Published in final edited form as:

J Expo Sci Environ Epidemiol. 2013 March ; 23(2): 151–155. doi:10.1038/jes.2012.103.

Environmental exposure to arsenic, AS3MT polymorphism and prevalence of diabetes in Mexico

Zuzana Drobná[1], **Luz M. Del Razo**[2], **Gonzalo G. García-Vargas**[3], **Luz C. Sánchez-Peña**[2], **Angel Barrera-Hernández**[2], **Miroslav Stýblo**[1], and **Dana Loomis**[4]

[1]Department of Nutrition, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

[2]Departamento de Toxicología, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, México, DF, México

[3]Facultad de Medicina, Universidad Juárez del Estado de Durango, Gómez Palacio, Durango, México

[4]IARC Monographs Section, IARC/WHO, Lyon Cedex, France

Abstract

Exposure to arsenic in drinking water is associated with increased prevalence of diabetes. We previously reported an association of diabetes and urinary concentration of dimethylarsinite ($DMAs^{III}$), a toxic product of arsenic methylation by arsenic (+3 oxidation state) methyltransferase (AS3MT). Here we examine associations between AS3MT polymorphism, arsenic metabolism and diabetes. Fasting blood glucose, oral glucose tolerance and self-reported diagnoses were used to identify diabetic individuals. Inorganic arsenic and its metabolites were measured in urine. Genotyping analysis focused on six polymorphic sites of AS3MT. Individuals with M287T and G4965C polymorphisms had higher levels of urinary $DMAs^{III}$ and were more frequently diabetic than the respective wild-type carriers, although the excess was not statistically significant. Odds ratios were 11.4 (95% confidence interval (CI) 2.2–58.8) and 8.8 (95% CI 1.6–47.3) for the combined effects of arsenic exposure >75th percentile and 287T and 4965C genotypes, respectively. Carriers of 287T and 4965C may produce more $DMAs^{III}$ and be more likely to develop diabetes when exposed to arsenic.

Keywords

biomonitoring; disease; emerging contaminants; metals

Correspondence: Professor Dana Loomis, IARC Monographs Section, IARC/WHO, 150 Cours Albert Thomas, 69372 Lyon Cedex 08, France. Tel.: + 33 (0)4 72 73 8909. Fax: + 33 (0)4 72 73 8575. loomisd@iarc.fr.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

Supplementary Information accompanies the paper on the Journal of Exposure Science and Environmental Epidemiology website (http://www.nature.com/jes)

INTRODUCTION

Chronic exposure to inorganic arsenic (iAs) in drinking water has been associated with cancer and it is suspected to cause other chronic diseases, including diabetes.[1] There is, however, a high degree of inter-individual variation in iAs metabolism and disease manifestation. Arsenic (+ 3 oxidation state) methyltransferase (AS3MT) is the key enzyme in the pathway for the methylation of iAs.[2] AS3MT polymorphism has been shown to account in part for inter-individual differences in iAs metabolism. The presence of variant alleles in intronic G12390C (rs3740393), C14215T (rs3740390) and G35991A (rs10748835) SNPs were shown to be significantly associated with the second methylation step in indigenous women in northern Argentina, that is, with lower percentage of total As (tAs) in urine represented by monomethylarsenic (MAs) and higher percentage represented by dimethylarsenic (DMAs). These polymorphisms were in a strong linkage and with higher allelic frequencies than those previously reported for other populations.[3] In addition, variation in the percentage of MAs in urine was linked to the presence of rs3740393 SNP in *AS3MT* gene and to individual cancer susceptibility.[4] A lower percentage of DMAs and a lower DMAs/ MAs ratio was detected in urine of Vietnamese carriers of variant alleles for G35991A (rs10748835) SNP in *AS3MT*.[5,6] Multiple studies showed that the most common exonic *AS3MT* SNP Met287Thr (rs11191439) is associated with higher percentage of MAs in urine of subjects exposed to iAs.[7,8] In addition, iAs-exposed carriers of the AS3MT(Met287Thr) variant have increased prevalence of DNA damage and skin lesions characteristic of iAs exposure.[9,10] Wood and associates reported differences in variable number of tandem repeats (VNTRs) in 5'-UTR region of *AS3MT*: transfection of HepG2 cells with a reporter gene constructs containing the VNTR variants (AB, A2B and A3B) indicated that the shorter VNTR (AB) was associated with higher activity of the reporter gene.[11]

