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RESEARCH ARTICLE

GSTO and *AS3MT* genetic polymorphisms and differences in urinary arsenic concentrations among residents in Bangladesh

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Abstract

We determined whether single nucleotide polymorphisms (SNPs) in the glutathione S-transferase omega (*GSTO*) and arsenic(III)methyltransferase (*AS3MT*) genes were associated with concentrations of urinary arsenic metabolites among 900 individuals without skin lesions in Bangladesh. Four SNPs were assessed in these genes. A pathway analysis evaluated the association between urinary arsenic metabolites and SNPs. *GSTO1 rs4925* homozygous wild type was significantly associated with higher monomethylarsonic acid (MMA) and dimethylarsinic acid urinary concentrations, whereas wild-type *AS3MT* rs11191439 had significantly lower levels of As^{III} and MMA. Genetic polymorphisms *GSTO and As3MT* modify arsenic metabolism as evidenced by altered urinary arsenic excretion.

Keywords: Pathway analysis, arsenic metabolism, drinking water

Introduction

According to the International Agency for Research on Cancer, arsenic (As) is classified as a known human carcinogen (IARC 1980). However, an individual's ability to metabolize As can modify the risk associated with chronic arsenic exposure (Ahsan et al. 2007, Steinmaus et al. 2010). According to the classical pathway, As is metabolized in humans by oxidative-reduction methylation reactions where arsenate (As^{V}) is reduced to arsenite (As^{III}) and then methylated to form monomethylarsonic acid (MMA^{V}), which undergoes second reduction to form monomethylarsonous acid (MMA^{III}) which can be further methylated to form dimethylarsinic acid (DMA^{V}) which can be further reduced to dimethylarsinous acid (DMA^{III}) (Challenger 1945). Glutathione is a reducing agent whereas S-adenosylmethionine is the primary methyl donor in these reactions (Vahter 2002).

The metabolism of As in humans is incomplete and all arsenic species (As^{V}, As^{III}, MMA, and DMA) are detected in urine. Urinary As profiles have been well documented in several populations and on average, the urinary As levels are composed of 10–30% inorganic As, 10–20% MMA, and 60–70% DMA (Vahter 1999). Observational studies in populations chronically exposed to arsenic from drinking water have shown that there is large inter-individual variability in the distribution of urinary arsenic metabolites (Vahter 1999, Loffredo et al. 2003, Steinmaus et al. 2005, Kile et al. 2009). Toxicological studies have shown that the toxicity of arsenic species differs with the trivalent monomethylated forms being the most toxic (Styblo et al. 2000). Epidemiological studies also suggest an individual's ability to methylate As influences susceptibility to As toxicity (Tseng 2009). Specifically, individuals with higher %MMA or ratio of MMA-to-DMA have a greater

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risk for skin cancer (Styblo et al. 2000, Vega et al. 2001), bladder cancer (Tseng 2009), hypertension (Chen et al. 2003), and peripheral vascular disease (Yu et al. 2000).

Understanding the factors associated with As metabolism is important to determine the characteristics that make one more susceptible to arsenic exposure. Age, gender, arsenic levels in drinking water, and urinary creatinine levels have been significantly associated with arsenic metabolism and urinary profiles (Ahsan et al. 2007, Lindberg et al. 2008), but it has also been shown that genetic differences influence As metabolism and urinary arsenic profiles (Marnell et al. 2003, Lindberg et al. 2007).

Several studies have examined the ability of genetic polymorphisms to modify arsenic metabolism. GSTO1 and GSTO2 are members of the glutathione s-transferase family, which are involved in metabolizing xenobiotics such as arsenic. *In vitro* studies have determined that glutathione-*S*-transferase omega (GSTO1), which is identical to monomethyl arsenate MMA^V reductase, is the rate-limiting enzyme involved in arsenic biotransformation (Zakharyan and Aposhian 1999, Zakharyan et al. 2001) and catalyzes the reduction of As^V to As^{III} and DMA^V to DMA^{III}. GSTO2 encodes a protein that shares 64% amino acid identity with GSTO1 (Whitbread et al. 2003) and has also been shown to catalyze the reduction of MMA^V and DMA^V (Schmuck et al. 2005). Additionally, it has been shown that the *Met287Thr* variant has higher enzyme (AS3MT) activity that may contribute to differences in arsenic metabolism, specifically higher levels of methylated arsenic compounds, and its toxicity (Wood et al. 2006).

