Arsenic methylation, *GSTO1* polymorphisms, and metabolic syndrome in arseniasis endemic area of southwestern Taiwan

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Abstract

Previous studies have shown that hair arsenic (As) levels are associated with an increased prevalence of metabolic syndrome (MetS), which is a strong predictor for type 2 diabetes. The objective of this study was to evaluate whether urinary arsenic methylation is related to MetS in the arseniasis endemic area of southwestern Taiwan, taking genetic factors into account. Subjects were from a community-based cohort recruited in 1990 from three villages in Putai township. In 2002-2003, 247 subjects were successfully followed, including urinary arsenic speciation measurements and single-nucleotide polymorphism determinations. We found that subjects with MetS drank from well water with higher As concentrations. Multiple logistic regression analysis showed that the odds ratio (OR) of MetS significantly increased with increasing dimethylarsinic acid ratios (dimethylarsinic acid levels/Total As) and decreasing monomethylarsonic acid ratios (monomethylarsonic acid levels/Total As). The highest OR (4.65, 95% CI: 2.22-9.73, $p<0.001$) was found in the higher secondary methylation index group with the *GSTO1* AA genotype compared to the reference group with lower secondary methylation index and the *GSTO1* AA genotype. Our findings suggested that a decreasing monomethylarsonic acid ratio is associated with an increased risk of MetS and that this risk might be marginally modified by the *GSTO1* genotype among As-exposed subjects.

Keywords: arsenic, metabolic syndrome, type 2 diabetes mellitus, risk factor, methylation

1. Introduction

Due to the lack of treated tap water and the high salinity of shallow well water during 1910-1960s, the local residents (before the 1970s) in an arseniasis endemic area of southwestern Taiwan typically consumed artesian well water with arsenic (As) concentrations as high as 0.7-0.93 mg/L (Kuo, 1964). Several studies have shown a dose-response relationship between As in drinking water and the prevalence of diabetes mellitus in southwestern Taiwan (Wang et al., 2003).

As is abundant in the environment (Smith et al., 2002) and its threat in the Western Pacific region has been noted (Suk et al., 2003). A great deal of new information is emerging from the extensive research on the health effects of chronic As toxicity (Guha Mazumder, 2008). Chronic exposure to high levels of inorganic As (iAs) is associated with a wide range of human ailments including cancer, arteriosclerosis, hypertension, and type 2 diabetes (T2D) (Fu et al., 2010). Recently it has been shown that even low levels of exposure to iAs in drinking water may play a role in the prevalence of T2D (Navas-Acien et al., 2008). Groundwater and industrial materials are important sources of As (Nordstrom, 2002). Epidemiological studies carried out in Taiwan (Chiou et al., 2006), Bangladesh (Nabi et al., 2005), and Mexico (Coronado-Gonzalez et al., 2007) have shown a strong diabetogenic effect of As in humans.

It is recognized that iAs contributes to oxidative stress in several organs and systems through generation of reactive oxygen species, which can function as signaling molecules to activate a number of cellular stress-sensitive pathways linked to insulin resistance and decreased insulin secretion (Izquierdo-Vega et al., 2006). T2D accounts for 90-95% of all cases of diabetes and is a major public health problem (Wild et al., 2004). The prevalence of T2D has been increasing substantially worldwide, including in Taiwan (Wang et al., 2005). Conventional risk factors have been identified, but they have not yet been sufficiently explained (Wang et al., 1997). Metabolic syndrome (MetS) has been demonstrated as a strong predictor for T2D incidence in middle-aged individuals (Wannamethee et al., 2005). Established risks factors for T2D include older age, obesity, physical inactivity, family history, and genetic polymorphisms (Navas-Acien et al., 2006). Moreover, As, an environmental toxicant, has been suggested to play an etiologic role in the development of diabetes (Tseng, 2004). Wang et al. (2007) has also reported a relationship between hair arsenic levels and MetS in a cross-sectional study in Taiwan.

The toxicity of As greatly differs among their chemical species, and methylated arsenicals are considered to be less toxic than iAs. Methylation is the major metabolic pathway for iAs in humans, and most of the iAs (arsenite [AsIII] and

arsenate [AsV]) are metabolized to monomethylarsonic acid (MMAV) and dimethylarsinic acid (DMAV) before excretion in the urine. Methylation of As involves a two-electron reduction of pentavalent (e.g., AsV and MMAV) to trivalent (e.g., AsIII and MMAIII [monomethylarsonous acid]) As species followed by the transfer of a methyl group from a methyl donor, such as *S*-adenosylmethionine (Thompson, 1993). In the biotransformation process of As, As (+3 oxidation state) methyltransferase (*AS3MT*) and glutathion *S*-transferase ω (*GSTO*) are required in a variety of animals including humans (Aposhian and Aposhian, 2006). GST is a phase II enzyme that can detoxify xenobiotics by catalyzing their conjugation with reduced glutathione. *GSTO1* is involved in the reduction activities of AsV, MMAV, and DMAV (Agusa et al., 2010). Human *AS3MT* is known to catalyze the methylation of arsenite (Fujihara et al., 2010). However, methylation of iAs is not necessarily a detoxification process, in that methylation is paradoxically both a detoxification and activation process (Thomas et al., 2007).