To investigate the role of AS3MT polymorphism in the metabolism of iAs and the development of diabetes, we conducted a cross-sectional study in the Zimapan and Lagunera regions in Mexico where levels of iAs in ground water were historically high. We focused on variation in the production of toxic methylated trivalent metabolites of iAs, methylarsonite (MAs^{III}) and dimethylarsinite ($DMAs^{III}$) and associations of diabetes indicators with AS3MT polymorphism. Six polymorphic sites (G12390C, rs3740393; C14215T, rs3740390; G35991A, rs10748835; Met287Thr, rs11191439; G4965C, rs17881215; VNTR) previously linked to differences in iAs metabolism or susceptibility to iAs toxicity[11–16] were examined.

MATERIALS AND METHODS

Details of population recruitment, data collection and analytical methods have been published elsewhere.[17] Briefly, we recruited individuals ≥5 years old who had resided in either region for ≥2 years and those giving consent were invited for an interview during which they completed a questionnaire on consumption and sources of drinking water, health history and potential occupational exposures to arsenic. Pregnant women, alcoholics and individuals with urinary tract diseases or occupational exposures to arsenic were excluded. Eligible subjects provided a sample of the water typically used for drinking at home and a

spot urine sample. Fasting blood was collected, followed by oral glucose tolerance test (OGTT) and sampling of venous blood 2 h after glucose administration. Body weight, height, blood pressure and waist-to-hip ratio were also recorded. All procedures involving human subjects were approved by Institutional Review Boards of Cinvestav-IPN and University of North Carolina at Chapel Hill.

The concentrations of iAs in drinking water and tAs in urine was determined by hydride generation-atomic fluorescence spectrometry[18] after acid digestion.[19] The concentration of iAs in water was expressed in ng As/ml or p.p.b.; tAs concentration in urine was expressed in ng As/ml. Hydride generation-cryotrapping-atomic absorption spectrometry was used for the oxidation state-specific analysis of iAs metabolites in urine.[20,21] To limit oxidation of unstable MAs^{III} and $DMAs^{III}$, aliquots of freshly collected urines were frozen in dry ice and analyzed within 6 h after collection. We have shown that only ~10% of $DMAs^{III}$, the most unstable metabolite of iAs, oxidizes in human urine under these conditions.[17] Pentavalent arsenicals were analyzed in aliquots of urine that were stored at −80 °C. Concentrations of arsenic species in urine were expressed in ng As/ml. Values of MAs^{III} and $DMAs^{III}$ that were below the limit of detection (LOD) of 0.1 ng As/ml were replaced with $LOD/2^{1/2}$ for statistical analysis.

Glucose concentrations were determined in the fasting and 2-h blood samples, using calibrated HemoCue Glucose 201RT glucometer (HemoCue, Lake Forest, CA, USA) that reports results in plasma glucose equivalent values. Subjects were classified as having diabetes if they had any of the following: fasting blood glucose level (FBG) ≥126 mg/dl, 2-h blood glucose level determined by OGTT (2HBG) ≥200 mg/dl, self-reported doctor diagnosis of diabetes or use of anti-diabetic medication.

DNA was isolated from blood using QIAamp DNA Blood Mini Kit (Qiagen) according to the manufacturer's protocol. The AS3MT single-nucleotide polymorphisms were analyzed in the Mammalian Genotyping Core (UNC, Chapel Hill, NC, USA), using predesigned or custom TaqMan assays (Applied Biosystems, Carlsbad, CA, USA). The ABI Dual 384-Well GeneAmp PCR System 9700 and ABI PRISM 7900HT Sequence Detection System from Applied Biosystems was used for genotyping and the ABI SDS software for data analysis. The VNTR variants (AB, A2B and A3B) and G4965C polymorph were identified by sequencing of a PCR-amplified promoter region.[22] For quality control, 10% of DNA samples were re-analyzed. The re-analysis found no systematic errors.