While many studies have investigated factors associated with arsenic profiles and percentages of urinary metabolites, few studies have reported on excretion of arsenic using urinary arsenic concentrations. The main objective of this study was to determine whether single nucleotide polymorphisms (SNPs) in the glutathione S-transferase omega (*GSTO*) and arsenic(III)methyltransferase (*AS3MT*) genes were associated with the excretion of arsenic measured as urinary concentrations of urinary arsenic species, As^V, As^{III}, MMA, and DMA. Given the complex relationship between urinary arsenic metabolites, we utilized a novel pathway analysis to simultaneously evaluate the association between the SNPs and all urinary arsenic metabolites among controls recruited in a large population-based case–control study of arsenic-related skin lesions in an arsenic-endemic region of Bangladesh.

Methods

Study population

A detailed description of participant selection and sample collection is published elsewhere (Breton et al. 2007). Briefly, a case–control study comprised of 900 cases and 900 controls was conducted in the Pabna region of Bangladesh from 2001–2003 to investigate the association between exposure to arsenic-contaminated drinking water and skin lesions. All participants were recruited for the study through the Dhaka Community Hospital and Pabna Community Clinic. Because this paper is investigating the effect of SNPs on the metabolism of arsenic and not a disease endpoint, the current analysis was limited to the 900 controls to eliminate any possible confounding by disease status. This restriction to controls ensures that the potential effects on arsenic metabolism are not due to the presence of disease. Participants were eliminated if they were missing data for any of the variables included in the path analysis, resulting in 842 controls. All protocols were approved by the Institutional Review Boards at Harvard School of Public Health and Dhaka Community Hospital, and informed consent was obtained from each participant prior to the conduct of the study.

Sample collection

At the time of enrollment, a water sample was collected from the tube well that each participant identified as their primary source of drinking water. The water samples were collected in 50 mL polypropylene centrifuge tubes, and two drops of pure nitric acid were added. The water samples were stored at room temperature and analyzed for arsenic using Environmental Protection Agency method 200.8 with inductively coupled plasma mass spectroscopy (ICP-MS) (Environmental Laboratory Services, North Syracuse, NY).

On the same day as the water sample collection, a spot urine sample (approximately 120 mL) was collected from each subject. Urine samples were placed in an icebox immediately upon collection, then transferred into 15-mL Falcon tubes, and then frozen at –20°C. Samples were shipped on dry ice to Taipei Medical University where they were analyzed for DMA, monomethylarsonic acid (MMA), As^{III}, and As^V. Total urinary arsenic was calculated by summing the concentrations of DMA, MMA, As^{III}, and As^V. A detailed description of the laboratory procedures have been described elsewhere (McCarty et al. 2007). Urinary creatinine concentrations were quantified using the kinetic Jaffe Method using a Hitachi 7170S autoanalyzer (Tokyo, Japan).

Genotyping

DNA was extracted from whole blood using the Puregene DNA Isolation kit (Gentra Systems, Minneapolis, MN). The following SNPs were detected by the Taqman method using the ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA) and were selected because they were functional (i.e. lead to an amino acid change) or were a tagging SNP: *GSTO1* (Ala140Asp, rs4925), *GSTO2* (Asn142Asp, rs156697), *GSTO2* (rs2297235), and *AS3MT* (Met287Thr, rs11191439). As part of quality control, 10% of the samples were analyzed in duplicate and two readers evaluated the output. All selected SNPs passed the Hardy–Weinberg equilibrium χ^2 test with p value

>0.05 (Rodriguez et al. 2009) and had a minor allele frequency >5%.

Statistical analyses

The arsenic and creatinine variables (DMA, MMA, As^{III}, As^{V}, water As, and creatinine) were natural log-transformed so that these variables were more approximately normally distributed. Age and body mass index (BMI) were treated as continuous covariates and education and gender were dichotomous variable. The Wilcoxon rank sum test was used to compare the urinary arsenic concentrations for each metabolite by genotype whereas linear regression was used to determine the effect of each SNP on urinary arsenic concentrations while adjusting for water As levels, age, sex, BMI, education, and urinary creatinine concentrations. We used the dominant genetic model where we combined the heterozygous and homozygous variants into one category due to the small number of variants for some SNPs.