In order to evaluate the changes in disease risk, we follow a community-based population with documented previous ingestion of As from artesian well water (Chen et al., 1995). We aimed to assess if *GSTO1* and *AS3MT* affected the As methylation patterns and evaluated the association among As methylation patterns, gene polymorphisms, and the occurrence of MetS.

2. Materials and Methods

2.1 Study site and subjects

Subjects were form a community-based cohort recruited in 1990 (Chen et al., 1995) consisting of 1297 men and women over 40-years-old. All subjects were from Putai village, a high arsenic exposure township in southwestern Taiwan, where the prevalence of black foot disease (BFD) was the highest and a median As concentration in the artesian well water ranging from 700 to 930 μg/L (Kuo, 1964). The soil and water from shallow wells in this area has a high salt content. The residents, therefore, have used artesian well water since the 1900s. During 1972 and thereafter, a tap water supply system was implemented in this BFD endemic area, which almost completely supplied the entire area with municipal water. In 1997 and 2002-3, we successfully followed 287 subjects from the residents, and 247 subjects with urinary arsenic speciation measurements and single-nucleotide polymorphism determinations.

2.2 Data and specimen collections

Informed consent from each participant and approval by the Human Subjects Review Board of National Health Research Institutes in Taiwan was obtained prior

100 to data collection. Demographic factors, artesian well water usage, personal and
101 family disease history of diabetes mellitus, dietary habits, and life style information
102 were acquired using standardized questionnaires at both baseline and follow-up.
103 Blood pressure was measured using a mercury sphygmomanometer with a suitable
104 cuff. Two measurements were carried out at least 30 min apart while subjects sat
105 calmly. If the difference between the two measurements exceeded 5% (i.e., >5
106 mmHg out of 100 mmHg), blood pressure was measured a third time. The average
107 of the two closest values was used for data analysis. The fasting blood samples were
108 stored frozen after collection and then keep in a -70^{0}C freezer until analysis.
109
110 **2.3 Specimen analysis**
111 We quantified four As species in urine collected at the 2002-2003 follow-up:
112 arsenite (AsIII), arsenate (AsV), monomethylarsonic acid (MMA), and
113 dimethylarsinic acid (DMA). Urinary As species were analyzed using
114 high-performance liquid chromatography (HPLC) coupled with flow injection
115 atomic absorption spectrometry. The HPLC system consisted of a solvent delivery
116 pump (PU-1580; Jasco, Tokyo, Japan) and a silica-based anion-exchange column
117 (Nucleosil 10 SB, 250 mm × 4.6 mm; Phenomenex, Torrance, CA, USA) with a
118 guard column packed with the same material. A flow injection analysis system

(FIAS-400; PerkinElmer, Waltham, MA, USA) was designed as the on-line interface to the continuous hydride generation system (Analyst 100; PerkinElmer, Waltham, MA, USA). The within-day and between-day precision (coefficient of variation, CV%) for AsIII, AsV, MMA, and DMA determinations ranged from 1.0 to 3.7%. The recoveries for AsIII, AsV, MMA, and DMA were 99.0, 98.9, 99.0, and 99.0%, while the detection limits were 0.75, 1.47, 1.19, and 0.76 μg/L, respectively.

Fasting plasma was analyzed in a central laboratory of the Kaohsiung Medical Center for blood glucose, cholesterol, triglycerides, low- and high-density lipoproteins, urine acid, and urine creatinine using a Beckmen Synchron LX20 System (Beckman Coulter, Brea, CA, USA). The CV% ranged between 0.4% and 3.4% with a mean of 2.8% and 2.2% for day-to-day and within-run measurements, respectively.

2.4 Genotype Analysis

Genomic DNA was extracted from buffy coat using a commercial kit (Gentra Puregene; Qiagen, Hilden, Germany). The genotype of *GSTO1* A140D was determined using polymerase chain reaction (PCR) followed by restriction enzyme digestion as described previously (Marahatta et al., 2006). For the *GSTO1* A140D genotype, a PCR containing 100 ng of DNA was incubated at 93^0C for 5 min