Associations of iAs and its metabolites in urine and of continuous variables for diabetes markers (2HBG and FBG) with AS3MT genotypes were analyzed by multiple linear regression. All continuous variables were approximately log-normally distributed, so log transformations were used. Associations of diabetes status with genotype were examined using logistic regression to estimate odds ratios (ORs) and 95% confidence intervals (CIs). Age in years, sex, hypertension (systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg or reported use of anti-hypertensive drugs) and obesity (BMI>30 kg/m^2) were included as covariates in models for diabetes and diabetes markers. No adjustment was made for urinary creatinine because of indications that creatinine concentration is associated with both diabetes status and the efficiency of iAs

methylation.[23–25] Interactions between AS3MT polymorphism and iAs exposure were examined in analyses of the separate and combined effects of genotype and iAs concentration >75th percentile of the exposure distribution (52 p.p.b.). Statistical analyses were performed in Stata version 11.2 for Mac OS X (StataCorp, College Station, TX, USA).

RESULTS

We enrolled 255 individuals with information on AS3MT SNPs (171 females and 84 males) in total. Data on G12390C, C14215T and G4965C SNPs and VNTR structure were not available for all participants. Characteristics of the population and genotype frequencies are given in Table 1. All SNPs were in Hardy– Weinberg equilibrium (Table 1). Two pairs of SNPs, M287T and G4965C, C14215T and G12390C were in linkage disequilibrium with r^2>0.7 (Supplementary eTable 1).

The concentrations of MAs^{III} and $DMAs^{III}$ in urine were elevated in individuals with variant M287T (TC + CC) and G4965C (GC + CC) genotypes compared with the respective wild-type (TT and GG) carriers (Table 2). The effects of these genotypes on $DMAs^{III}$ were more pronounced than on MAs^{III}. In contrast, the levels of both MAs^{III} and $DMAs^{III}$ were reduced in individuals with the variant genotypes of G35991A, and the level of MAs^{III} was also reduced in individuals with variant VNTR A2B (Table 2). When diabetes markers were analyzed as continuous variables, carriers of the M287T (TC + CC) and G4965C (GC + CC) genotypes had elevated levels of 2HBG and FBG compared with individuals with the wild-type genotypes (Table 2). There was little indication of consistent association of any other genotype with diabetes (data not shown) or with 2HBG or FBG (Table 2). The VNTR–A2B structure was, however, associated with higher DMAs/MAs ratio (β 1.17, 95% CI 0.2–2.3). A3B structure was not found.

Individuals with the C allele in M287T or G4965C were also more likely to be classified as diabetic than the wild-type carriers: OR 2.70 (95% CI 0.93–7.80) and OR 2.24 (95% CI 0.69–7.25), respectively, although the associations did not reach the conventional level of statistical significance. Other polymorphisms were less strongly associated with diabetes and the ORs were more imprecise (Table 3). We repeated the analysis for G35991A using a co-dominant model with AG located midway between AA and GG. The resulting ORs were 0.88 (95% CI 0.53–1.44) for AG and 0.77 (95% CI 0.21–2.82) for GG. ORs based on classification of diabetes using only 2HBG ≥200 mg/dl or FBG ≥126 mg/dl in addition to self-reported diagnosis or medication use were similar in magnitude and precision (Supplementary eTables 2 and 3).

Analyses of the interaction of M287T genotype and exposure to iAs in drinking water (Figure 1) suggested increasing associations of diabetes with variant (TC + CC) genotype and exposure at >75th percentile of concentration (52 p.p.b.) separately (ORs 3.47 and 5.68, respectively) and in combination (OR 11.41). The pattern of separate and combined associations was similar for G4965C, with ORs of 1.97 and 4.60 for variant (GC + CC) genotype and exposure beyond the 75th percentile, respectively, and 8.75 for variant genotype and high exposure combined. The combined effect of high arsenic exposure and M287T (TC + CC) genotype was greater than additive, whereas for the combination of high

arsenic and G4965C (GC + CC) the effect was approximately multiplicative. For both polymorphisms, the separate effect of high exposure and the combined effect of high exposure and variant genotype were statistically significant, although the 95% CIs were wide as a result of small numbers (Figure 1).

