participants in Project Jeebon are shown in Table 1. Among 1613 participants in this study, 1562 had maternal blood samples for genotyping, and 1369 of them passed the genotyping data QC process. After excluding participants with missing drinking water samples and missing urinary arsenic metabolite data, we had a final sample of 1364 participants at visit 1. Due to loss of contact, miscarriage, or stillbirth, the final sample at visit 2 included 1228 participants. The median and interquartile range (IQR) of DW-As were 2.2 (1.0–34.0) μg/L. The median and the IQR of adjusted urinary arsenic concentration for visits 1 and 2 were 79.9 (43.2–185.1) and 92.2 (52.2–202.2) mg/g-creatinine, respectively.

Triangle plots (Fig. 2) depict the ratios between iAs%, MMA%, and DMA%, as they summed to 1. The position of each individual dot on the equilateral triangle shows the arsenic methylation efficiency of the corresponding individual.

Table 2 shows the top-hit SNPs that were associated with DMA% by study visit (Model 1). Among 60 SNPs that passed QC processes, we identified that one more T allele in rs9527 was associated with −2.98 unit change in DMA% ($P_{FDR} = 0.008$) and one more C allele in rs1046778 was associated 1.64 unit increase in DMA% ($P_{FDR} = 0.008$) at visit 1. Rs3740393 ($\beta = 2.54\%$, $P_{FDR} = 0.002$) and rs1046778 ($\beta = 1.97\%$, $P_{FDR} = 0.0023$) were significantly associated with DMA% at visit 2. These SNPs are intronic variants in *As3MT*.

Further, variants of *As3MT* and *GSTO2* showed suggestive evidence of SNP-arsenic interaction in the early gestation. Rs156697 (intronic variant/missense in *GSTO2*, $\beta = 1.9\%$ vs. -0.2% for low and high arsenic group, $P_{GxE} = 0.022$), rs1046778 ($\beta = 0.7\%$ vs. -2.5%, $P_{GxE} = 0.042$) and rs2297235 (upstream variant in 5′ UTR of *GSTO2*, $\beta = 1.7\%$ vs. -0.5%, $P_{GxE} = 0.047$) showed different effect sizes conditional on arsenic exposure levels. In mid-to-late gestation, we found evidence suggestive of SNP-arsenic interaction effects in *N6AMT1*, *As3MT*, and *GSTO2* polymorphisms, including rs1048546 (intronic variant of *N6AMT1*, $\beta = -1.8\%$ vs. 1.4%, $P_{GxE} = 0.001$), rs9527 ($\beta = 0.1\%$ vs. -3.8%, $P_{GxE} = 0.029$), rs2705671 ($\beta = -2.4\%$ vs. 1.4%, $P_{GxE} = 0.048$), and rs156697 ($\beta = 1.9\%$ vs. -0.4%, $P_{GxE} = 0.028$). However, all of these interaction effects were not statistically significant after adjusting for FDR. Tables 3 and 4 show the regression coefficients and adjusted p-values of Models 2 and 3 by study visit.

In Model 3, we found in the early gestational period, the individual effect of rs1046778 and rs156697 had significant SNP-arsenic interactions. The association between rs156697 and DMA% was stronger in those participants in the low exposure group ($\beta = 1.9\%$ vs. -0.2% for low and high exposure group, $p = 0.022$). The association between rs1046778 and DMA% was stronger in those participants in the high exposure group ($\beta = 0.7\%$ vs. 2.5% for low and high exposure group, $p = 0.042$). However, after adjusting for FDR, the interaction effect was no longer statistically significant.

We observed similar suggestive evidence of interaction effects in mid-to-late gestation. The regression coefficient between rs1046778 and DMA% were 0.9% vs. 3.0% for low and high exposure groups, respectively, in Model 2 ($p = 0.040$), but the p-value for the interaction term in Model 3 was 0.16 (therefore excluded from Table 4). Conversely, the association between rs156697 and DMA% was stronger among participants in the lower exposure group, which was consistent throughout gestation. Rs1048546 of *N6AMT1* affected DMA% differently by water arsenic level only in the mid-to-late gestational period ($\beta = -1.8\%$ vs. 1.4% for low and high exposure group, $p = 0.075$). However, these associations were not statistically significant after adjusting for FDR.