138 followed by 33 cycles at 93^{0}C for 60 sec, 51^{0}C for 60 seconds, and 72^{0}C for 30
139 seconds, with a final 4-min extension at 72^{0}C. The A140D polymorphism was
140 detected by *Cac* 8I digestion, with the result of a single 254 bp band indicating the
141 presence of the D allele, while double bands (186 and 68 bp) indicating the A allele.
142 For the *GSTO2* N142D genotype, PCR conditions included an initial step at 95^{0}C for
143 5 min followed by 35 cycles of 93^{0}C for 60 sec, 62^{0}C for 60 sec, and 72^{0}C for 30 sec,
144 with a final 4-min extension at 72^{0}C. A fragment of 185 bp was amplified and
145 analyzed using *Mbo* I. The presence of the variant D allele resulted in digested
146 bands of 122 and 63 bp. All PCR products were analyzed using 3.5% NuSieve
147 (Cambrex, Charles City, IA, USA) agarose gel electrophoresis. Primers for *GSTO1*
148 A140D were 5'-GAACTTGATGCACCCTTGGT-3' (forward) and
149 5'-TGATAGCTAGGAGAAATAATTAC-3' (reverse). Primers for *GSTO2* N142D
150 were 5'-AGGCAGAACAGGAACTGGAA-3' (forward) and
151 5'-GAGGGACCCCTTTTTGTACC-3' (reverse).
152 The *AS3MT* M287T genotype was determined using a commercial TaqMan
153 SNP Genotyping Assay (Assay ID C_31979150_10; Applied Biosystems, Foster
154 City, CA, USA). Reactions were conducted following the instructions of the
155 manufacturer.
156

157 **2.5 Data analysis**

158 Adult Treatment Panel III (2001) (Alberti et al., 2006; Saely et al., 2006)
159 criteria were adopted to define MetS: fasting plasma glucose (≥ 110 mg/dL),
160 triglycerides (≥ 150 mg/dL), high density lipoprotein (≤ 40 mg/dL for men and ≤ 50
161 mg/dL for women), increased systolic (≥ 130 mmHg) or diastolic (≥ 85 mmHg)
162 blood pressure, and waist girth (≥ 90 cm for men and ≥ 80 cm for women). MetS
163 was defined as the presence of three or more of the risk factors mentioned above. We
164 also calculated insulin sensitivity (Si), which was the inverse of the homeostasis
165 model assessment index, defined as 22.5EXP[-ln(glucose)]/insulin (Cohen et al.,
166 2006).

167 The total urine As (TotAs) in the present study was defined as the sum of AsIII,
168 AsV, MMA, and DMA. The cumulative arsenic exposure (CAE) (mg/L-y) was
169 defined as the sum of the products, derived by multiplying the As concentration in
170 the well water by the duration of water consumption during consecutive periods of
171 living at different villages (Chen et al., 1995). Primary methylation index (PMI) and
172 secondary methylation index (SMI) were defined as MMA divided by (AsIII + AsV)
173 and as DMA divided by MMA, respectively. Because of the distribution of As was
174 skewed to the right, As concentrations were log-transformed and calculate as
175 geometric means (GM). We examined the differences between (Student's *t*-test) and

among the groups (analysis of variance, ANOVA) using GM. A chi-square test was performed for categorical variables. The univariate and multivariate logistic regression analyses were used to evaluate MetS status in relation to risk factors, with the multivariate model adjusted for significant risk factors identified by univariate analysis. All analyses were performed using SPSS v15 (SPSS, Chicago, IL, USA).

3. Results

Among the 247 followed subjects, 111 were found to have MetS. **Table 1** shows the occurrence of MetS according to demographic factors, life style, and MetS-related risk factors. The mean age of the group with MetS (64.34 ± 7.93) was significantly higher than that of the group without MetS (60.36 ± 9.00) ($p < 0.001$). Insulin sensitivity showed a significant difference the groups without and with MetS (0.95 ± 0.47 vs. 0.51 ± 0.37, respectively; $p < 0.001$). We also found that the As concentration in the well water and betel nut chewing significantly correlated with the risk of MetS after adjustment for age. **Table 2** shows MetS according to As metabolites and genotypes. MMA levels were significantly lower in the group with MetS than those without MetS. Subjects without MetS tended to have a higher incidence of the AA genotype, with borderline significance after adjustment for age. **Table 3** shows the Pearson correlation coefficients for Si and As metabolites. We

