Arsenic Metabolism, Genetic Susceptibility, and Risk of Premalignant Skin Lesions in Bangladesh

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Abstract

We conducted a case-control study to investigate interindividual variability in susceptibility to health effects of inorganic arsenic due to arsenic metabolism efficiency, genetic factors, and their interaction. A total of 594 cases of arsenic-induced skin lesions and 1,041 controls was selected from baseline participants in a large prospective cohort study in Bangladesh. Adjusted odds ratios (OR) for skin lesions were estimated in relation to the polymorphisms in the *glutathione S-transferase ω1* and *methylenetetrahydrofolate reductase* genes, the percentage of monomethylarsonous acid (%MMA) and dimethylarsinic acid (%DMA) in urine, and the ratios of MMA to inorganic arsenic and DMA to MMA. Water arsenic concentration was positively associated with %MMA and inversely associated with %DMA. The dose-response relationship of risk of skin lesion with %MMA was more apparent than those with other methylation indices; the ORs for skin lesions in relation to increasing %MMA quartiles were 1.00 (reference), 1.33 [95% confidence interval (95% CI), 0.92-1.93], 1.68 (95% CI, 1.17-2.42), and 1.57 (95% CI, 1.10-2.26; *P* for trend = 0.01). The ORs for skin lesions in relation to the *methylenetetrahydrofolate reductase* 677TT/1298AA and 677CT/1298AA diplotypes (compared with 677CC/1298CC diplotype) were 1.66 (95% CI, 1.00-2.77) and 1.77 (95% CI, 0.61-5.14), respectively. The OR for skin lesions in relation to the *glutathione S-transferase ω1* diplotype containing all at-risk alleles was 3.91 (95% CI, 1.03-14.79). Analysis of joint effects of genotypes/diplotypes with water arsenic concentration and urinary %MMA suggests additivity of these factors. The findings suggest that arsenic metabolism, particularly the conversion of MMA to DMA, may be saturable and that differences in urinary arsenic metabolites, genetic factors related to arsenic metabolism, and their joint distributions modulate arsenic toxicity. (Cancer Epidemiol Biomarkers Prev 2007;16(6):1270–8)

Introduction

Worldwide, more than 100 million people, including ~70 million in Bangladesh and neighboring West Bengal, India, are chronically exposed to arsenic, a class I human carcinogen, through contaminated drinking water (1). Given the magnitude and immenseness of the problem, the WHO has designated the arsenic contamination of drinking water in Bangladesh as the largest ever mass poisoning event in human history (2). High levels of arsenic exposure from drinking water have been associated with elevated risks of a wide range of cancer and noncancer outcomes (3-5). The literature suggests that individual variability in susceptibility to the health effects of arsenic exposure may be due to the variation in host characteristics, including lifestyle factors, nutritional status, and arsenic metabolism (6-10). However, evidence of susceptibility due to the variation in genetic susceptibility has not been well established.

Arsenic in drinking water is present as inorganic arsenic (InAs), namely arsenite (As^{III}) and arsenate (As^{V}). As^{III} is the predominant form to which people are exposed in Bangladesh from groundwater. Methylation of $InAs^{III}$, which is primarily hepatic, first generates monomethylarsonic acid (MMA^{V}). After the reduction of MMA^{V} to monomethylarsonous acid (MMA^{III}), a second methylation can occur to generate dimethylarsinic acid (DMA^{V}; ref. 11). It is unclear to what extent DMA^{V} is reduced to dimethylarsinous acid (DMA^{III}) *in vivo*, as DMA^{III} is an unstable intermediate (12). The health implications of these methylation steps have yet to be fully understood, especially with regard to whether specific methylated arsenic species increase or decrease disease susceptibility. The composition of urinary arsenic metabolites varies from person to person and has been interpreted to reflect arsenic methylation efficiency (13, 14), with a typical profile of urinary arsenic metabolites consisting of 10% to 30% for InAs, 10% to 20% for MMA, and 60% to 80% for DMA (14, 15). The ratios of MMA/InAs and DMA/MMA in urine are indicative of efficiency for the first and second methylation steps, respectively. Limited evidence from human studies suggests that increased proportions of MMA and InAs species in urine may be associated with a higher risk of cancer (7-9).

The enzymatic regulation of arsenic metabolism has only recently (albeit partially) been understood. Glutathione S-transferase ω1 (GSTO1), also known as MMA reductase, is capable of catalyzing the reduction of MMA^{V} to MMA^{III} (16). One-carbon metabolism, the biochemical pathway responsible for the methylation of arsenic, is a folate-dependent pathway that uses *S*-adenosylmethionine as the methyl donor. Methylenetetrahydrofolate reductase (MTHFR) is an important enzyme in one-carbon metabolism; it catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which ultimately promotes the formation of *S*-adenosylmethionine (17). Functional polymorphism(s) involving the *MTHFR* and *GSTO1* gene is a common cause of varying enzyme activity. Whether variants in the *MTHFR* and *GSTO1* genes influence arsenic toxicity has also not been investigated in epidemiologic studies.

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Cutaneous abnormalities are well-known early signs of chronic InAs poisoning. Unlike arsenic-related internal cancers that could have long latencies, these premalignant skin lesions may appear with shorter periods of arsenic exposure (18). They give rise to the majority of arsenic-induced basal and squamous cell skin cancers (19-21). Using baseline data and biospecimen samples from participants in a large prospective cohort study, we investigated the determinants of arsenic methylation capacity and the associations of risk of premalignant skin lesions with arsenic methylation capacity, the polymorphisms in *MTHFR* and *GSTO1* gene, and their joint distributions in a case-control study in the Bangladeshi population.

Materials and Methods

Study Design and Population. The current case-control study was conducted within the Health Effects of Arsenic Longitudinal Study, a large prospective cohort study of 11,746 men and women established in year 2000 in Araihazar, Bangladesh. Details of the study and methods for Health Effects of Arsenic Longitudinal Study have been described elsewhere (22, 23). Briefly, between October 22, 2000 and May 19, 2002, a total of 11,746 participants (5,042 men and 6,704 women) was recruited into the Health Effects of Arsenic Longitudinal Study, with a 97.5% response rate. A total of 11,224 participants (95.6% of all participants) provided urine samples.

Trained interviewers and physicians visited potential study participants in their homes to do in-person interviews, including a full dietary instrument (24). In addition, participants were clinically assessed for skin lesions across the entire body (22). We instituted a structured protocol, similar to the quantitative assessment of the extent of body surface involvement in burn patients (25), to quantify the size, shape, and extent of skin lesion involvement. At baseline, 699 confirmed cases of premalignant skin lesions were identified among the 11,224 cohort members who provided urine samples (6, 22). The physicians were blind to the arsenic concentrations in the tube wells.