Path analysis is an intuitive statistical method when the link between exposure and outcome are mediated by other variables. Path analysis is used to establish how well a statistical model accounts for existing correlations among variables (outcome and covariates) in observed data and is particularly well suited for analysis of variables with complex relationships. Path analysis was used to estimate the relationship among urinary arsenic metabolites (As3, As5, MMA, and DMA) and SNPs in the *GSTO* and *AS3MT* genes controlling for current As exposure.

This path analysis included 842 participants due to missing data on any of the included variables. In this path analysis, the relationship between the urinary arsenic metabolites and SNPs was estimated using raw data, not covariance matrices, in Proc CALIS in SAS 9.1.3 (SAS Institute Inc, Cary, NC). The paths incorporated factors that significantly ($p < 0.10$) predicted the natural log of the DMA, MMA, As^{III}, and As^{V} variables. The process of selecting the model involved simultaneously fitting a series of linear regressions and selecting the model which best fit the observed covariances among the urinary arsenic metabolites and covariates. Ultimately, we chose the most biologically plausible model that conformed to standard path analysis goodness of fit indices. Fit is based on criteria described in "A Step by Step Approach to Using SAS for Factor Analysis and Structural Equation Modeling, 2007" that included a χ^2 test (χ^2 p value >0.05), root mean square error of approximation (RMSEA <0.05), root mean square residuals (RMR <0.05), Bentler's comparative fit index (CFI > 0.9), Bentler and Bonnet's Normed Fit Index (NFI > 0.9), and Bentler and Bonnet's non-normed fit index (NNFI > 0.9).

Results

Demographic information is presented in Table 1. The majority of participants were male and 52% had completed primary school or less. There was high variability in the water arsenic concentrations of the participants with an average arsenic concentration of 11.4 ug/L and a range from 0.5 to 1190 ug/L.

Table 1. Characteristics of study population.

Characteristic	Controls n = 896
Age, mean (SD)	33.3 (12)
Body mass index (BMI), mean (SD)	20.3 (3)
Water As level, ug/L, median (range)	11.4 (0.5–1190)
Missing	24
Years drinking from current well, median (range)	8.0 (0–60)
Missing	5
Urinary AsV, ug/L, median (range)	0.97 (ND–269.2)
Urinary AsIII, ug/L, median (range)	3.51 (ND–366.8)
Urinary MMA, ug/L, median (range)	6.03 (ND–320.5)
Urinary DMA, ug/L, median (range)	40.04 (ND–2135.6)
Gender, *n* (%)	
Male	553 (62)
Female	343 (38)
Education, *n* (%)	
Illiterate	135 (15)
Able to write name	219 (24)
Primary	117 (13)
Middle school	290 (32)
Secondary or more	134 (15)
Missing	1 (1)
GSTO1-1, rs4925, *n* (%)	
HZ+Variant	295 (33)
Wildtype	577 (64)
Missing	24 (3)
GSTO2-1, rs156697, *n* (%)	
HZ+Variant	437 (49)
Wildtype	404 (45)
Missing	55 (6)
GSTO2-2, rs2297235, *n* (%)	
HZ+Variant	279 (31)
Wildtype	583 (65)
Missing	34 (4)
AS3MT, rs11191439, *n* (%)	
HZ+Variant	97 (11)
Wildtype	792 (88)
Missing	7 (1)

SD, standard deviation; ND, not detectable; HZ, heterozygous; DMA, dimethylarsinic acid; MMA, monomethylarsonic acid.

The concentrations of creatinine-adjusted urinary arsenic species were compared for the homozygous wild-type genotype and the combined heterozygous and variant genotypes (Table 2). The homozygous wild type for one of the SNPs in the *GSTO2* gene (rs2297235) had statistically higher As^{III}, MMA, and DMA urinary concentrations, while the homozygous wild-type SNP in the *AS3MT* gene had lower concentrations of urinary As^{III} and MMA.

The effect estimates of related demographic, environmental, and biological predictors of urinary arsenic concentrations are shown in Table 3, and these variables

Table 2. Creatinine-adjusted urinary concentrations (μg/g) by genotype among controls.