found that Si was associated with MMA, MMA%, PMI, and SMI. TotAs showed a high correlation with other As metabolites, but not with CAE. **Table 4** shows the results of multiple logistic regression analysis for MetS in relation to tertiles of As methylation patterns by three models. The upper groups of MMA% (MMA/TotAs), DMA% (DMA/TotAs), PMI, and SMI showed significant differences as compared to their reference groups. MMA% and PMI were negatively associated with MetS (OR = 0.35, 95% CI: 0.18-0.66 and 0.39, 95% CI: 0.20-0.76 for MMA% > 0.11 and PMI > 0.91 compared to MMA% < 0.06 and PMI < 0.44, respectively). DMA% and SMI were also associated with increased risk of MetS (OR = 2.01, 95% CI: 1.05-3.86 and 2.61, 95% CI: 1.35-5.08 for DMA% > 0.82 and SMI > 12.03, respectively). **Table 5** shows the arsenic methylation patterns linked to MetS. Three models for logistic regression were performed and four As methylation patterns, MMA%, DMA%, PMI, and SMI, were considered. We found the MetS OR for subjects with a lower PMI and higher SMI was significantly higher (3.71, 95% CI: 1.90-7.21) than that for subjects with a higher PMI and lower SMI. Consistently, the MetS OR for subjects with a lower MMA% and higher DMA% was significantly higher (3.63, 95% CI: 1.90-6.96) than that for subjects with a higher MMA% and lower DMA%. **Table 6** shows the arsenic methylation patterns and *GSTO1* genotypes linked to MetS. The analysis of the four As methylation patterns and

214 *GSTO1* genotypes were performed using three logistic regression models. Subjects
215 with a lower PMI, lower MMA%, and the AD+DD genotype showed a significantly
216 higher MetS risk of around 3–4 fold (ORs were 4.00 and 3.24 for lower PMI and
217 lower MMA%, respectively). Subjects with both a higher SMI and higher DMA%
218 showed a significantly higher MetS risk (ORs were 3.87 and 3.54 for higher SMI
219 and higher DMA%, respectively).
220
221 **4. Discussion**
222 The positive association between arsenic exposure and increased rates of
223 diabetes has been shown in Taiwan; however, its association with MetS, an
224 important risk factor and predictor for cardiovascular diseases including T2D
225 (especially for middle-age subjects), is seldom studied. A cross-sectional study in
226 Taiwan (Wang et al., 2007) indicated the increasing prevalence of MetS from the 2nd
227 tertile (0.034 μg/g) of hair arsenic levels (OR = 2.54, p = 0.015) after adjustment for
228 age, gender, occupation, and life style. To our knowledge, the present study is the
229 first to report the relation between arsenic methylation patterns and MetS, with
230 genotype and other risk covariates taken into account.
231 The excretion of methylated As metabolites in urine is the result of a number of
232 factors, though our knowledge of the importance of these factors is limited.

233 According to a report from the National Research Council (1999), it can be
234 concluded that the level of arsenic has little influence on methylation efficiency. The
235 rates of As metabolites in our study are close to those of a previous study (Vahter,
236 2000), which indicated that most individuals have an average of 10–30% iAs,
237 10–20% MMA, and 60–70% DMA (for this study, 17%, 10%, and 73% for iAs,
238 MMA, and DMA, respectively). In one study, a 500-μg/L increase in the
239 concentration of As metabolites in urine corresponded to a 2% increase in urine
240 MMA and a 3% decrease in DMA (Hopenhayn-Rich et al., 1996). We found an
241 association between *AS3MT* and MMA that was similar to the results of a recent
242 study (Ahusa et al., 2010). MMA%, PMI, and SMI are affected by *AS3MT* (MM vs.
243 MT genotype: 0.10 vs. 0.22, 0.82 vs. 1.75, and 18.25 vs. 3.45, respectively; data not
244 shown). Some studies have shown that MMA is more cytotoxic (Petrick et al., 2000)
245 and genotoxic (Mass et al., 2001) than AsIII and AsV, suggesting that the oxidation
246 state of methylated arsenicals is important for the manifestation of their toxic and/or
247 genotoxic effects. A recent study (Chung et al., 2009) showed that MMM% might be
248 a potential predictor of cancer risk and that changes in MMA% are linked to
249 individual cancer susceptibility related to *AS3MT*. In the present study, however, we
250 found that Si increased with increasing MMA, MMA%, PMI, and SMI. We also
251 found that lower PMI and MMA% and higher SMI and DMA% seemed to be risk

252 factors for MetS. When looking at the As methylation pattern and the genotypes of
253 *GSTO1* (**Table 5**), As methylation patterns seems to be dominate factors of MetS
254 risk, as apposed to *GSTO1*. Up to now, a clear cut-off level for As exposure or
255 As-induced health effects has not been established. The biological exposure index of
256 35 μg/g creatinine is based on an estimated risk for lung cancer. The thresholds of
257 different As metabolites for developing MetS are not clear and await further
258 investigation.