At the time of the selection of the cases and controls for the present study (December 2002), the recruitment of cohort members was completed and roster lists of 11,746 overall and 11,224 participants with urine samples were created. Clinical data with skin lesion status were cleaned and available for 9,285 cohort members with urinary samples available, of whom 594 were confirmed cases of skin lesion. A 10% random sample of the 11,224 cohort members who provided urine samples was selected as potential controls in this study. Among the 1,123 subjects, 12 did not complete physical examination and 70 were also cases of skin lesions. The final study population for this study thus included 594 cases and 1,041 controls; 569 cases and 959 controls provided blood samples and thus have genotyping information for at least one polymorphism of interest. Among the 594 cases, 352 (283 men and 69 women) had melanosis only, whereas the remaining 242 (206 men and 36 women) had both hyperkeratosis and melanosis.

The study protocol and field procedures were approved by the Columbia University Institutional Review Board and by the Ethical Committee of the Bangladesh Medical Research Council.

Arsenic Exposure Assessment

Water Arsenic and Time-Weighted Arsenic. Arsenic exposure was assessed based on arsenic concentrations in water and urine. Water samples from all wells in the study area were collected in 50 mL acid-washed tubes. Water arsenic concentrations were analyzed by graphite furnace atomic absorption (26). Because the graphite furnace atomic absorption method used for testing water samples has a detection limit of 5 μg/L, water samples found to have arsenic concentration at or below the detection limit were reanalyzed by inductively coupled plasma-mass spectrometry, which has a detection limit of 0.1 μg/L (27).

In addition to information on the primary well, we also collected usage information on any other wells and at least one previous well from which each participant might drink. The average duration of drinking from wells with known arsenic exposure levels was 10.0 and 8.3 years for men and women, respectively, accounting for an average of 25% of lifetime for both sexes. We derived a time-weighted arsenic measure (TWA) as a function of drinking durations and well water arsenic concentrations [TWA in μg/L = $\Sigma C_i T_i$ / ΣT_i, where C_i and T_i denote the well water arsenic concentration and drinking duration for the ith well (6)]. Eighty-six percent of study participants used one well as their exclusive source of drinking water. For participants who reported drinking water from a second well, the average concentration of the two wells was considered for the same drinking duration in TWA calculation.

Table 1. Descriptions for the studied SNPs and their primers and probes used for genotyping

Gene	*MTHFR*	*MTHFR*	*GSTO1*	*GSTO1*	*GSTO1*
Chromosomal location	1p36.3	1p36.3	10q25.1	10q25.1	10q25.1
Accession no.	AY 338232	AY 338232	NM_004832	NM_004832	NM_004832
SNP ID	rs1801133	rs1801131	rs4925	rs11509438	rs11509437
Seq 5′- to SNP	agcacttgaaggagaagg-tgtctgcgggag	Agatgtggggsgakgag-ctgaccagtgaag	Ttattagaagccaaaata-aagaagactatg	aaactgaaactgtggatggc-agccatgaag	aatttcgtaaagaatttac-caagctagagg
Seq 3′- to SNP	cgatttcatcatcacgcagc-ttttctttga	Aagtgtctttgaagtcttyg-ttctttacct	Tggcctaaaagaagaattt-cgtaaagaatt	aagatcccacagtctcagcc-ctgcttacta	taattatttctcctagctatc-atcagagta
Amino acid change	Alanine 222 Valine	Alanine 429 Glutamic Acid	Alanine 140 Aspartic Acid	Glutamic Acid 208 Lysine	
Forward primer	5′-tctctctgcccagtcc-ctgt-3′	5′-gagcgggagggcaga-agaag-3′	5′-attattctctgtctagg-tgccat-3′	5′-agtttctctctttgggca-agttc-3′	5′-attattctctgtctaggt-gccat-3′
Reverse primer	5′-aagtgatgcccatgtc-ggtg-3′	5′-ttggttctcccgagag-gtaa-3′	5′-aagcaagcccatgaca-aagtctg-3′	5′-cctcagggctgttctgta-agtag-3′	5′-aagcaagcccatgaca-aagtctg-3′
PCR product	249 bp	309 bp	340 bp	389 bp	340 bp
Probe type	Reverse	Forward	Reverse	Forward	Forward
Probe sequence	5′-gctgcgtgatgatgaa-atcg-3′	5′-gggggaggagctgacc-agtgaag-3′	5′-gtaaattctttacgaaatt-cttcttttaggcca-3′	5′-gaaactgtggatggcag-ccatgaag-3′	5′-cgtaaagaatttaccaa-gctagagg-3′
AcycloPrime dye used	G/A	C/A	G/T	G/A	A/T
Base extension step: no. cycles	20	20	30	20	25

Table 2. Characteristics of cases and controls

	Cases* (n = 594)	Controls* (n = 1,041)	P
Gender			
Male (%)	82.3	40.1	<0.01
Mean age, y (SD)	44.4 (9.8)	36.6 (9.8)	<0.01
Mean BMI (SD)	18.9 (2.6)	20.0 (3.4)	<0.01
Mean education level, y (SD)	2.7 (3.5)	3.5 (3.9)	<0.01
Cigarette smoking status			
Ever-smokers in men, %	82.6	76.3	0.02
Ever-smokers in women, %	15.2	6.7	<0.01
Drinking duration of baseline well, y	8.9 (5.9)	7.1 (5.1)	
Baseline well arsenic, μg/L	159.2 (157.1)	92.6 (106.4)	<0.01
Drinking duration with known arsenic levels, y	11.9 (8.2)	9.1 (6.9)	
Time-weighted well arsenic, μg/L	158.3 (155.5)	90.5 (101.9)	
Mean total urinary arsenic, μg/L (SD)	205.5 (242.2)	133.0 (147.6)	<0.01
Mean urinary creatinine, g/L (SD)	59.5 (45.8)	59.9 (46.3)	0.88
Mean urinary %InAs (SD)	15.4 (6.2)	15.8 (7.2)	0.28
Mean urinary %MMA (SD)	14.8 (5.0)	12.6 (4.9)	<0.01
Mean urinary %DMA (SD)	69.0 (8.6)	70.7 (8.6)	<0.01
Mean PMI MMA/(As^{III} + As^{V}) (SD)	1.10 (0.53)	0.95 (0.81)	<0.01
Mean SMI (DMA/MMA) (SD)	5.42 (2.74)	6.77 (3.66)	<0.01

*Data were missing on BMI for cases and controls, respectively, 5 and 7 subjects; on drinking duration and TWA for 38 and 15 subjects; on PMI for 1 and 3 subjects (due to that %InAs = 0); on SMI for 1 and 6 subjects (due to that %MMA = 0); and on betel nut use for 3 and 1 subjects.