DISCUSSION

We have recently reported that exposure to iAs in drinking water is associated with an increased risk of diabetes among Zimapan and Lagunera residents; we have also found positive correlation between $DMAs^{III}$ concentration in urine and the diabetes indicators.[17] Here, we provide evidence that individuals with the variant AS3MT/287T and 4965C genotypes have higher levels of $DMAs^{III}$ in urine. Our data show that these individuals also have higher levels of 2HBG and FBG, and suggest they are more likely to be classified as diabetic by common clinical criteria. Taken together, these findings suggest that carriers of the 287T and 4965C variant genotypes are more likely to develop diabetes when exposed to iAs, possibly due to enhanced production of its toxic metabolite, $DMAs^{III}$. Consistent with the results in this study our recent data indicate that *in vitro* methylation of iAs by recombinant AS3MT/287T produces significantly more $DMAs^{III}$ than methylation by wild-type AS3MT/287M.[26]

Strengths of this study include the analysis of unstable, but highly toxic, methylated trivalent metabolites of iAs in freshly collected urines and the use of multiple criteria to classify diabetes status. The setting in an area with moderately elevated levels of iAs in drinking water (mean concentration 43 p.p.b.) can also be considered a strength in that most previous studies have been conducted in areas with higher exposures, leaving open questions about the effects of iAs at lower concentrations that are more typical of population exposures throughout the world.

The cross-sectional design used in this study is commonplace in this area of research, but nevertheless limits interpretation in several respects. Prevalent cases of diabetes may differ from incident cases in clinical characteristics of the disease or in the metabolism of arsenic. In addition, there are indications that iAs exposures may have changed recently due to changes in water supply and consumption, but we did not have data to account for those changes.[17] Selective migration can also be a source of bias in cross-sectional studies and it is common for men in the areas we studied to emigrate for work. The study population was predominantly female as a result, but there is no reason to believe that men or women with particular genotypes or exposures were more likely to leave the area.

Interpretation of the study results is also limited by the small number of diabetes cases among people with variant genotypes. This resulted in wide confidence intervals for several associations, particularly those related to gene–environment interactions.

Our study builds on the previous evidence linking iAs exposure to diabetes and is the first to identify specific genotypes that can increase individual susceptibility to the diabetogenic effects associated with this exposure.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was funded by US EPA/STAR grant no. 832735 and by NIH grant no. 5R01 ES015326-02 to MS. Support was also provided by the UNC Nutrition Obesity Research Center funded by the National Institute of Diabetes and Digestive and Kidney Diseases (grant no. DK056350). We thank Erika Hernández-Castellanos and Olga L Valenzuela (Departamento de Toxicología, Cinvestav, Arturo Cebrián and Jaime Duarte (Facultad de Medicina, Universidad Juárez del Estado de Durango), and the staff of Jurisdicción Sanitaria 5 de Zimapán, Hidalgo, for their enthusiastic support and help with subject recruitment, interviews, medical exams and sample collection. We also thank Professor William R Cullen (University of British Columbia) who provided us with methylated trivalent arsenic standards and Dr. David J Thomas (US EPA) and Dr. John Buse for their suggestions and comments regarding the data analysis and interpretation.