259 Several factors can influence the toxicity of As towards organs involved in
260 glucose metabolism and can determine the progression of insulin resistance, such as
261 personal susceptibility with respect to genetics, nutritional uptake, health status,
262 detoxification capability, interactions with other trace elements, and the other
263 well-recognized risk factors for diabetes mellitus. Although drinking water is the
264 main source of As uptake, up to 44% of ingested As may come from food sources
265 (Del Razo et al., 2002). Another study has indicated that diet is the largest exposure
266 source for most individuals, with an average intake of about 50 μg/d from food
267 (Tchounwou et al., 2003). Other uptake routes are usually much smaller, but may
268 become significant in areas of As contamination. For accumulative As, we measured
269 individual As exposure and then multiplied the number of years lived in a specific
270 area times the average As level in the well water. The measurement, however, was

271 based on one measurement for the period and assumed constant water As
272 concentrations throughout the period. Das et al. (1989) proposed that selenium (Se)
273 and As counteract each other in regards to glucose metabolism and that the joint
274 effect of high As and low Se could play a role in developing T2D. The variation of
275 exposure to Se, consumption of As via food, and other nutrients were not considered
276 in this study.
277 Induction of oxidative stress and interference of signal transduction or gene
278 expression by As or by its methylated metabolites are the most possible causes to
279 As-induced T2D through mechanisms of induced insulin resistance (Tseng, 2004).
280 In subjects with chronic As exposure, oxidative stress is increased. The expression
281 levels of tumor necrosis factor α (TNFα) and interleukin-6 (IL-6), both cytokines,
282 are well known for their effect on the induction of insulin resistance. Fu et al. (2010)
283 indicated that low levels of As provoke a cellular adaptive oxidative stress response
284 that increases antioxidant levels, dampens reactive oxygen species signaling
285 involved in glucose-stimulated insulin secretion, and thus disturbs *β*-cell function. It
286 was reported that the methylation of As in individuals via drinking water (in Taiwan)
287 is associated with genetic polymorphism in GST (Chiou et al., 1997). Between
288 individuals, there are a lot of differences in As biomethylation. As such, it is likely
289 that there are genetic polymorphisms affecting the regulation of As

methyltransferases. It is possible that As metabolism is also affected by the documented polymorphism in enzymes involved in the remethylation of homocysteine.

Current available evidence (including in vitro, in vivo, and animal studies) are inadequate to establish the cause-effect relationship between As and MetS. Due to the short biological half-life of arsenic, hair or nails may be a more valid source for estimating individual As exposure. Further studies should focus on the measurement of appropriate samples (e.g., hair) that more accurately represent long-term As exposure.

5. Conclusions

We found that the group of subjects with MetS had a higher As concentration in their well water. *AS3MT* polymorphisms were associated with MMA%, PMI, and SMI. Multiple logistic regression analysis showed that the OR of MetS significantly decreased with increasing MMA% and decreasing DMA%. We found that subjects with lower PMI and higher SMI levels had a significantly increased OR of MetS. The high OR (4.65, 95% CI: 2.22-9.73, $p<0.001$) was found in the higher SMI group with the AA genotype compared to the reference group with lower SMI and the AA genotype. Our findings suggested that decreasing MMA% and PMI as well

309 increasing DMA% and SMI are associated with an increased risk of MetS and that
310 this risk might be marginally modified by the *GSTO1* genotype among As-exposed
311 subjects.
312
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Table 1. Occurrence of metabolic syndrome according to demographic factors, life style, and metabolic syndrome parameters (n = 247)

Characteristics	Metabolic Syndrome		*p*-value[a]	*p*-value[b]
	No (n = 136)	Yes (n = 111)		
	Mean ± SD	Mean ± SD		
Continuous variable				
Age (y)	60.36 ± 9.00	64.34 ± 7.93	< 0.001***	
Residing yrs (y)	42.29 ± 14.08	46.66 ± 12.46	0.011*	0.97
Drinking yrs (y)	19.95 ± 12.90	25.31 ± 12.32	0.001**	0.13
As concentration in well water (μg/L)[c]	569.94 ± 321.51	684.39 ± 245.93	0.005**	0.03*
CAE (mg/L-y)	13.96 ± 9.03	17.71 ± 8.62[c]	0.001**	0.13
Glucose (mg/dL)	97.77 ± 42.64	127.39 ± 56.85	< 0.001***	< 0.001***
Waist girth (cm)	80.73 ± 9.18	89.77 ± 10.56	< 0.001***	< 0.001***
Systolic blood pressure (mmHg)	128.42 ± 19.51	146.32 ± 20.24	< 0.001***	< 0.001***
Diastolic blood pressure (mmHg)	77.39 ± 12.65	84.78 ± 14.09	< 0.001***	< 0.001***
Triglycerides (mg/dL)	98.40 ± 49.27	181.34 ± 102.80	< 0.001***	< 0.001***
High density lipoprotein (mg/dL)	45.11 ± 12.57	33.57 ± 7.85	< 0.001***	< 0.001***
Insulin sensitivity[d]	0.95 ± 0.47	0.51 ± 0.37	< 0.001***	< 0.001***
	Frequency (%)	Frequency (%)		
Categorical variable				
Gender				
Female	79 (58.09)	62 (55.86)	0.72	0.81
Male	57 (41.91)	49 (44.14)		
Alcohol drinking				
No	126 (92.65)	98 (89.09)	0.33	0.27
Yes	10 (7.35)	12 (10.91)		
Betel nut chewing				
No	126 (95.45)	98 (89.91)	0.09	0.045*
Yes	6 (4.55)	11 (10.09)		
Smoking				
No	105 (80.15)	78 (72.90)	0.19	0.28
Yes (including ever)	26 (19.85)	29 (27.10)		
Secondary Smoking				
No	92 (71.32)	81 (75.00)	0.52	0.69
Yes	37 (28.68)	27 (25.00)		
Education				
No	36 (26.47)	40 (36.04)		
Primary	71 (52.21)	57 (51.35)	0.11	0.46
High school or higher	29 (21.32)	14 (12.61)		
Exercise				
No	75 (55.15)	59 (53.15)	0.75	0.47
Yes	61 (44.85)	52 (46.85)		