Total Urinary Arsenic and Urinary Arsenic Metabolites. Urine samples were stored in coolers until their transfer to −20°C freezers and were batch shipped on dry ice to Columbia University for analysis. Total urinary arsenic concentration was measured by graphite furnace atomic absorption using the Analyst 600 graphite furnace system (with a detection limit of 2 μg/L) as described previously (28). Urinary creatinine was analyzed using a method based on the Jaffe reaction for adjustment of urinary total arsenic concentration (29).

Urinary arsenic metabolites were distinguished using a method described by Reuter et al. (30). This method uses high-performance liquid chromatography separation of arsenobetaine, arsenocholine, As^{V}, As^{III}, MMA, and DMA followed by detection by inductively coupled plasma-mass spectrometry with dynamic reaction cell. Although, as mentioned above, MMA and DMA do exist in two different valence states, we did not attempt to distinguish valence in this study.

Because As^{III} can oxidize to As^{V} during sample transport, storage, and preparation, we express total InAs (i.e., As^{III} + As^{V}). The percentage of InAs, MMA, and DMA in total arsenic was calculated after subtracting arsenobetaine and arsenocholine (i.e., nontoxic organic arsenic from dietary sources) from the total. In addition, we constructed two methylation indices: primary methylation index (PMI), namely the ratio of MMA to InAs, and the secondary methylation index (SMI), namely the ratio of DMA to MMA.

Genotyping for the *MTHFR* and *GSTO1* Gene Variants and Construction of Haplotype Pairs (Diplotypes). Single nucleotide polymorphisms (SNP) of interest in the present analysis included two SNPs in the *MTHFR* gene (rs1801133 and rs1801131) and three SNPs in the *GSTO1* gene (rs4925, rs11509438, and rs11509437). The details of the SNPs are shown in Table 1. High-throughput DNA extraction was completed in 96-well format using the QIAmp DNA 96 DNA Blood kit (Qiagen). Replica plates were made with 12.5 ng DNA in 2.5 μL per well. In the first step, the genomic DNA was amplified by PCR using appropriate primers. After PCR amplification, the primers and deoxynucleotide triphosphates in 10 μL PCR product were digested with the 10 μL shrimp alkaline phosphatase cocktail containing 1.0 μL (1 unit/μL) of shrimp alkaline phosphatase, 0.1 μL of *Escherichia coli* exonuclease I (10 units/μL; U.S. Biochemical), 1.0 μL of 10× shrimp alkaline phosphatase buffer, and 7.9 μL of DNase and RNase-free water for 45 min at 37°C followed by heating at 95°C for 15 min for enzyme deactivation. Then, in the third step, single nucleotide extension was carried out in the presence of the appropriate allele-specific dideoxynucleotide triphosphates fluorescence labeled with either R110 or TAMRA (Perkin-Elmer Life Science). For single nucleotide extension reactions, both the forward and reverse probes were initially tested to select the better probe based on clear signal clustering. Reaction mixture (13 μL/well) containing 0.025 μL AcycloPrime enzyme, 0.5 μL terminator dye, 1 μL reaction buffer, 0.25 μL extension probe (10 pmol/μL), and 11.225 μL water was added to 7 μL of digested PCR product to make 20 μL reaction volume. Thermocycling was done using heating at 95°C for 3 min followed by optimum number of cycles of 95°C for 15 s and 55°C for 30 s. Finally, the fluorescence was measured with Wallac 1420 Multilabel Counter Victor 3 (Perkin-Elmer Life and Analytical Sciences). In addition to our assay-specific quality control samples, 10% of the samples were run in duplicate after relabeling to keep laboratory researchers blinded to its identity. Concordance based on the κ statistics was >0.92. Call rates for the SNPs of interest ranged from 93.1% to 97.3%. Distributions of age, sex, skin lesions status, and arsenic exposure levels did not differ between those with and those without interpretable genotype calls; P values for comparisons between the two groups were all >0.12 based on χ^2 test and t test.

Haplotype pairs (diplotypes) were constructed using the PHASE 2.1 software,[6] which is based on Bayesian statistical methods. PHASE is reported to have lower error rates than either the maximum likelihood (expectation maximization algorithm) or the parsimony method (Clark algorithm; ref. 31). Only participants with known genotypes on all studied *MTHFR* and *GSTO1* polymorphisms were included in the haplotype construction for each gene. Pairwise linkage disequilibrium between any two alleles out of the polymorphic sites in the same gene was evaluated by normalized disequilibrium (D′) estimated using the program expectation maximization estimation of haplotype frequencies and linkage disequilibrium calculation.[7]

Statistical Analysis. We first did descriptive analysis to compare cases and controls with regard to distribution of sociodemographic characteristics, arsenic exposure, methylation indices, and genotypes/diplotypes (i.e., haplotype pairs) of the *MTHFR* and *GSTO1* genes. Hardy-Weinberg equilibrium

[6] http://www.stat.washington.edu/stephens/software.html
[7] http://epi.mdanderson.org/~qhuang/Software/pub.htm

was tested by comparing the observed and expected genotype frequencies among cases and controls separately.

Determinants of arsenic metabolite profiles were evaluated in the controls using multiple linear regression. To assess the associations of risk of skin lesions with arsenic metabolite indices, *MTHFR*, and *GSTO1* genotypes/diplotypes, unconditional logistic regression was done with adjustments for age, gender, body mass index (BMI), and smoking status. Additional analysis was conducted with more adjustments for well water arsenic concentration and water consumption. Tests for trend were conducted using each urinary arsenic metabolite index as a continuous variable in the model. We conducted stratified analysis by sex, and the results did not suggest confounding effect by sex (data not shown). Odds ratios (OR) for the joint effect of genetic factors (genotypes and diplotypes) and arsenic exposure as well as %MMA were estimated using subjects exposed to the lowest arsenic levels and carrying the low-risk genotypes/diplotypes as the reference group. Tests for interaction were done using the cross-product term representing product of genetic factor (number of at-risk allele) with %MMA or arsenic exposure, both expressed as continuous variables.