REFERENCES

1. Navas-Acien A, Silbergeld EK, Streeter RA, Clark JM, Burke TA, Guallar E. Arsenic exposure and type 2 diabetes: a systematic review of the experimental and epidemiological evidence. Environ Health Perspect. 2006; 114:641–648. [PubMed: 16675414]
2. Thomas DJ, Li J, Waters SB, Xing W, Adair BM, Drobna Z, et al. Arsenic (+3 oxidation state) methyltransferase and methylation of arsenicals. Exp Biol Med. 2007; 232:3–13.
3. Engström K, Vahter M, Mlakar SJ, Concha G, Nermell B, Ragib R, et al. Polymorphisms in arsenic(+ III oxidation state) methyltransferase (AS3MT) predict gene expression of AS3MT as well as arsenic metabolism. Environ Health Perspect. 2011; 119:182–188. [PubMed: 21247820]
4. Chung CJ, Hsueh YM, Bai CH, Hunag YK, Huang YL, Yang MH, et al. Polymorphisms in arsenic metabolism genes, urinary arsenic methylation profile and cancer. Cancer Causes Control. 2009; 20:1653–1661. [PubMed: 19680750]
5. Agusa T, Iwata H, Fujihara J, Kunito T, Takeshita H, Minh TB, et al. Genetic polymorphisms in AS3MT and arsenic metabolism in residents of the Red River Delta, Vietnam. Toxicol Appl Pharmacol. 2009; 236:131–141. [PubMed: 19371612]
6. Fujihara J, Fujii Y, Agusa T, Kunito T, Yasuda T, Moritani T, et al. Ethnic differences in five intronic polymorphisms associated with arsenic metabolism within human arsenic (+ 3 oxidation state) methyltransferase (AS3MT) gene. Toxicol Appl Pharmacol. 2009; 234:41–46. [PubMed: 18976679]
7. Hernández A, Xamena N, Sekaran C, Tokunaga H, Sampayo-Reyes A, Quinteros D, et al. High arsenic metabolic efficiency in AS3MT287Thr allele carriers. Pharmacog Genom. 2008; 18:349–355.
8. Lindberg AL, Kumar R, Goessler W, Thirumaran R, Gurzau E, Koppova K, et al. Metabolism of low-dose inorganic arsenic in a Central European population: Influence of sex and genetic polymorphisms. Environ Health Perspect. 2007; 115:1081–1086. [PubMed: 17637926]
9. Sampayo-Reyes A, Hernandez A, El-Yamani N, Lopez-Campos C, Mayet-Machado E, Rincon-Castaneda CB, et al. Arsenic induces DNA damage in environmentally exposed Mexican children and adults. Influence of GSTO1 and AS3MT polymorphisms. Tox Sci. 2010; 117:63–71.
10. Valenzuela OL, Drobná Z, Hernández-Castellanos E, Sanchez-Pena LC, Garcia-Vargas GG, Borja-Aburto VH, et al. Association of AS3MT polymorphisms and the risk of premalignant arsenic skin lesions. Toxicol Appl Pharmacol. 2009; 239:200–207. [PubMed: 19538983]
11. Wood TC, Salavagionne OE, Mukherjee B, Wang L, Klumpp AF, Thomae BA, et al. Human arsenic methyltransferase (AS3MT) pharmacogenetics. Gene resequencing and functional genomics studies. J Biol Chem. 2006; 281:7364–7373. [PubMed: 16407288]
12. Engström KS, Broberg K, Concha G, Nermell B, Warholm M, Vahter M. Genetic polymorphisms influencing arsenic metabolism: Evidence from Argentina. Environ Health Perpect. 2007; 115:599–605.