The C allele in rs4919694 (intronic variant in *CNNM2*) was negatively associated with DMA% in both study visits, with a stronger effect size in the higher exposure group. Among the ten haplotypes identified from the candidate SNP panel, we found that *As3MT* (rs9527|rs3740400), and *As3MT/CNNM2* (rs3740393|rs11191439|rs10748835|rs1046778|rs4919694|rs11191527) haplotypes were associated with arsenic methylation (Tables S4 and S5), but we did not find interaction effects conditional on arsenic exposure.

4. Discussion

We assessed gene-environment interaction for two key determinants of arsenic metabolism: 1) genetic variants and 2) drinking water exposure level. We found suggestive evidence that the effects of SNPs on arsenic methylation efficiency were modified by water arsenic exposure level. Moreover, these associations may vary by stage of pregnancy. Our findings are important to understand the risk of arsenic-related negative health effects.

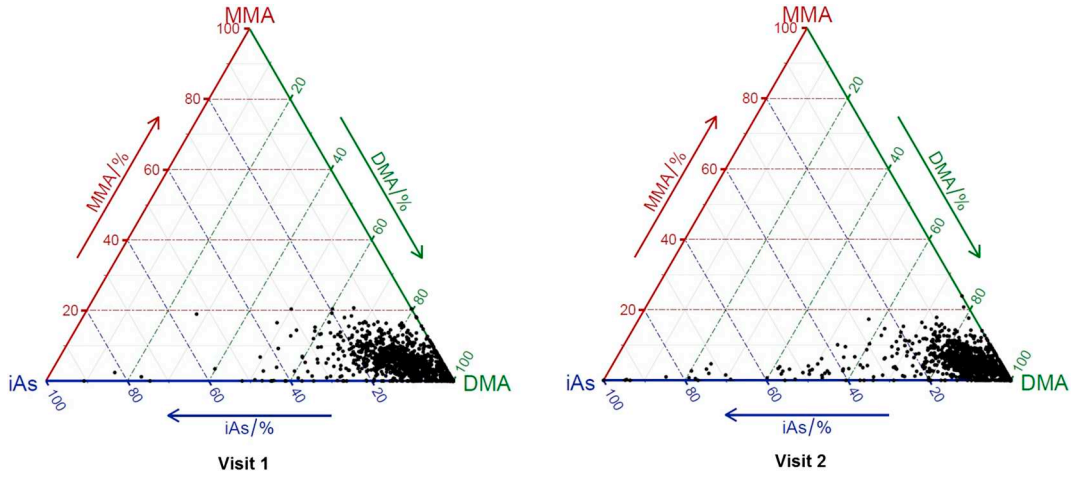

Fig. 2. Triangle plots of the proportions of urinary arsenic metabolites for visit 1 and visit 2*.
*Average levels of the urinary arsenic profile were iAs% = 9.82%, MMA% = 5.36%, DMA% = 84.80% at visit 1 (N = 1606), and iAs% = 8.7%, MMA% = 5.2%, DMA% = 86.01% at visit 2 (N = 1443). The average level of urinary arsenic level of visit 1 were iAs% = 9.80%, MMA% = 5.28%, DMA% = 84.91% for participants having data for both visits (N = 1443).

Table 2
Top-hit SNPs associated with DMA% during pregnancy with *p*-values after adjusting for multiple comparisons, in early (visit 1) and mid-to-late (visit 2) gestational periods.

Visit	CHR	Gene	SNP	Position	Coded/non-coded allele	Coded allele freq.	β[a]	$P_{unadjusted}$	P_{FDR}
Visit 1	10	*As3MT*	rs9527	102863821	T/C	0.08	−2.98	< 0.001	0.008
	10	*As3MT*	rs1046778	102901727	C/T	0.35	1.64	< 0.001	0.008
	10	*As3MT*	rs3740393	102876898	C/G	0.18	1.56	0.005	0.093
	10	*CNNM2*	rs4919694	102939221	C/T	0.10	−1.94	0.007	0.108
	10	*As3MT*	rs11191439	102878966	C/T	0.06	−2.23	0.013	0.155
	12	*SLCO1B1*	rs2291075	21178691	T/C	0.23	1.02	0.048	0.479
	10	*As3MT*	rs10748835	102900499	A/G	0.44	0.74	0.086	0.733
	10	*GSTO2*	rs156697	104279427	G/A	0.33	0.76	0.101	0.758
Visit 2	10	*As3MT*	rs3740393	102876898	C/G	0.18	2.54	< 0.001	0.002
	10	*As3MT*	rs1046778	102901727	C/T	0.35	1.97	< 0.001	0.003
	10	*As3MT*	rs10748835	102900499	A/G	0.44	1.25	0.001	0.198
	10	*As3MT*	rs3740400	102869708	G/T	0.44	1.18	0.018	0.271
	10	*As3MT*	rs9527	102863821	T/C	0.08	−1.99	0.028	0.279
	10	*As3MT*	rs11191439	102878966	C/T	0.06	−2.17	0.028	0.279
	10	*CNNM2*	rs4919694	102939221	C/T	0.10	−1.43	0.079	0.591
	8	*GSR/GR*	rs2253409	30689449	G/C	0.30	−0.91	0.084	0.591
	10	*GSTO1*	rs11509438	104267301	A/G	0.30	1.38	0.089	0.591
	12	*SLCO1B1*	rs2291075	21178691	T/C	0.23	0.84	0.144	0.706