[a]Student's T test for continuous variables, chi-square test for categorical variables
[b]age-adjusted
[c]102 subjects had records available
[d]insulin sensitivity, which was the inverse of the homeostasis model assessment index, was defined as 22.5EXP[-ln(glucose)]/insulin
*p<0.05, **p<0.01, ***p<0.001

Table 2. Metabolic syndrome according to arsenic patterns and genotypes (n = 247)

Characteristics	Metabolic Syndrome		p-value[b]	p-value[c]
	No (n = 136)	Yes (n = 111)		
	Mean ± SD	Mean ± (SD)		
Arsenic metabolites[a]				
iAs%	17.2 ± 14.4	16.8 ± 16.2	0.84	0.98
oAs%	82.6 ± 14.1	83.4 ± 15.8	0.84	0.98
AsIII%	10.1 ± 11.6	10.4 ± 13.1	0.73	0.98
AsV%	7.3 ± 7.3	6.9 ± 6.5	0.85	0.99
MMA%	10.6 ± 9.2	9.4 ± 8.8	0.08#	0.06#
DMA%	72.3 ± 18.4	73.5 ± 20.0	0.49	0.37
PMI	0.92 ± 0.75	0.75 ± 0.71	0.08#	0.06#
SMI	16.12 ± 31.21	19.98 ± 32.71	0.35	0.42
AsIII (μg/g creatinine)	3.79 ± 4.60	4.12 ± 6.15	0.64	0.79
AsV (μg/g creatinine)	1.78 ± 1.93	1.47 ± 1.14	0.11	0.29
iAs (μg/g creatinine)	5.57 ± 4.84	5.58 ± 6.08	0.98	0.97
MMA (μg/g creatinine)	4.51 ± 5.92	3.08 ± 2.95	0.02*	0.03*
DMA (μg/g creatinine)	32.79 ± 28.94	35.52 ± 36.98	0.53	0.42
oAs (μg/g creatinine)	37.30 ± 30.92	38.60 ± 38.26	0.77	0.63
TotAs (μg/g creatinine)	42.87 ± 33.06	44.19 ± 40.94	0.78	0.66
	Frequency (%)	Frequency (%)		
Genotypes				
GST01 A140D				
AA	92 (68.15)	66 (60.00)		
AD	37 (27.41)	31 (28.18)	0.08	0.06
DD	6 (4.44)	13 (11.82)		
GST02 N142D				
NN	68 (50.75)	53 (47.75)		
ND	53 (39.55)	48 (43.24)	0.84	0.61
DD	13 (9.70)	10 (9.01)		
AS3MT M287T				
MM	130 (97.74)	109 (99.09)	0.63[d]	0.29
MT	3 (2.26)	1 (0.91)		

[a]iAs: inorganic arsenic, AsIII+AsV; oAs: organic arsenic, MMA+DMA; TotAs: total arsenic, iAs+oAs; PMI: MMA/(AsIII+AsV); SMI: DMA/MMA; (As metabolites)%: (As metabolites)/TotAs
[b]Student's T test for continuous variables, chi-square test for categorical variables
[c]age-adjusted
[d]Fisher's Exact Test
#$p<0.1$, *$p<0.05$

Table 3. Pearson correlation coefficients for insulin sensitivity and arsenic metabolites (n = 247)

	Si	CAE	TotAs	MMA	DMA	iAs	MMA%	DMA%	PMI	SMI
Si	1.0									
CAE[a]	-0.03	1.0								
TotAs	0.05	0.11$^{\#}$	1.0							
MMA	0.14*	0.09	0.40***	1.0						
DMA	0.04	0.09	0.97***	0.26***	1.0					
iAs	0.03	0.18**	0.49***	0.26***	0.36***	1.0				
MMA%	0.14*	0.02	0.23***	0.62***	-0.33***	-0.08	1.0			
DMA%	-0.03	-0.002	0.37***	-0.19***	0.48***	-0.23***	-0.64***	1.0		
PMI	0.14*	0.04	0.11$^{\#}$	0.66***	0.06	-0.22***	0.60***	-0.03	1.0	
SMI	0.14*	-0.03	0.45***	-0.23***	0.52***	0.15**	-0.41***	0.35***	-0.30***	1.0