13. Hernández A, Xamena N, Surrallés J, Sekaran C, Tokunaga H, Quinteros D, et al. Role of the Met(287)Thr polymorphism in the AS3MT gene on the metabolic arsenic profile. Mutat Res. 2008; 637:80–92. [PubMed: 17850829]
14. Hwang YH, Chen YH, Su YN, Hsu CC, Chen YH, Yuan TH. Genetic polymorphism of As3MT and delayed urinary DMA excretion after organic arsenic intake from oyster ingestion. J Environ Monit. 2010; 12:1247–1254. [PubMed: 20532380]
15. Gomez-Rubio P, Meza-Montenegro MM, Cantu-Soto E, Klimecki WT. Genetic association between intronic variants in AS3MT and arsenic methylation efficiency is focused on a large linkage disequilibrium cluster in chromosome 10. J Appl Toxicol. 2010; 30:260–670. [PubMed: 20014157]
16. Meza MM, Yu L, Rodriguez YY, Guild M, Thompson D, Gandolfi AJ, et al. Developmentally restricted genetic determinants of human arsenic metabolism: Association between urinary methylated arsenic and Cyt19 polymorphisms in children. Environ Health Perspect. 2005; 113:775–781. [PubMed: 15929903]
17. Del Razo LM, García-Vargas GG, Valenzuela OL, Castellanos EH, Sanchez-Pena LC, Currier JM, et al. Exposure to arsenic in drinking water is associated with increased prevalence of diabetes: a cross-sectional study in the Zimapán and Lagunera Regions in Mexico. Environ Health. 2011; 10:73. [PubMed: 21864395]
18. Le XC, Ma M. Short-column liquid chromatography with hydride generation atomic fluorescence detection for the speciation of arsenic. Anal Chem. 1998; 70:1926–1933. [PubMed: 9599587]
19. Devesa V, Del Razo LM, Adair B, Drobna Z, Waters SB, Hughes MF, et al. Comprehensive analysis of arsenic metabolites by pH-specific hydride generation atomic absorption spectrometry. J Anal At Spectrom. 2004; 19:1460–1467.
20. Hernández-Zavala A, Matoušek T, Drobná Z, Paul DS, Walton F, Adair BM, et al. Speciation of arsenic in biological matrices by automated hydride generation-cryotrapping-atomic absorption spectrometry with multiple microflame quartz tube atomizer (multiatomizer). J Anal At Spectrom. 2008; 23:342–351. [PubMed: 18677417]
21. Matoušek T, Hernández-Zavala A, Svoboda M, Langrova L, Adair BM, Drobna Z, et al. Oxidation state specific generation of arsines from methylated arsenicals based on L- cysteine treatment in buffered media for speciation analysis by hydride generation - automated cryotrapping - gas chromatography- atomic absorption spectrometry with the multiatomizer. Spetrochim Acta Part B. 2008; 63:396–406.
22. Drobná Z, Waters SB, Walton FS, LeCluyse EL, Thomas DJ, Styblo M. Interindividual variation in the metabolism of arsenic in cultured primary human hepatocytes. Toxicol Appl Pharmacol. 2004; 201:166–177. [PubMed: 15541756]
23. de Fine Olivarius N, Andreasen AH, Vestbo E, Holstein-Rathlou NH, Jorgensen LG, Mogensen CE. Urinary creatinine concentration is inversely related to glycaemic control and the presence of some diabetic complications in patients with newly diagnosed Type 2 diabetes. J Diabetes Complications. 2006; 20:45–50. [PubMed: 16389167]
24. Nermell B, Lindberg AL, Rahman M, Berglund M, Persson LA, El Arifeen S, et al. Urinary arsenic concentration adjustment factors and malnutrition. Environ Res. 2008; 106:212–218. [PubMed: 17900556]
25. Basu A, Mitra S, Chung J, Guha Mazumder DH, Ghosh N, Kalman D, et al. Creatinine, diet, micronutrients, and arsenic methylation in West Bengal, India. Environ Health Perspect. 2011; 119:1308–1313. [PubMed: 21652291]
26. Ding L, Saunders RJ, Drobna Z, Walton FS, Xun P, Thomas DJ, et al. Methylation of arsenic by recombinant human wild-type arsenic (+3 oxidation state) methyltransferase and its methionine 287 threonine (M287T) polymorh. Chem Res Toxicol. 2012; 264:121–130.

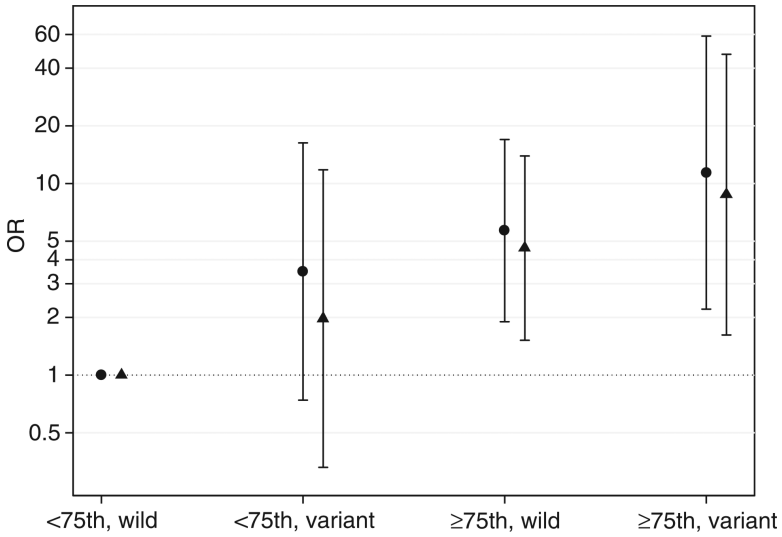

Figure 1.