Freq: frequency; Chr: chromosome; SNP: single nucleotide polymorphism; SE: standard error; $P_{unadjusted}$: original *p*-values; P_{FDR}: Benjamini and Hochberg (2000) step-up FDR control.
[a] β is in the unit of (%); it represents the change in DMA% for one more coded allele controlling for water arsenic level.

Table 3
SNP-arsenic interaction effects on DMA% during early gestation identified in Bangladeshi pregnant women, and *p*-values under multiple testing adjustment.

			Model 2				Model 3				
			Low As group	High As group							
Gene	SNP	Coded allele	β_{SNP} (SD)[a]	β_{SNP} (SD)	P_{GxE}	P_{GxE_FDR}	β_{SNP} (SD)	P_{SNP}	β_{GxE} (SD)	P_{GxE}	P_{GxE_FDR}
GSTO2	rs156697	G	1.9 (0.8)	−0.2 (0.6)	0.022	0.269	1.7 (0.6)	0.002	−0.6 (0.2)	0.005	0.312
As3MT	rs1046778	C	0.7 (0.7)	2.5 (0.5)	0.042	0.401	0.8 (0.6)	0.139	0.5 (0.2)	0.01	0.312
GSTO2	rs2297235	G	1.7 (0.9)	−0.5 (0.7)	0.047	0.401	1.4 (0.7)	0.029	−0.5 (0.2)	0.027	0.359
CNNM2	rs4919694	C	0.4 (1.3)	−3.6 (0.8)	0.008	0.243	−0.5 (0.9)	0.601	−0.7 (0.3)	0.034	0.359
PEMT	rs897453	T	−2.4 (1.0)	0.6 (0.7)	0.014	0.269	−1.7 (0.7)	0.020	0.5 (0.3)	0.054	0.391
TXNRD1	rs11111979	C	−1.5 (0.7)	1.2 (0.5)	0.003	0.179	−0.7 (0.5)	0.183	0.4 (0.2)	0.063	0.391
As3MT	rs3740393	C	0.5 (0.9)	2.6 (0.7)	0.068	0.496	0.9 (0.7)	0.194	0.5 (0.2)	0.068	0.391
As3MT	rs11191439	C	0.5 (1.6)	−4.0 (1.0)	0.019	0.269	−0.7 (1.2)	0.559	−0.7 (0.4)	0.098	0.515

Freq: frequency; Chr: chromosome; SNP: single nucleotide polymorphism; SE: standard error; $P_{unadjusted}$: original *p*-values; P_{FDR}: Benjamini and Hochberg (2000) step-up FDR control.
We present only top hits with $P_{GXE} < 0.1$ in Model 3.
[a] β is in the unit of (%), it represents the change in DMA% for one more coded allele controlling for water arsenic level.

Table 4
SNP-arsenic interactions on DMA% during mid-to-late pregnancy period identified in Bangladeshi pregnant women, and *p*-values under multiple testing adjustment.