[a] n = 238

$^{\#}p<0.1$, $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$

Table 4. Multiple logistic regression analysis for metabolic syndrome in relation to tertiles of previously exposed arsenic methylation patterns (n = 247)

Characteristics	OR	95% CI	*p*-value	OR[a]	95% CI	*p*-value	OR[b]	95% CI	*p*-value
As concentration in well water (μg/L)[c]									
< 700	1.00			1.00			1.00		
700 - 767.65	1.56	(0.85 - 2.84)	0.15	1.35	(0.72 - 2.53)	0.34	1.25	(0.66 - 2.39)	0.49
> 767.65	1.51	(0.81 - 2.80)	0.19	1.33	(0.71 - 2.51)	0.38	1.24	(0.65 - 2.37)	0.52
CAE									
< 12.60	1.00			1.00			1.00		
12.60 - 18.90	1.56	(0.83 - 2.93)	0.17	1.04	(0.51 - 2.15)	0.91	1.01	(0.48 - 1.89)	0.98
> 18.90	2.71	(1.42 - 5.17)	0.003**	1.96	(0.83 - 4.65)	0.13	1.73	(0.72 - 4.19)	0.22
AsIII%									
< 5.2	1.00			1.00			1.00		
5.2 - 8.9	1.02	(0.54 - 1.91)	0.95	1.05	(0.55 - 2.00)	0.89	1.09	(0.56 - 2.11)	0.80
> 8.9	0.94	(0.52 - 1.71)	0.84	0.88	(0.48 - 1.63)	0.69	0.78	(0.41 - 1.49)	0.45
AsV%									
< 3.1	1.00			1.00			1.00		
3.1 - 6.4	0.87	(0.46 - 1.63)	0.66	0.91	(0.47 - 1.77)	0.78	0.86	(0.43 - 1.71)	0.67
> 6.4	0.81	(0.45 - 1.46)	0.48	0.80	(0.43 - 1.46)	0.46	0.80	(0.43 - 1.49)	0.48
MMA%									
< 5.8	1.00			1.00			1.00		
5.8 - 11.3	0.96	(0.51 - 1.80)	0.89	0.97	(0.50 - 1.89)	0.92	0.91	(0.45 - 1.81)	0.78
> 11.3	0.36	(0.19 - 0.68)	0.001**	0.34	(0.18 - 0.64)	0.001**	0.35	(0.18 - 0.66)	0.001**
DMA%									
< 72.4	1.00			1.00			1.00		
72.4 - 81.9	1.57	(0.84 - 2.94)	0.16	1.59	(0.84 - 2.99)	0.15	1.60	(0.83 - 3.08)	0.17
> 81.9	1.83	(0.99 - 3.38)	0.06#	1.94	(1.03 - 3.65)	0.04*	2.01	(1.05 - 3.86)	0.04*
PMI									
< 0.44	1.00			1.00			1.00		
0.44 - 0.91	0.86	(0.47 - 1.60)	0.64	0.83	(0.44 - 1.58)	0.56	0.82	(0.42 - 1.60)	0.57
> 0.91	0.40	(0.21 - 0.76)	0.005**	0.39	(0.20 - 0.74)	0.004**	0.39	(0.20 - 0.76)	0.006**
SMI									
< 6.06	1.00			1.00			1.00		
6.06 - 12.03	1.23	(0.65 - 2.33)	0.52	1.29	(0.68 - 2.45)	0.44	1.23	(0.63 - 2.39)	0.54
> 12.03	2.44	(1.31 - 4.56)	0.005**	2.71	(1.41 - 5.21)	0.003**	2.61	(1.35 - 5.08)	0.005**

[a]adjusted by age

[b]adjusted by age and betel nut chewing

[c]n = 238

#$p<0.1$, *$p<0.05$, **$p<0.01$

Table 5. Arsenic methylation patterns and metabolic syndrome (n = 247)