		AsV		AsIII		MMA		DMA	
SNP	*n*	Median (IQR[a])	*p* value	Median (IQR)	*p* value	Median (IQR)	*p* value	Median (IQR)	*p* value
GSTO1-1, rs4925									
HZ+Variant	295	1.97 (0.5, 7.1)	0.75	5.61 (1.2, 15.7)	0.24	11.55 (4.4, 26.9)	**0.05**	72.33 (39.5, 156.4)	0.12
Wildtype	577	2.12 (0.6, 6.9)		6.90 (1.8, 18.5)		13.40 (5.7, 30.9)		82.61 (46.2, 154.9)	
GSTO2-1, rs156697									
HZ+Variant	437	2.16 (0.7, 7.2)	0.45	6.17 (1.7, 16.4)	0.20	12.61 (5.5, 28.4)	0.37	75.82 (41.3, 149.3)	0.14
Wildtype	404	2.00 (0.5, 6.9)		7.10 (2.0, 19.5)		13.70 (5.9, 29.4)		85.28 (47.2, 160.6)	
GSTO2-2, rs2297235									
HZ+Variant	279	1.98 (0.5, 7.1)	0.67	5.38 (1.0, 15.3)	**0.03**	10.88 (4.9, 25.6)	**0.03**	70.25 (39.5, 146.0)	**0.02**
Wildtype	583	2.11 (0.6, 7.2)		7.32 (1.9, 18.9)		13.78 (5.8, 31.3)		83.62 (47.1, 158.9)	
AS3MT, rs11191439									
HZ+Variant	97	2.29 (0.5, 8.3)	0.86	8.70 (4.3, 22.3)	**0.01**	18.08 (6.6, 34.6)	**0.01**	77.54 (41.9, 167.1)	0.82
Wildtype	792	2.04 (0.6, 6.8)		6.32 (1.2, 16.2)		12.27 (5.2, 27.4)		80.55 (44.0, 152.9)	

Wilcoxon rank sum test was used to compare the urinary concentrations by genotype.
[a]IQR: Interquartile range defined as the range from the 25th percentile to the 75th percentile.
DMA, dimethylarsinic acid; HZ, heterozygous; MMA, monomethylarsonic acid; SNP, single nucleotide polymorphism.
Bold values indicate statistically significant values ($p < 0.05$).

were included in the final models. Our findings were similar after adjusting for water arsenic levels, age, sex, BMI, education, urinary creatinine levels and years of drinking from current well (Table 4). The results of the linear regression models show that the heterozygous and homozygous variant genotypes in the *GSTO* genes were associated with lower urinary arsenic concentrations, specifically MMA, DMA, and total As, indicating an overall decrease in urinary arsenic excretion. Conversely, the heterozygous and homozygous variant *Met287Thr* had higher urinary arsenic concentrations, specifically As^{III} and MMA, compared to the wild type.

In addition to investigating the effect of the SNPs on each urinary arsenic metabolite separately, path analysis was used to investigate the relationship between the SNPs and the urinary metabolites in one model. The path analysis tested Model 1 shown in Figure 1 (does not include SNPs) and Model 2 in Figure 2 (includes SNPs). Similar to the regression models, each urinary arsenic metabolite was modeled with demographic variables (e.g. sex, age, BMI, and education), water arsenic concentrations, urinary creatinine levels, current well use, and the relevant SNPs as predictors. In addition, the urinary arsenic concentrations of the other metabolites were included as predictors (e.g. MMA concentration was a predictor of DMA concentration). The standardized path coefficients for the main effects of the urinary metabolites in Model 1 are shown in Figure 1 and are all statistically significant ($p < 0.05$). Water arsenic levels were significantly associated with each of the urinary metabolites with the standardized path coefficients ranging from 0.16 to 0.27, showing the greatest effect on MMA. In addition, Model 2 (Figure 2) shows the significant relationships between the SNPs and the urinary metabolites in the path analysis are consistent with those found in the linear regression models. In Figure 2, all standardized path coefficients for the included SNPs are significant at $p < 0.05$, except the interaction between *GSTO* and water As for DMA which has a p value = 0.08. Unlike the regression models, the path analysis allows us to show the significant associations among the urinary metabolites as well.

The goodness of fit indices are listed in Table 5 and indicate that Model 2 (with SNPs) fits the data quite well. Specifically, a non-significant model χ^2 p value for Model 2 ($p = 0.69$) indicates that the proposed model fits the data better than a model that does not include SNPs (χ^2 p value = 0.01 for Model 1). Additional goodness of fit indices include the NFI, NNFI, and the CFI, and all these indices were greater than 0.9 indicating that both models provided a good fit to the data. However, the Akaike information criterion (AIC) statistic is smaller for Model 2 (–13.7504 vs. –0.7746), which again supports a better fit with the SNPs included in the model.