Table 3. Relationship of sociodemographic characteristics, arsenic exposure, and lifestyle variables with urinary arsenic metabolites in controls

	*n**	PMI = MMA/InAs		SMI = DMA/MMA		%DMA		%MMA	
		Adjusted means (SD)	*P* for trend	Adjusted means (SD)	*P* for trend	Adjusted means (SD)	*P* for trend	Adjusted means (SD)	*P* for trend
Sociodemographic and lifestyle variables †									
BMI ‡									
<18.2	341	0.91 (0.05)	0.20	6.32 (0.23)	0.40	69.60 (0.54)	0.22	13.17 (0.31)	0.20
18.2-20.8	340	0.94 (0.06)		6.32 (0.24)		69.58 (0.56)		13.34 (0.33)	
>20.8	350	0.99 (0.06)		6.57 (0.24)		70.40 (0.56)		12.71 (0.33)	
Age (y) ‡									
17-30	344	0.81 (0.06)	<0.01	6.43 (0.27)	0.80	69.12 (0.63)	0.02	12.72 (0.36)	0.11
30-40	367	0.96 (0.05)		6.28 (0.24)		69.72 (0.55)		13.13 (0.32)	
41-65	320	1.09 (0.05)		6.49 (0.23)		70.75 (0.53)		13.38 (0.31)	
Sex									
Women	614	0.89 (0.06)	0.05	7.63 (0.27)	<0.01	72.15 (0.63)	<0.01	11.33 (0.36)	<0.01
Men	417	1.05 (0.05)		5.16 (0.19)		67.91 (0.45)		14.95 (0.26)	
Cigarette smoking status									
Never	671	1.00 (0.04)	0.61	6.43 (0.19)	0.90	70.17 (0.45)	0.68	13.18 (0.26)	0.46
Past	52	0.97 (0.11)		6.12 (0.49)		68.60 (1.14)		13.77 (0.66)	
Current	308	0.96 (0.06)		6.53 (0.24)		70.64 (0.56)		12.81 (0.32)	
Betel nut use									
Never	628	0.99 (0.05)	0.40	6.41 (0.22)	0.39	69.79 (0.53)	0.70	13.37 (0.30)	0.59
Ever	403	0.96 (0.05)		6.32 (0.21)		69.82 (0.49)		13.14 (0.28)	
Well arsenic (μg/L) ‡									
0.1-23	344	1.04 (0.06)	0.06	7.06 (0.25)	<0.01	72.01 (0.59)	<0.01	12.43 (0.34)	0.01
24-101	337	0.90 (0.06)		6.13 (0.24)		69.43 (0.56)		13.25 (0.32)	
102-864	350	0.91 (0.06)		6.01 (0.25)		68.13 (0.59)		13.54 (0.35)	
Urine arsenic (μg/L) ‡									
5-53	341	0.99 (0.06)	0.74	6.15 (0.27)	0.34	69.47 (0.64)	0.76	13.16 (0.37)	0.76
54-128	340	0.90 (0.05)		6.55 (0.24)		70.47 (0.55)		12.80 (0.32)	
>129	350	0.95 (0.06)		6.51 (0.27)		69.64 (0.63)		13.26 (0.36)	
Urinary creatinine (g/L) ‡									
4-32	339	0.80 (0.06)	<0.01	5.64 (0.25)	<0.01	66.41 (0.59)	<0.01	13.73 (0.34)	<0.01
33-68	343	1.03 (0.06)		6.37 (0.24)		70.57 (0.55)		13.40 (0.32)	
69-359	349	1.02 (0.06)		7.20 (0.25)		72.60 (0.59)		12.10 (0.35)	
Genetic variables of interest †									
MTHFR 677 (rs1801133)									
CC	733	0.96 (0.03)	0.46	6.80 (0.13)	0.15	70.76 (0.30)	0.39	12.55 (0.17)	0.30
CT	183	0.91 (0.06)		6.53 (0.26)		70.69 (0.61)		12.76 (0.34)	
TT	11	0.94 (0.24)		5.39 (1.04)		66.45 (2.48)		14.34 (1.39)	
MTHFR 1298 (rs1801131)									
CC	135	0.92 (0.07)	0.41	6.83 (0.30)	0.40	70.50 (0.72)	0.85	13.10 (0.40)	0.57
CA	432	0.93 (0.04)		6.79 (0.17)		70.77 (0.40)		12.45 (0.22)	
AA	332	0.98 (0.04)		6.58 (0.19)		70.48 (0.46)		12.65 (0.25)	
GSTO1 (rs4925)									
CC	628	0.92 (0.03)	0.24	6.60 (0.14)	0.35	12.65 (0.18)	0.86	70.61 (0.33)	0.88
CA	267	1.01 (0.05)		7.07 (0.21)		12.46 (0.28)		70.83 (0.50)	
AA	30	0.92 (0.14)		6.03 (0.64)		13.07 (0.84)		70.23 (1.50)	
GSTO1 (rs11509437)									
AA	706	0.94 (0.02)	0.17	6.64 (0.13)	0.36	12.80 (0.17)	0.07	70.45 (0.31)	0.35
AT	154	0.89 (0.04)		6.95 (0.27)		12.03 (0.36)		71.43 (0.64)	
TT	15	0.83 (0.12)		6.77 (0.87)		12.31 (1.15)		69.65 (2.05)	
GSTO1 (rs11509438)									
GG	715	0.91 (0.13)	0.20	6.41 (0.90)	0.37	13.12 (1.20)	0.08	69.41 (2.12)	0.32
GA	164	0.91 (0.04)		6.54 (0.31)		12.56 (0.41)		70.67 (0.72)	
AA	15	0.96 (0.03)		6.25 (0.19)		13.32 (2.54)		69.80 (0.44)	

*Analysis excluded 10 participants with unknown status of betel nut use (n = 3) and BMI (n = 7). Among the 1,031 participants with values for all the sociodemographic and lifestyle variables, 926, 899, 925, 875, and 894 have information on carrier status of *MTHFR* 677 (rs1801133), *MTHFR* 1298 (rs1801131), *GSTO1* (rs4925), *GSTO1* (rs11509437), and *GSTO1* (rs11509438), respectively.

†Means according to sociodemographic and lifestyle variables were adjusted for one another. Means according to genetic variables were adjusted for sociodemographic and lifestyle variables.

‡Cut points were determined based on tertiles in the controls.