			Model 2				Model 3				
			Low As group	High As group							
Gene	SNP	Coded allele	β_{SNP} (SD)[a]	β_{SNP} (SD)	P_{GxE}	P_{GxE_FDR}	β_{SNP} (SD)	P_{SNP}	β_{GxE} (SD)	P_{GxE}	P_{GxE_FDR}
N6AMT1	rs1048546	T	−1.8 (0.7)	1.4 (0.7)	0.001	0.075	−1.2 (0.7)	0.077	0.6 (0.2)	0.005	0.359
As3MT	rs11191439	C	0.1 (1.5)	−3.7 (1.3)	0.062	0.289	0.1 (1.3)	0.932	−1.0 (0.5)	0.019	0.375
As3MT	rs9527	T	0.1 (1.3)	−3.8 (1.3)	0.029	0.289	−0.2 (1.2)	0.837	−0.9 (0.4)	0.028	0.375
N6AMT1	rs2705671	G	−2.4 (1.4)	1.4 (1.3)	0.048	0.289	−2.0 (1.2)	0.100	1.0 (0.4)	0.032	0.375
GSTO2	rs156697	G	1.9 (0.7)	−0.4 (0.7)	0.028	0.289	1.5 (0.7)	0.018	−0.5 (0.2)	0.033	0.375
CNNM2	rs4919694	C	0.4 (1.2)	−2.7 (1.1)	0.063	0.289	0.2 (1.1)	0.854	−0.7 (0.4)	0.039	0.375
N6AMT1	rs1997605	G	−2.1 (1.2)	0.9 (1.1)	0.055	0.289	−1.7 (1.0)	0.092	0.7 (0.4)	0.057	0.375
N6AMT1	rs16983411	G	−1.2 (0.8)	1.3 (0.8)	0.025	0.289	−0.6 (0.7)	0.365	0.5 (0.3)	0.059	0.375
As3MT	rs11191527	T	−1.1 (0.9)	2.2 (0.9)	0.011	0.289	−0.3 (0.8)	0.690	0.5 (0.3)	0.070	0.400
MTR	rs1805087	G	0.8 (0.8)	−1.5 (0.8)	0.044	0.289	0.4 (0.7)	0.581	−0.5 (0.3)	0.082	0.400

Freq: frequency; Chr: chromosome; SNP: single nucleotide polymorphism; SE: standard error; $P_{unadjusted}$: original *p*-values; P_{FDR}: Benjamini and Hochberg (2000) step-up FDR control.
We present only top hits with $P_{GXE} < 0.1$ in Model 3.
[a] β is in the unit of (%); it represents the change in DMA% for one more coded allele controlling for water arsenic level.

The SNP-arsenic interactions for *As3MT* polymorphisms have been investigated in both animal models and human cohort studies (Hernández et al., 2014; Huang et al., 2017a; Huang et al., 2017b; Pierce et al., 2013; Wu et al., 2014). Among them, the Health Effects of Arsenic Longitudinal Study (HEALS) and BEAR pregnancy cohort both provide some evidence about the association between genetic variants and specific health outcomes, as well as the SNP-arsenic interaction. For example, Pierce et al. reported that the individual effects of rs9527 (*As3MT*) and rs11191527 (*CNNM2*) are associated with skin lesions, and participants with higher arsenic exposure have a stronger association (Pierce et al., 2013). This effect modification is hypothesized to result, under higher exposure levels, in genetic variants producing enzymes in different amounts or activity levels, resulting in higher proportions of unmethylated iAs accumulating in the body. Drobna et al. reported that rs3740393 (*As3MT*) is significantly associated with placental weight and marginally associated with birth measures, and the effect was modified by infant sex, but not exposure level (Drobna et al., 2016). A 7-year follow-up study, HEALS, reported that rs3794624 (*CYBA*) is associated with annual pulse pressure (a cardiovascular function indicator), and water arsenic level modifies this association (Farzan et al., 2015). In our evaluation of the interaction effect of genetic variation on pregnant womens' arsenic methylation efficiency, we have found similar suggestive evidence that the drinking water arsenic level may modify this association. Tables S2 and S3 show the result sorted in the order of significance level of the interaction analysis of Models 2 and 3.

Interestingly, although the individual effect of SNPs varied by study visits, the regression coefficients conditional on high and low exposure group were more stable over study visits. This indicates that arsenic exposure is likely to be an effect modifier between genetic variants and arsenic methylation efficiency. Our results imply that the altered arsenic metabolism conditional on water exposure may be the mechanism underlying the effect of SNP-arsenic interaction on health outcomes (Thomas et al., 2007). This effect modification may occur due to accumulated intermediates in arsenic metabolism. Arsenic (III) methyltransferase catalyzes the transfer of a methyl group from S-adenosyl-L-methionine (AdoMet) to trivalent arsenical to become MMA (Hayakawa et al., 2005; Marafante et al., 1985; Styblo et al., 1999). Meanwhile, arsenic methylation efficiency is associated with an oxidative stress biomarker in children and adults, which suggests that the negative health effects related to poor arsenic metabolism may be induced by oxidative stress (Xu et al., 2008).