Both pathway models explained 4, 22, 40 (39% for Model 1), and 67% of the variability in As^{V}, As^{III}, MMA, and DMA, respectively (Table 5). In addition to calculating the total variability explained, we were able to calculate both the direct and indirect effects of each variable on an outcome. We demonstrate this in Table 6 for Model 2 where we calculate the direct and indirect effects of the urinary arsenic metabolites on DMA. As expected, MMA had the greatest effect on DMA, and As^{V} had the smallest. While the direct effects were larger, the indirect effects were notable, especially for those further down the pathway from DMA. The indirect effects indicate that the urinary metabolites should be considered simultaneously as they may impact each other through intermediates. Water had the greatest direct effect on urinary MMA concentrations compared to the other metabolites which is consistent with the previous findings that MMA (V) reductase is the rate-limiting enzyme in arsenic biotransformation.

Table 3. Univariate effect estimates of related factors on urinary arsenic metabolite concentrations among controls only.

		LN(AsV)			LN(AsIII)			LN(MMA)			LN(DMA)		
Effect	*n*	β	SE	*p* value	β	SE	*p* value	β	SE	*p* value	β	SE	*p* value
Ln Water As level, μg/L	872	0.27	0.05	**<0.0001**	0.47	0.07	**<0.0001**	0.40	0.04	**<0.0001**	0.26	0.02	**<0.0001**
Age, years	896	0.0009	0.01	0.93	0.04	0.01	**0.0008**	0.01	0.007	**0.04**	0.01	0.003	**0.005**
Sex													
Female	553	−0.23	0.23	0.32	0.37	0.30	0.21	−0.22	0.17	0.19	0.16	0.08	0.06
Male	343	Ref			Ref			Ref			Ref		
BMI	896	−0.05	0.03	0.16	−0.03	0.04	0.51	−0.06	0.02	**0.02**	−0.005	0.01	0.71
Education				**0.04**			**<0.0001**			0.08			**0.002**
Illiterate	135	0.77	0.41	0.06	1.90	0.53	0.0003	0.68	0.29	0.02	0.51	0.15	0.0006
Able to write name	219	1.16	0.37	0.002	1.46	0.47	0.002	0.37	0.26	0.16	0.39	0.13	0.004
Primary	117	0.74	0.43	0.08	−0.09	0.55	0.87	−0.07	0.31	0.82	0.17	0.15	0.26
Middle School	290	0.63	0.35	0.08	0.51	0.45	0.26	0.26	0.25	0.31	0.16	0.13	0.21
Secondary or more	134	Ref			Ref			Ref			Ref		
Urinary creatinine levels, mg/dL	896	0.005	0.002	**0.02**	0.02	0.003	**<0.0001**	0.01	0.001	**<0.0001**	0.01	0.0007	**<0.0001**
Drinking from current well, years	891	0.02	0.01	0.19	0.04	0.02	**0.03**	−0.00007	0.01	0.99	0.002	0.005	0.72

BMI, body mass index; DMA, dimethylarsinic acid; LN, natural logarithm; MMA, monomethylarsonic acid; SE, standard error; β, percent change in the urinary metabolite.
Bold values indicate statistically significant values ($p < 0.05$).

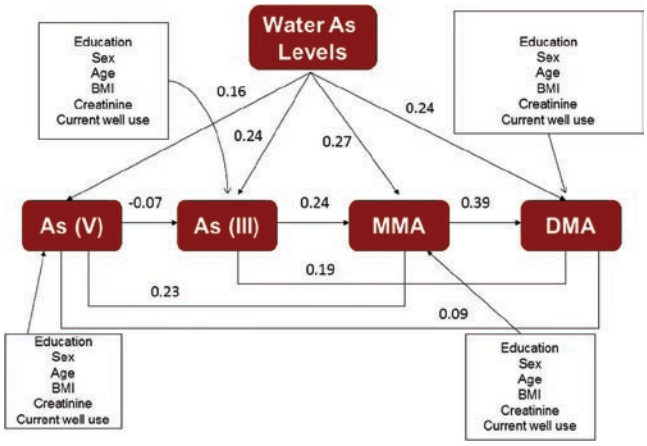

Figure1. Arsenicmetabolismpathwayillustratingtherelationships between water arsenic levels and urinary arsenic metabolites while adjusting for covariates (Model 1). The standardized path coefficients (*r*) are listed for each path and effects of the SNPs. Only the SNPs that were significant at the 0.05 level remained in the path analysis.