Table 4. Associations of urinary arsenic metabolites, *MTHFR*, and *GSTO1* genotypes/diplotypes with risk of skin lesions

	*n**		ORs for skin lesions (95% CI) †	ORs for skin lesions (95% CI) ‡	*P* for trend ‡
	Cases	Controls			
%InAs					
0-11.1 (8.4)	153	255	1.0	1.0	0.99
11.2-14.7 (12.9)	149	260	1.02 (0.74-1.42)	1.00 (0.71-1.40)	
14.8-18.7 (16.7)	140	269	0.91 (0.66-1.26)	0.79 (0.56-1.11)	
18.9-69.3 (24.6)	152	257	1.27 (0.92-1.76)	1.02 (0.72-1.45)	
PMI, MMA/InAs					
0-0.63 (0.46)	92	315	1.0	1.0	0.11
0.64-0.90 (0.77)	153	256	1.52 (1.08-2.14)	1.43 (1.00-2.04)	
0.91-1.22 (1.05)	159	248	1.32 (0.94-1.86)	1.35 (0.95-1.93)	
0.23-19.57 (1.75)	189	219	1.44 (1.02-2.03)	1.38 (0.96-1.98)	
%MMA					
0-9.7 (7.5)	89	320	1.0	1.0	0.01
9.8-12.8 (11.4)	132	276	1.30 (0.92-1.85)	1.33 (0.92-1.93)	
12.9-16.4 (14.5)	170	240	1.74 (1.23-2.46)	1.68 (1.17-2.42)	
16.5-33.7 (20.2)	203	205	1.91 (0.35-2.70)	1.57 (1.10-2.26)	
%DMA					
27.9-65.0 (58.6)	181	227	1.0	1.0	0.09
65.1-70.8 (68.1)	163	247	0.80 (0.59-1.09)	0.93 (0.67-1.29)	
70.9-76.0 (73.3)	129	279	0.63 (0.46-0.87)	0.80 (0.57-1.12)	
76.1-100.0 (80.3)	121	288	0.64 (0.46-0.89)	0.83 (0.58-1.18)	
SMI (DMA/MMA)					
1.5-3.9 (3.1)	205	202	1.0	1.0	0.01
4.0-5.5 (4.7)	160	247	0.77 (0.56-1.04)	0.88 (0.64-1.21)	
5.6-7.6 (6.4)	134	273	0.63 (0.46-0.86)	0.78 (0.56-1.09)	
7.7-32.3 (10.9)	94	313	0.52 (0.37-0.73)	0.64 (0.44-0.91)	
MTHFR 677 (rs1801133)					
CC	416	739	1.00	1.00	0.23
CT	124	185	1.14 (0.86-1.53)	1.14 (0.84-1.54)	
TT	12	11	1.55 (0.60-3.99)	1.56 (0.57-4.30)	
MTHFR 1298 (rs1801131)					
CC	63	135	1.00	1.00	0.09
CA	250	436	1.07 (0.73-1.56)	1.11 (0.75-1.66)	
AA	234	336	1.36 (0.93-2.01)	1.37 (0.91-2.06)	
MTHFR diplotypes					
Other/other	224	325	1.00	1.00	0.06
CC/other	246	432	0.77 (0.59-1.00)	0.80 (0.61-1.05)	
CC/CC	61	131	0.73 (0.49-1.08)	0.72 (0.48-1.09)	
Other/other	122	223	1.00	1.00	0.35
CA/other	268	448	1.12 (0.83-1.51)	1.19 (0.87-1.63)	
CA/CA	141	217	1.21 (0.86-1.71)	1.19 (0.83-1.72)	
Other/other	399	699	1.00	1.00	0.22
TA/other	120	178	1.16 (0.87-1.56)	1.17 (0.86-1.59)	
TA/TA	12	11	1.54 (0.60-3.98)	1.55 (0.57-4.27)	
MTHFR diplotypes					
CC CC	61	131	1.00	1.00	0.12
CC CA	197	351	1.06 (0.72-1.58)	1.13 (0.75-1.71)	
CA CA	141	217	1.26 (0.83-1.92)	1.25 (0.80-1.94)	
CC TA	49	81	1.01 (0.60-1.70)	1.01 (0.58-1.74)	
CA TA	71	97	1.58 (0.97-2.57)	1.66 (1.00-2.77)	
TA TA	12	11	1.72 (0.63-4.67)	1.77 (0.61-5.14)	
GSTO1 (rs4925)					
CC	377	633	1.00	1.00	0.39
CA	149	270	1.00 (0.77-1.31)	0.98 (0.74-1.29)	
AA	24	30	1.65 (0.88-3.07)	1.73 (0.91-3.30)	
GSTO1 (rs11509437)					
AA	453	713	1.00	1.00	0.11
AT	82	155	0.83 (0.60-1.14)	0.85 (0.60-1.19)	
TT	4	16	0.30 (0.09-0.95)	0.45 (0.14-1.44)	
GSTO1 (rs11509438)					
GG	450	722	1.00	1.00	0.22
GA	84	165	0.87 (0.63-1.20)	0.88 (0.63-1.24)	
AA	5	16	0.36 (0.12-1.05)	0.52 (0.18-1.52)	
GSTO1 diplotypes					
Other/other	424	674	1.00	1.00	0.27
CTA/other	77	140	0.92 (0.65-1.28)	0.92 (0.65-1.32)	
CTA/CTA	4	16	0.30 (0.10-0.97)	0.45 (0.14-1.44)	
Other/other	345	568	1.00	1.00	0.24
AAG/other	138	234	1.03 (0.78-1.36)	1.02 (0.76-1.37)	
AAG/AAG	22	28	1.67 (0.88-3.19)	1.84 (0.94-3.60)	
Other/other	41	66	1.00	1.00	0.80
CAG/other	185	330	0.89 (0.55-1.44)	0.84 (0.51-1.38)	
CAG/CAG	279	434	0.95 (0.60-1.52)	0.87 (0.54-1.41)	
GSTO1 diplotypes					
CTA CTA	4	16	1.00	1.00	0.15
CTA AAG	15	22	3.69 (0.95-14.39)	2.24 (0.55-9.10)	

(*Continued on the following page*)

Table 4. Associations of urinary arsenic metabolites, *MTHFR*, and *GSTO1* genotypes/diplotypes with risk of skin lesions (Cont'd)

	*n**		ORs for skin lesions (95% CI)†	ORs for skin lesions (95% CI)‡	*P* for trend‡
	Cases	Controls			
CAG CTA	62	118	2.89 (0.87-9.56)	2.03 (0.61-6.83)	
CAG CAG	279	434	3.26 (1.02-10.40)	2.19 (0.68-7.06)	
CAG AAG	123	212	3.14 (0.97-10.17)	2.15 (0.66-7.07)	
AAG AAG	22	28	5.21 (1.41-19.28)	3.91 (1.03-14.79)	

*Data were missing on PMI for cases and controls, respectively, 1 and 3 subjects; on SMI for 1 and 6 subjects; on *MTHFR* 677 (rs1801133) for 42 and 106 subjects; on *MTHFR* 1298 (rs1801131) for 47 and 134 subjects; on *MTHFR* 677 (rs1801133) for 42 and 106 subjects; on *MTHFR* diplotypes for 62 and 153 subjects; on *GSTO1* (rs4925) for 44 and 108 subjects; on *GSTO1* (rs11509438) for 55 and 138 subjects; on *GSTO1* (rs11509437) for 55 and 157 subjects, and on *GSTO1* diplotypes for 88 and 210 subjects.