We found that rs4919694 (*CNNM2*) was strongly associated with arsenic methylation efficiency. *CNNM2* encodes a member of the ancient conserved domain-containing protein family that contains a cyclin box motif and has structural similarity to the cyclins; it plays an important role in magnesium homeostasis by mediating the epithelial transport and renal reabsorption of MgII (de Baaij et al., 2012). Its function is not directly associated with the arsenic methylation pathway. Thus, its association with As methylation efficiency is more likely due to its high LD with *As3MT* polymorphisms, as the *CNNM2* gene is within 800 kilobasepairs of *As3MT* (Engström et al., 2013). In the haplotype analysis, we found that the *As3MT/CNNM2* haplotype is significantly associated with arsenic methylation efficiency at both visit 1 and visit 2, which supports the above interpretation. We did not identify a significant gene-environment effect for this SNP.

Glutathione is an important antioxidant. Its reduced state can donate a reducing equivalent to other molecules, and be involved in the reducing reactions of arsenic methylation (Kobayashi et al., 2005; Waters et al., 2004). There is debate on whether SNPs in the *GST* family are associated with arsenic methylation efficiency (Kile et al., 2005; McCarty et al., 2007; Steinmaus et al., 2007; Yang et al., 2015). One study found that SNPs in *GSTT1* and *GSTM1* are associated with MMA and DMA levels (Yang et al., 2015), while another study's findings did not support this conclusion (Kile et al., 2013). There is also no agreement about the SNP-environment interactions of these genes. In our screening of the *GST* family, we did not find association in the regression analysis of Model 1, but found that one more G allele of SNP rs156697 (*GSTO2*) was linked to a 1.9% increase in DMA% in the low exposure group and −0.2% change of DMA% in the high exposure group. The interaction effect is not significant after adjusting for FDR, and the *GSTO1/GSTO2* haplotype was not associated with DMA%.

We found no evidence that other SNPs in the candidate panel were significantly associated with arsenic methylation efficiency during pregnancy. Since other genes are not directly involved in the arsenic methylation pathway, it was not surprising that the previously reported associations did not replicate in pregnant women in our study. In addition, we provided vitamin supplements with sufficient folate, which may also result in the lack of effect of *MTHFR*, *MTR*, and *DNMT3a* (involved in the folate pathway (Schlawicke Engstrom et al., 2009)) on arsenic methylation efficiency.

Although we found limited evidence that variants in genes encoding enzymes involved in arsenic metabolism alter arsenic methylation efficiency, the magnitudes of the effects were small. Previous evidence indicated that maternal arsenic methylation efficiency influences the risk of low birth weight (Gelmann et al., 2013; Vahter and Concha, 2001). We performed an exploratory analysis to assess the association between maternal genetic variants and birth outcomes, but did not find

any significant result (data not shown).

We recognize several limitations of our study. First, we could not differentiate MMA^{III} and DMA^{III} from MMA^{V} and DMA^{V}, respectively. While all metabolites appeared in the urine, it is not yet possible to capture the precise amount of trivalent forms (Concha et al., 2002). The proportions of total MMA and total DMA in urine may not precisely represent the toxicity of arsenic metabolites (Styblo et al., 2000). Second, we may need a larger sample size to detect SNP-arsenic interactions. Third, the generalizability of this study is limited because of the homogeneity of the participants' race and demographics.

In spite of these limitations, we had a well-established cohort with comprehensive information on their demographics, lifestyle, and medical condition during pregnancy. We have individual-level measures of water arsenic exposure, and repeated urinary arsenic metabolites measures covered different time points during pregnancy. Our statistical methods were well-established, and two models were used to compare the consistency of the analytical result.

5. Conclusion

Understanding the determinants of maternal arsenic methylation during pregnancy is important in predicting arsenic-related morbidities. Our study evaluated the association between genetic polymorphisms and arsenic methylation efficiency, and found potential SNP-arsenic interaction effects during pregnancy. After evaluating the SNP-arsenic interaction effect on arsenic methylation efficiency, we found the association patterns were slightly different between the early and mid-to-late gestational periods. In a future study we will evaluate the association between arsenic methylation efficiency and low birth weight.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2019.01.042.

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