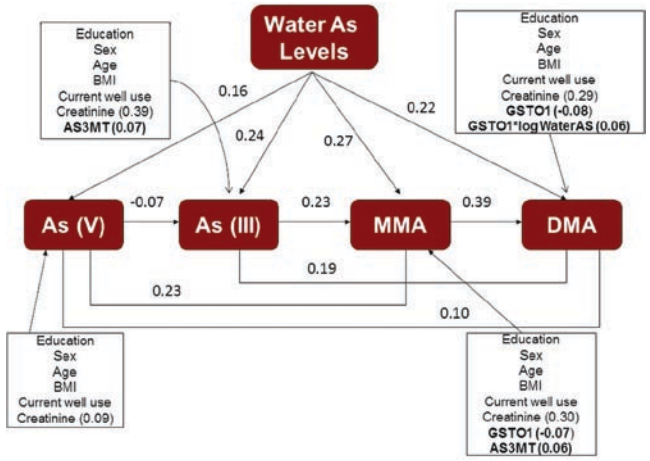

Figure2. Arsenicmetabolismpathwayillustratingtherelationships between water arsenic levels and urinary arsenic metabolites, including the main effects of genetic SNPs and covariates (Model 2). The standardized path coefficients (*r*) are listed for each path and effects of the SNPs. Only the SNPs that were significant at the 0.05 level remained in the path analysis.

Discussion

While the physiological role of *GSTO1* enzyme is not fully understood, the function of the rs4925 variant (*Ala140Asp*) has been characterized and thioltransferase activity has been shown to be significantly lower for this variant compared to the wild type. In contrast, no difference in the kinetic parameters of MMA^{V} reductase activity was observed between the variant and wildtype (Tanaka-Kagawa et al. 2003). Our findings show that this variant is associated with decreased excretion of arsenic measured as lower urinary arsenic concentrations, indicating that thioltransferase activity may play a role in arsenic metabolism or this variant may be highly correlated with another SNP that is involved in the metabolism of arsenic.

Similar to other Asian populations (Fujihara et al. 2008), the frequency of the *Met287Thr* variant in the *AS3MT* gene in this study population was very low (0.25%), but the combined heterozygous and variant group had significantly higher concentrations of urinary As^{III} and MMA. This finding is consistent with another study that found increased arsenic methylation (higher %MMA) among males living in Chile with the polymorphism (Hernandez et al. 2008a, Hernandez et al. 2008b).

Table 4. Effect of genetic single nucleotide polymorphisms on urinary arsenic metabolite concentrations among controls only*.

	LN(AsV)			LN(AsIII)			LN(MMA)			LN(DMA)			LN(Total As)		
Effect	β	SE	*p* value	β	SE	*p* value	β	SE	*p* value	β	SE	*p* value	β	SE	*p* value
GSTO1-1 (Ala140Asp)															
HZ+Variants	−0.06	0.24	0.80	−0.22	0.29	0.44	−0.44	0.16	**0.005**	−0.19	0.07	**0.005**	−0.18	0.06	**0.005**
Wildtype	ref			ref			ref			ref			ref		
GSTO2-1															
HZ+Variants	0.23	0.24	0.34	0.06	0.28	0.82	−0.10	0.14	0.50	−0.14	0.07	**0.03**	−0.12	0.06	0.06
Wildtype	ref			ref			ref			ref			ref		
GSTO2-2															
HZ+Variants	−0.10	0.25	0.70	−0.37	0.29	0.21	−0.40	0.15	**0.009**	−0.23	0.07	**0.0009**	−0.21	0.06	**0.001**
Wildtype	ref			ref			ref			ref			ref		
AS3MT															
HZ+Variants	−0.20	0.37	0.59	1.13	0.44	**0.01**	0.66	0.23	**0.005**	0.12	0.10	0.24	0.18	0.10	0.06
Wildtype	ref			ref			ref			ref			ref		

*Adjusted for water arsenic levels, age, sex, BMI, education, urinary creatinine levels, and years of drinking from current well.
DMA, dimethylarsinic acid; HZ, heterozygous; LN, natural logarithm; MMA, monomethylarsonic acid; SE, standard error; β, percent change in the urinary metabolites compared to the wildtype.
Bold values indicate statistically significant values ($p < 0.05$).