†ORs were adjusted for sex and age.

‡ORs were adjusted for sex, age, BMI, smoking status, well arsenic concentration, and water consumption per day. Subjects with missing on BMI were additionally excluded from the analysis.

Results

The distributions of age, sex, and arsenic-related variables of the controls included in the present study did not differ from those of the rest of participants without skin lesions (n = 9,683) in the cohort (P > 0.20). A total of 83% of the cases in the cohort (n = 714) was included in the study, and their distributions of age, sex, and urinary arsenic level did not differ from those of the cases not included in the study (n = 121; P > 0.20). Average well arsenic concentration was somehow lower in cases included in the study compared with that in the rest of cases (194 versus 159 μg/L; P = 0.05). Consistent with our previous cross-sectional analysis of the overall cohort (6, 10), cases were more likely to be men, of older age, slightly leaner, less educated, and more likely to be smokers compared with controls (Table 2). Well water and urinary arsenic concentrations were significantly higher for the cases compared with controls. Urinary %MMA was significantly higher among cases, whereas urinary %DMA was higher among controls.

Compared with women, men had a lower average of %DMA and SMI and a higher average of %MMA and PMI in urine (Table 3). Well water arsenic level was inversely proportional to %DMA and SMI and positively associated with %MMA and PMI. Urinary creatinine was positively associated with %DMA and inversely associated with %MMA. Cigarette smoking and use of betel nut were not significantly associated with any of the methylation indices among our study population. After adjustment for total arsenic exposure in water and urine, mean arsenic methylation indices (urinary %MMA, %DMA, PMI, and SMI) did not differ significantly by genotype status for the *MTHFR* and *GSTO1* genes.

The %MMA in urine and PMI were positively associated with the risk of skin lesions, whereas SMI was inversely and %InAs was not associated with risk of skin lesions (Table 4). Increasing %MMA was significantly associated with an increasing ORs for skin lesions (P for trend < 0.01), whereas the trend for increasing %DMA in relation to a decreasing risk of skin lesions was apparent, although not statistically significant (P for trend = 0.09).

All SNPs were in Hardy-Weinberg equilibrium (P > 0.05) in cases and controls. Pairwise linkage disequilibrium analysis suggests strong linkage disequilibrium among the SNPs of the same gene (all D′ > 0.98). The variant T allele of the *MTHFR* codon 677 and the wild-type A allele of codon 1298 were related to an elevated risk of skin lesions after adjustment for well arsenic concentration and other potential covariates (Table 4). The ORs associated with the TA/TA diplotype was the highest [1.55; 95% confidence interval (95% CI), 0.57-4.27] compared with diplotypes not containing the TA haplotype. The *GSTO1* rs4925 AA genotype was related to an elevated risk of skin lesions, whereas the variant alleles of rs11509438 and rs11509437 were related to a reduced risk of skin lesions. Individuals with the AAG/AAG diplotype (i.e., the diplotype containing the high-risk alleles for all three SNPs) had elevated risk (OR, 1.84; 95% CI, 0.94-3.60) for arsenic-related skin lesions.

Table 5 presents the joint effects of the *MTHFR* genotypes/diplotypes with higher arsenic exposure and higher %MMA on the risk of skin lesions. The risk of skin lesions in relation to high levels of arsenic exposure and %MMA was most pronounced among individuals with the at-risk variant alleles and diplotypes, especially among homozygous carriers. The OR for skin lesions in relation to both higher arsenic exposure and TT genotype of *MTHFR* codon 677 was 5.73 (95% CI, 1.10-29.77), and the OR in relation to both higher %MMA and TT genotype was 3.99 (95% CI, 1.05-15.13). The joint effects of higher arsenic exposure and %MMA with *MTHFR* codon 1298 were less pronounced. The OR in relation to both the *MTHFR* TA/TA diplotype and higher arsenic exposure was 6.31 (95% CI, 1.24-32.26), much greater than the ORs in relation to the TA/TA diplotype alone (OR, 1.07; 95% CI, 0.28-4.15) or arsenic exposure alone (OR, 2.11; 95% CI, 1.57-2.82). Similarly, the OR in relation to the TA/TA diplotype and higher %MMA was 3.91 (95% CI, 1.06-14.41), much greater than the ORs in relation to the TA/TA diplotype alone (OR, 0.41; 95% CI, 0.07-2.37) or higher %MMA alone (OR, 1.36; 95% CI, 1.02-1.81). The test for interaction does not suggest that the association between *MTHFR* genotypes and risk for skin lesions differed by levels of %MMA or arsenic exposure levels.

The results on joint effects of the *GSTO1* genotypes/diplotypes with higher arsenic exposure and higher %MMA on the risk of skin lesions are presented in Table 6. For *GSTO1* rs11509437, genotypes TT and AT were combined as the reference group because of the rarity of TT. Similarly, for *GSTO1* rs11509438, genotypes AA and GA were combined. The ORs for skin lesions in relation to the joint effects of both higher arsenic exposure and different *GSTO1* genotypes, and also of both higher %MMA and different *GSTO1* genotypes, although elevated (ORs ranged from 1.63 to 3.39), were mostly not statistically significant at P < 0.05. The OR in relation to the joint effects of both higher arsenic exposure and the *GSTO1* AAG/AAG diplotype was 3.38 (95% CI, 1.30-8.79), greater than the ORs in relation to the AAG/AAG diplotype alone (OR, 1.94; 95% CI, 0.78-4.85) or arsenic exposure alone (OR, 2.24; 95% CI, 1.66-3.03). On the other hand, the OR in relation to the AAG/AAG diplotype and a lower %MMA was 2.64 (95% CI, 1.08-6.45), greater than the ORs in relation to the AAG/AAG diplotype and a higher %MMA (OR, 1.61; 95% CI, 0.64-4.05) or a higher %MMA alone (OR, 1.50; 95% CI, 1.11-2.03). The test for interaction suggests that the association between *GSTO1* genotypes and risk of skin lesions differed by levels of %MMA.