Interaction of wild-type and variant genotypes of M287T (circle) and G4965C (triangle) polymorphisms and exposure to iAs in water categorized at the 75th percentile (52 p.p.b.).

Table 1

Population characteristics and genotype frequencies.

	N *(mean)*	% *(SD)*
Population	255	100
Female	171	67
Age (years)	(34)	(18)
iAs concentration in drinking water (p.p.b.)	(43)	(48)
FBG (mg/dl)	(92)	(39)
2HBG (mg/dl)	(105)	(52)
BMI>30kg/m2	86	34
Hypertensive	95	37
M287T		
rs11191439		
TT	223	87
CT	29	11
CC	3	1
G35991A		
rs10748835		
GG	76	30
GA	134	53
AA	45	18
G12390C		
rs3740393		
GG	128	55
GC	95	41
CC	11	5
C14215T		
rs3740390		
CC	125	53
CT	97	41
TT	15	6
G4965C		
rs17881215		
GG	199	88
GC	27	12
CC	1	0
VNTR		
AB	173	72
A2B	68	28

Table 2

Association of the trivalent methylated metabolites of iAs and diabetes markers with genetic polymorphism.

Genotype	*rs number*[c]	*In(MAs^{III})*		*In(DMAs^{III})*		*In(2HBG)*		*In(FBG)*	
		β[a]	*95% CI*	β[a]	*95% CI*	β[b]	*95% CI*	β[b]	*95% CI*
M287T	*rs11191439*								
	TT (referent)								
	TC/CC	0.11	(–0.22, 0.45)	0.47	(0.03, 0.91)	0.14	(0.01, 0.29)	0.19	(0.07, 0.31)
G35991A	*rs10748835*								
	AA/AG (referent)								
	GG	–0.16	(–0.41, 0.10)	–0.21	(–0.53, 0.12)	0.03	(–0.05, 0.13)	–0.01	(–0.10, 0.08)
VNTR	AB (referent)								
	A2B	–0.16	(–0.43, 0.10)	0.04	(–0.31, 0.40)	–0.01	(–0.11, 0.10)	–0.04	(–0.14, 0.06)
G12390C	*rs3740393*								
	GG (referent)								
	GC/CC	–0.11	(–0.31, 0.10)	–0.22	(–0.58, 0.15)	–0.09	(–0.18, –0.01)	–0.02	(–0.11, 0.06)
C14215T	*rs3740390*								
	CC (referent)								
	CT/TT	–0.09	(–0.30, 0.11)	–0.31	(–0.68, 0.05)	–0.08	(–0.16, 0.01)	–0.06	(–0.15, 0.02)
G4965C	*rs17881215*								
	GG (referent)								
	GC/CC	0.30	(–0.01, 0.61)	0.63	(0.07, 1.19)	0.19	(0.04, 0.33)	0.22	(0.09, 0.35)

Abbreviations: *β*, regression coefficient; CI, confidence interval.

[a] Adjusted for age and sex.

[b] Adjusted for age, sex, hypertension and obesity.

[c] rs numbers were cited from NCBI SNP data base (http://www.ncbi.nlm.nih.gov/projects/SNP/).

Table 3

Association of diabetes status classified by 2HBG ≥ 200mg/dl, FBG ≥ 126mg/dl or self-reported diagnosis or medication with AS3MT polymorphism.

Polymorphism	*Diabetic*	*Non-diabetic*	*OR* [a]	*95% CI*
M287T				
TT	17	206	1.00	
TC/CC	7	25	2.70	(0.93, 7.80)
G35991A				
AA/AG	17	162	1.00	
GG	7	69	0.92	(0.35, 2.42)
VNTR				
AB	17	157	1.00	
2AB	6	62	0.57	(0.20, 1.64)
G12390C				
GG	13	115	1.00	
GC/CC	8	98	0.82	(0.31, 2.17)
C14215T				
CC	14	111	1.00	
CT/TT	7	105	0.63	(0.23, 1.70)
G4965C				
GG	16	184	1.00	
GC/CC	6	22	2.24	(0.69, 7.25)

Abbreviations: CI, confidence interval; OR, odds ratio.

[a] Adjusted for age, sex, hypertension and obesity.