Table 5. Model Fit Indices (SAS Proc CALIS), n = 837.

Index (criterion for "good fit")	Model 1	Model 2
χ^2 test *p* value (>0.05)	0.0126	0.6908
RMSEA (<0.05)	0.0337	0.0000
RMR (<0.05)	0.0346	0.0265
CFI (>0.9)	0.9955	1.0000
NFI (>0.9)	0.9911	0.9977
NNFI (>0.9)	0.9707	1.0077
AIC (smaller is better)	−0.7746	−13.7504
SBC (smaller is better)	−76.4517	−65.7785
R^2 values for DMA, MMA, As(III), As(V)	0.67, 0.39, 0.22, 0.04	0.67 0.40, 0.22, 0.04

Model 1: no genetic single nucleotide polymorphisms in the model
Model 2: significant genetic single nucleotide polymorphisms included in model
Independence Model $\chi^2 = 3513.2$, df = 105, *p* value <0.001.
CFI, comparative fit index ; DMA, dimethylarsinic acid; MMA, monomethylarsonic acid; NFI, normed fit index; NNFI, non-normed fit index; RMR, root mean square residuals; RMSEA, root mean square error of approximation. AIC, Akaike information criterion; SBC, Schwarz Bayesian criterion.

Table 6. The direct and indirect effects of urinary arsenic metabolites on DMA.

Effect of	Indirect[a]	Direct	Total
MMA		0.39	0.39
As(III)	0.23 × 0.39 = 0.09	0.19	0.28
As(V)	(−0.07 × 0.23 × 0.39) + (0.23 × 0.39) + (0.07 × 0.19) = 0.07	0.10	0.17

[a]Indirect effects are calculated by multiplying the partial correlation coefficients for each path and summing them together.
DMA, dimethylarsinic acid; MMA, monomethylarsonic acid.

Unlike our study, Hernandez et al. did not find significant differences in urinary As^{III} levels.

In addition to investigating the main effect of the SNPs, we also investigated whether there was effect modification by gender. While the stratified results appeared different for males and females, the interaction terms were not statistically significant indicating that the gender difference was not significant. While seafood may contain organic arsenicals, our analytical approach focused on urinary metabolites that are not related to seafood consumption (Hsueh et al. 2002). Smoking status was also not included in the analysis because it was strongly correlated with gender as women in Bangladesh do not smoke. While we excluded individuals with arsenic-related skin lesions from this analysis we were unable to exclude other-arsenic related diseases because individual medical histories only collected information on broad categories of chronic diseases. Also, we were unable to evaluate heritability in this study even though it is plausible that some participants were related to each other given our recruitment in small rural villages because we did not collect information on participant's genealogy.

There are several strengths of this study. Namely, it is a large population-based study which was confined to controls to eliminate potential confounding by disease status. However, these controls were only defined as not having arsenic-induced skin lesions, and they may have had other diseases which could influence metabolic processes. The path analysis is a statistically efficient method for evaluating the relationship between highly collinear variables and allows us to calculate both direct and indirect effects of independent variables. Although the fit indices for the model indicated that the model fit the data quite well, there may be additional variables that were not included in the model. For example, there may be other SNPs in these investigated genes or other genes that are involved in the metabolism of arsenic or other environmental and health factors that may explain more of the variability in the urinary arsenic concentrations.

Unlike the linear regression models, the use of path analysis allows us to include several correlated urinary arsenic metabolites in the same model. While the effect

estimates resulting from the different analyses cannot be directly compared, the results are consistent showing that the same significant associations are observed between the genetic polymorphisms and the urinary metabolites for both statistical methods.

Conclusions

SNPs in the *GSTO* and *AS3MT* genes are associated with urinary arsenic metabolite concentrations among individuals not exhibiting skin lesions. These genes play a role in the metabolism and excretion of arsenic which may lead to increased risk of arsenic-related diseases. While this study shows significant findings, the results cannot be generalized to individuals with arsenic-related or other chronic health conditions.

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Declaration of interest

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