Table 5. Joint effect of the *MTHFR* genotypes/diplotypes and arsenic exposure on risk of skin lesions

MTHFR genotype/diplotype	Joint effect of *MTHFR* genotype/diplotype and time-weighted well arsenic concentration					Joint effect of *MTHFR* genotype/diplotype and urinary %MMA				
	TWA levels	Means of TWA	*n* (cases/controls)	Adjusted ORs for skin lesions*	*P* for interaction	%MMA	Means of %MMA	*n* (cases/controls)	Adjusted ORs for skin lesions†	*P* for interaction
MTHFR 677 (rs1801133)										
CC	<73	25.34	155/396	1.00	0.35	<13	9.38	150/415	1.00	0.61
CT	<73	29.52	50/108	1.13 (0.74-1.74)		<13	9.73	54/107	1.32 (0.85-2.05)	
TT	<73	22.06	4/8	1.08 (0.28-4.19)		<13	10.13	2/6	0.44 (0.07-2.89)	
CC	≥73	204.92	252/318	2.15 (1.64-2.82)		≥13	17.24	266/324	1.36 (1.01-1.82)	
CT	≥73	204.59	71/68	2.59 (1.69-3.96)		≥13	17.60	70/78	1.38 (0.89-2.13)	
TT	≥73	195.45	8/3	5.73 (1.10-29.77)		≥13	17.76	10/5	3.99 (1.05-15.13)	
MTHFR 1298 (rs1801131)										
CC	<73	28.54	20/70	1.00	0.38	<13	9.22	24/74	1.00	0.28
AC	<73	25.53	97/235	0.99 (0.64-1.49)		<13	9.50	98/247	0.97 (0.53-1.77)	
AA	<73	28.25	88/188	1.35 (0.87-2.08)		<13	9.53	87/191	1.18 (0.64-2.18)	
CC	≥73	196.79	42/59	2.14 (1.22-3.74)		≥13	18.18	39/61	1.00 (0.49-2.05)	
AC	≥73	196.90	148/187	2.23 (1.49-3.35)		≥13	17.08	152/189	1.28 (0.71-2.33)	
AA	≥73	215.20	140/136	2.80 (1.84-4.26)		≥13	17.31	147/145	1.60 (0.87-2.94)	
MTHFR diplotypes										
Other/other	<73	25.79	149/371	1.00	0.39	<13	9.37	147/392	1.00	0.64
TA/other	<73	29.97	49/102	1.21 (0.78-1.89)		<13	9.74	53/104	1.29 (0.83-1.99)	
TA/TA	<73	22.06	4/8	1.07 (0.28-4.15)		<13	10.12	2/6	0.41 (0.07-2.37)	
Other/other	≥73	203.63	241/304	2.11 (1.57-2.82)		≥13	17.29	252/307	1.36 (1.02-1.81)	
TA/other	≥73	202.74	68/68	2.47 (1.59-3.83)		≥13	17.50	67/74	1.49 (0.97-2.29)	
TA/TA	≥73	195.45	8/3	6.31 (1.24-32.26)		≥13	17.77	10/5	3.91 (1.06-14.41)	

*ORs were adjusted for age and gender, smoking status, BMI, and water consumption per day. Cut points for TWA levels and %MMA were determined based on median values in the controls.
†ORs were adjusted for age and gender, smoking status, BMI, water consumption per day, and well arsenic concentration.

Discussion

In this large population-based case-control study in Bangladesh, we investigated the determinants of arsenic metabolism, the role of arsenic metabolism in arsenic-induced skin lesions, as well as the influence of genetic susceptibility to skin lesion risk. We found that differences in urinary arsenic metabolites, genetic factors related to arsenic metabolism, and their joint distributions explained some of the variations in the risk of arsenic-related skin lesions.

Several studies have indicated that the proportion of MMA in urine is positively associated whereas the proportion of DMA is inversely associated with the risk of skin and bladder cancer (7-9). Several smaller studies have indicated that arsenic metabolic process is influenced by demographic (sex and age) characteristics (13, 32, 33). The present study of

Table 6. Joint effect of the *GSTO1* genotypes/diplotypes and arsenic exposure on risk of skin lesions

GSTO1 genotype/diplotype	Joint effect of *GSTO1* genotype/diplotype and time-weighted well arsenic concentration					Joint effect of *GSTO1* genotype/diplotype and urinary %MMA				
	TWA levels	Means of TWA	*n* (cases/controls)	Adjusted ORs for skin lesions*	*P* for interaction	%MMA	Means of %MMA	*n* (cases/controls)	Adjusted ORs for skin lesions†	*P* for interaction
GSTO1 (rs4925)										
CC	<73	26.19	145/344	1.00	0.99	<13	9.53	133/354	1.00	0.07
CA	<73	26.94	51/147	0.93 (0.61-1.42)		<13	9.38	64/158	1.11 (0.73-1.67)	
AA	<73	31.75	10/18	1.98 (0.79-4.93)		<13	9.62	12/17	2.89 (1.18-7.08)	
CC	≥73	207.47	222/268	2.08 (1.54-2.82)		≥13	17.34	244/279	1.31 (0.87-1.96)	
CA	≥73	205.22	96/114	2.32 (1.58-3.41)		≥13	17.34	85/112	1.43 (1.05-1.94)	
AA	≥73	159.25	14/9	3.39 (1.29-8.88)		≥13	16.35	12/13	1.63 (0.66-4.00)	
GSTO1 (rs11509437)										
TT/AT	<73	22.81	31/94	1.00	0.40	<13	9.26	33/97	1.00	0.05
AA	<73	27.45	171/386	1.51 (0.96-2.36)		<13	9.51	173/398	1.50 (0.95-2.37)	
TT/AT	≥73	201.46	53/70	2.89 (1.70-4.92)		≥13	17.08	53/74	1.73 (1.01-2.97)	
AA	≥73	207.72	272/301	3.31 (2.13-5.16)		≥13	17.32	280/315	1.88 (1.20-2.95)	
GSTO1 (rs11509438)										
AA/GA	<73	23.45	35/98	1.00	0.59	<13	9.24	33/110	1.00	0.02
GG	<73	27.53	170/395	1.27 (0.81-1.98)		<13	9.57	171/395	1.58 (0.99-2.52)	
AA/GA	≥73	199.14	52/76	2.32 (1.34-4.01)		≥13	17.25	56/71	1.96 (1.11-3.45)	
GG	≥73	205.17	270/301	2.83 (1.82-4.39)		≥13	17.26	279/327	1.85 (1.17-2.91)	
GSTO1 diplotypes										
Other/other	<73	26.42	132/308	1.00	0.83	<13	9.57	122/310	1.00	0.08
AAG/other	<73	26.66	51/124	1.12 (0.74-1.69)		<13	9.30	62/135	1.22 (0.81-185)	
AAG/AAG	<73	34.08	9/17	1.94 (0.78-4.85)		<13	9.57	12/16	2.64 (1.08-6.45)	
Other/other	≥73	207.17	203/239	2.24 (1.66-3.03)		≥13	17.35	223/258	1.50 (1.11-2.03)	
AAG/other	≥73	206.96	85/102	2.30 (1.56-3.39)		≥13	17.17	76/99	1.39 (0.93-2.08)	
AAG/AAG	≥73	164.51	13/8	3.38 (1.30-8.79)		≥13	16.20	10/12	1.61 (0.64-4.05)	