		Metabolic syndrome										
		No (n = 136)	Yes (n = 111)	OR	95% CI	*p*-value	OR[a]	95% CI	*p*-value	OR[b]	95% CI	*p*-value
		Frequency (%)										
PMI	SMI											
High	Low	58 (67.44)	28 (32.56)	1.00			1.00			1.00		
Low	Low	23 (65.16)	14 (37.84)	1.26	(0.57 - 2.82)	0.57	1.30	(0.58 - 2.93)	0.52	1.41	(0.61 - 3.26)	0.43
High	High	21 (56.76)	16 (43.24)	1.58	(0.72 - 3.48)	0.26	1.7	(0.76 - 3.84)	0.19	2.17	(0.92 - 5.09)	0.08[#]
Low	High	34 (39.08)	53 (60.92)	3.23	(1.73 - 6.02)	<0.001[***]	3.41	(1.79 - 6.50)	<0.001[***]	3.71	(1.90 - 7.21)	<0.001[***]
MMA/TotAs	DMA/TotAs											
High	Low	64 (68.09)	30 (31.91)	1.00			1.00			1.00		
Low	Low	169 (61.54)	10 (38.46)	1.33	(0.54 - 3.28)	0.53	1.35	(0.55 - 3.32)	0.52	1.27	(0.51 - 3.18)	0.61
High	High	16 (51.61)	15 (48.39)	2.00	(0.88 - 4.57)	0.10	2.05	(0.89 - 4.70)	0.09[#]	1.96	(0.82 - 4.65)	0.13
Low	High	40 (41.67)	56 (58.33)	2.99	(1.65 - 5.41)	<0.001[***]	3.32	(1.78 - 6.19)	<0.001[***]	3.63	(1.90 - 6.96)	<0.001[***]

[a]adjusted by age

[b]adjusted by age and betel nut chewing

[#]$p<0.1$, [***]$p<0.001$

Table 6. Arsenic methylation patterns and *GSTO1* genotypes linked to metabolic syndrome (n = 247)

		Metabolic syndrome										
		No (n = 135)	Yes (n = 110)	OR	95% CI	*p*-value	OR[a]	95% CI	*p*-value	OR[b]	95% CI	*p*-value
		Frequency (%)										
PMI	*GSTO1*											
High	AA	48 (66.67)	24 (33.330	1.00			1.00			1.00		
High	AD + DD	30 (60.00)	20 (40.00)	1.33	(0.63 - 2.82)	0.45	1.29	(0.60 - 2.76)	0.52	1.32	(0.61 - 2.90)	0.48
Low	AA	44 (51.16)	42 (48.84)	1.91	(1.00 - 3.65)	0.05$^{\#}$	1.94	(1.00 - 3.77)	0.05*	2.03	(1.03 - 4.01)	0.04*
Low	AD + DD	13 (35.14)	24 (64.86)	3.69	(1.60 - 8.50)	<0.001***	3.96	(1.65 - 9.47)	0.002**	4.00	(1.65 - 9.71)	0.002**
SMI	*GSTO1*											
Low	AA	57 (74.03)	20 (25.97)	1.00			1.00			1.00		
Low	AD + DD	23 (51.11)	22 (48.89)	2.73	(1.26 - 5.92)	0.01*	2.78	(1.27 - 6.07)	0.01*	2.91	(1.29 - 6.57)	0.01*
High	AA	35 (43.21)	46 (56.79)	3.75	(1.91 - 7.34)	<0.001***	4.25	(2.10 - 8.60)	<0.001***	4.65	(2.22 - 9.73)	<0.001***
High	AD + DD	20 (47.62)	22 (52.38)	3.14	(1.42 - 6.92)	0.005**	3.15	(1.39 - 7.14)	0.006**	3.87	(1.62 - 9.26)	0.002**
MMA%	*GSTO1*											
High	AA	55 (71.43)	22 (28.57)	1.00			1.00			1.00		
High	AD + DD	24 (51.06)	23 (48.94)	2.40	(1.13 - 5.10)	0.02*	2.40	(1.12 - 5.14)	0.02*	2.35	(1.07 - 5.15)	0.03*
Low	AA	37 (45.68)	44 (54.32)	2.97	(1.54 - 5.75)	0.001**	3.28	(1.65 - 6.52)	<0.001***	3.36	(1.66 - 6.79)	<0.001***
Low	AD + DD	19 (47.50)	21 (52.50)	2.76	(1.25 - 6.11)	0.01*	2.87	(1.26 - 6.53)	0.01*	3.24	(1.37 - 7.66)	0.007**
DMA%	*GSTO1*											
Low	AA	57 (71.25)	23 (28.750	1.00			1.00			1.00		
Low	AD + DD	22 (56.41)	17 (43.59)	1.92	(0.86 - 4.25)	0.11	1.93	(0.87 - 4.30)	0.11	1.86	(0.83 - 4.18)	0.13
High	AA	35 (44.87)	43 (55.13)	3.05	(1.58 - 5.88)	<0.001***	3.50	(1.75 - 6.99)	<0.001***	3.44	(1.69 - 6.99)	<0.001***
High	AD + DD	21 (43.75)	27 (56.25)	3.19	(1.51 - 6.73)	0.002**	3.32	(1.53 - 7.19)	0.002**	3.54	(1.59 - 7.87)	0.002**

[a]adjusted by age

[b]adjusted by age and betel nut chewing

$^{\#}p<0.1$, $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$