*ORs were adjusted for age and gender, smoking status, BMI, and water consumption per day.
†ORs were adjusted for age and gender, smoking status, BMI, water consumption per day, and well arsenic concentration.

arsenic-related skin lesions confirms these inferences in arsenic toxicity. Consistent with previous studies (33, 34), cigarette smoking was not significantly related to %MMA and %DMA in urine. The modifying effect of smoking on the risk of arsenic-related disease is therefore likely due to mechanisms not directly related to arsenic metabolism. BMI was positively related to %MMA and inversely related to %DMA; however, these associations were not statistically significant. Specific dietary food/nutrient intakes may be more relevant to arsenic metabolism. In this regard, previous studies from our group found that plasma folate concentrations were positively associated with %DMA and negatively associated with %MMA (17) and that folic acid supplementation (400 μg/d for 12 weeks) significantly increased %DMA and decreased both %MMA and %InAs (35). Consistent with these previous studies (17, 35), we found that urinary creatinine was a strong predictor of %MMA and %DMA.

Our study shows that %DMA in urine is inversely associated with arsenic exposure, especially at the high dose range, suggesting that either methylation of arsenic to DMA is saturable or, alternatively, arsenic inhibits the arsenic methyltransferase enzyme. This may have profound public health implications because methylation of arsenic, especially the conversion of MMA to DMA, has long been considered a detoxification step. Our observation that, at a given level of arsenic exposure, %MMA was most strongly associated with the increased risk of arsenic-induced skin lesions suggests that %MMA, rather than InAs itself, may be largely responsible for the induction of skin lesions. Research efforts for mitigating arsenic-induced health effects may focus on enhancing MMA methylation and excretion from the body. In addition, the fact that %MMA tends to accumulate with increasing arsenic exposure indicates that the need for mitigation efforts for highly exposed populations is especially warranted.

Our findings also suggest that variations in the *MTHFR* and *GSTO1* genes modulate health effects of arsenic exposure. The consistent relationships of the risk of skin lesions with genotypes and diplotypes in the *MTHFR* and *GSTO1* genes provide novel data on the genetic susceptibilities in this study population. However, variations in these two genes were not significantly associated with urinary arsenic methylation indices. The relationship between the *MTHFR* polymorphisms and arsenic methylation status may depend on other elements in the one-carbon metabolism pathway. In addition, the effect of *MTHFR* genotype on risk of skin lesions may not be through its direct effect on levels of %MMA. The *MTHFR* gene also plays a role in the synthesis, repair, and methylation of DNA, processes that are central to maintaining the integrity of the genome (36). Alternatively, the small sample size of subjects with *MTHFR* 677TT genotype suggests a lack of power in detecting the association. The *GSTO1* gene is hypothesized to be related to the reduction of MMA^V to MMA^{III}. We found that the association between *GSTO1* genotypes and risk of skin lesions differed by level of %MMA (Table 6). This finding suggests that differences in one's ability to reduce MMA^V to MMA^{III} may be related to the risk of skin lesions, depending on the overall level of %MMA. Future studies are needed to study effects of different species of MMA. Although genetic makeup is not modifiable, these findings underscore the need of considering interindividual variability in developing and evaluating appropriate interventions to mitigate arsenic-induced health effects. These findings also reiterate that arsenic-induced health effects may be especially deleterious in subsets of the population carrying susceptible variants of genes relevant to arsenic metabolism. Based on the risk estimates observed in this study, the proportion of skin lesions in our study population that is attributable to the *MTHFR* 677TT/1298AA and 677CT/1298AA diplotypes was estimated to be 7.5%. The corresponding attributable proportion for the *GSTO1* at-risk diplotype was 8.9%.

Several aspects should be taken into consideration when interpreting the results of the present study. First, urine samples were taken at one point in time. If urinary arsenic metabolites are not a stable measure, then an assessment based on one spot urine sample may not be reflective of the individual's usual level. The literature, including our own study among the parent cohort members, suggests that arsenic methylation efficiency of an individual as measured in urine is remarkably stable over time (37). In particular, among 98 subjects from our parent cohort participating in the placebo arm of a double-blind randomized folate supplementation trial (35) with three urine samples collected over a period of 3 months, the intraclass correlations for urinary arsenic metabolites were all >0.65. Of particular note, the intraclass correlation estimates for %MMA, %DMA, and SMI were all >0.82. Second, the controls were not matched to cases on sex and age that may influence the risk of arsenic-related skin lesions. However, the advantage of our sampling is that the controls were representative of the overall cohort members, who were recruited from the general population in a defined region from which cases were derived. Results were similar in stratified analysis by sex and also in the analysis of 505 pairs of cases and controls who were randomly selected from the study population and were matched by sex and age (data not shown).

Our study has several notable strengths. This study included sample sizes that are several orders of magnitude higher than all of the previous epidemiologic studies of topics related to arsenic metabolism. We did comprehensive assessments of the interrelationships among arsenic methylation, genetic susceptibility, and arsenic-induced skin lesion risk in one large population. In addition, the study population is ethnically homogeneous, which prevented population stratification bias that may arise due to different ethnic composition of cases and controls. Our study population also offered a unique opportunity to investigate the study objectives among a population with current (or recent) exposure, limiting the sources of recall and exposure assessment errors.

In conclusion, we found that the proportion of MMA in urine was a strong predictor of the risk of arsenic-induced skin lesions. We also observed a dose-dependent saturation (or inhibition) of the conversion of MMA to DMA. The effect of MMA on skin lesion risk and the effect of arsenic doses were influenced to some extent by carrier status of at-risk genotypes and diplotypes of arsenic metabolism genes. These findings have implications for public health interventions, evaluations, and policy.

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