

## Genetic Polymorphisms Influencing Arsenic Metabolism in Human: Evidence from Vietnam

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**Abstract**—We report the association of levels of urinary arsenic (As) compounds (arsenite (As<sup>III</sup>), arsenate (As<sup>V</sup>), monomethylarsonic acid (MMA<sup>V</sup>), dimethylarsinic acid (DMA<sup>V</sup>), and arsenobetaine (AB)) with genetic polymorphisms of candidate genes, glutathione *S*-transferase omega 1 (*GSTO1*) and 2 (*GSTO2*), mu 1 (*GSTM1*), and theta 1 (*GSTT1*), arsenic (+3 oxidation state) methyltransferase (*AS3MT*), methylenetetrahydrofolate reductase (*MTHFR*), and DNA repair gene *XPB* in 100 subjects from Ha Nam Province in the Red River Delta, Vietnam. No significant relationship between As levels and genetic polymorphisms of *GSTT1* and *MTHFR* was observed. *GSTM1* null was found to be associated with increased percentage of urinary DMA<sup>V</sup>. High concentrations of As<sup>V</sup> in urine were observed in individuals with Asn142Asp genotype of *GSTO2*. Subjects with gene polymorphisms of Glu155del in *GSTO1* and Met287Thr in *AS3MT* had higher ratios of urinary MMA<sup>V</sup>/(As<sup>III</sup> + As<sup>V</sup>) indicating higher capacity for primary As methylation. We found that intronic polymorphisms of G12390C wild-type homozygosity and G35991A variant-type homozygosity in *AS3MT* were associated with lower DMA<sup>V</sup>/MMA<sup>V</sup> and DMA<sup>V</sup> percentages in urine, respectively. Percentages of As<sup>III</sup> in the urine of subjects with Lys751Gln genotype in *XPB* were higher than those with wild type. From our findings, it can be concluded that genetic polymorphisms in *GSTM1*, *GSTO1*, *GSTO2*, *AS3MT*, and *XPB* may be responsible for As metabolism and toxicity in Vietnamese.

Keywords: arsenic, genetic polymorphism, methylation capacity, Vietnam

### INTRODUCTION

It has been generally accepted that inorganic arsenic (As) is oxidatively methylated

(Challenger, 1945; Cullen and Reimer, 1989). In some recent reports, a reductive methylation pathway has also been proposed (Hayakawa *et al.*, 2005; Naranmandura *et al.*, 2006). In both pathways, glutathione-S-transferase omega (*GSTO*) and arsenic (+3 oxidation state) methyltransferase (*AS3MT*) participate in the reduction of pentavalent arsenicals to trivalent forms (Zakharyan *et al.*, 2005) and in the methylation of As species (Lin *et al.*, 2002; Wood *et al.*, 2006), respectively.

There are wide variations in the susceptibility of As toxicity among individuals and population, which is probably related to genetic polymorphisms in metabolism of As (Meza *et al.*, 2005; Schmuck *et al.*, 2005; Wood *et al.*, 2006; Schläwicke *et al.*, 2007; Lindberg *et al.*, 2007; Steinmaus *et al.*, 2007). A few studies have indicated that two other GSTs, *GST mu 1* (*GSTM1*) and *GST theta 1* (*GSTT1*), have little effect on As metabolism (Chiou *et al.*, 1997; Schläwicke *et al.*, 2007; Steinmaus *et al.*, 2007). Polymorphisms in other genes such as methylenetetrahydrofolate reductase (*MTHFR*) that reduces methylenetetrahydrofolate to methyltetrahydrofolate regenerating methionine from homocysteine in the one-carbon metabolism may indirectly influence As methylation. This genetic polymorphism is associated with higher percentage of monomethylated As in urine (Lindberg *et al.*, 2007). Ahsan *et al.* (2003) reported that DNA repair gene *XPD* influences the extent of hyperkeratosis caused by As exposure.

Recently, we investigated the status of human exposure to As in Vietnam (Agusa *et al.*, 2004, 2005, 2006, 2007). However, there is no available report on the association of gene polymorphism with As metabolism in Vietnamese. In the present study, we evaluated the influence of genetic factors on As metabolism in Vietnamese.

## MATERIALS AND METHODS

Groundwater ( $n = 28$ ) and human urine ( $n = 100$ ) and blood ( $n = 100$ ) were collected from rural areas of Hoa Hau (HH) Commune and Liem Thuan (LT) Commune in Ha Nam Province located in the Red River Delta, Vietnam in March, 2006. We obtained informed consent from all the subjects and collected the samples in an ethical manner. This study was approved by the Ethical Committee in Ehime University. All samples were preserved in the *es*-BANK (Tanabe, 2006), Center for Marine Environmental Studies (CMES), Ehime University at  $-25^{\circ}\text{C}$  until analyses of As and genotyping.

Groundwater samples were acidified with nitric acid. Concentration of total As in groundwater was measured with an inductively coupled plasma-mass spectrometry (ICP-MS). Urine was filtered with syringe filter (0.20  $\mu\text{m}$  mixed cellulose ether) and then diluted by Milli-Q water. Five arsenicals (dimethylarsinic acid ( $\text{DMA}^{\text{V}}$ ), monomethylarsonic acid ( $\text{MMA}^{\text{V}}$ ), arsenite ( $\text{As}^{\text{III}}$ ), arsenate ( $\text{As}^{\text{V}}$ ), and arsenobetaine (AB)) in urine were determined with a high performance liquid chromatograph connected with ICP-MS using an anion exchange column (Mandal *et al.*, 2001; Agusa *et al.*, 2005). Sum of all As compounds and  $\text{As}^{\text{III}} + \text{As}^{\text{V}}$  detected by this method are represented as SAs and IAs, respectively. Urinary

creatinine level was measured by SRL Inc. (Tokyo, Japan). Concentrations of As compounds in urine are expressed on creatinine basis.

Genomic DNA was extracted from blood collected from all the subjects. Using a polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) method, genotyping of polymorphisms in *GSTO1* Asn140Asp, *GSTO2* Asn142Asp, *AS3MT* Met287Thr, C12390G (intron), T14215C (intron), and A35991G (intron), *MTHFR* Ala222Val, and *XPD* Lys751Gln was performed (Fujihara *et al.*, 2007b). Genetic polymorphisms in *GSTT1* and *M1* (wild or null) were identified by allele specific multiplex PCR. The Glu155del in *GSTO1* was detected using confronting two-pair primers analysis (CTPP) (Fujihara *et al.*, 2007a).

All statistical analyses were performed with StatView (version 5.0, SAS® Institute, Cary, NC, USA) and SPSS (version 12, SPSS, Chicago, IL, USA). One half of the value of the respective limits of detection were substituted for those values below the limit of detection and used in statistical analysis. All data were tested for goodness of fit to a normal distribution with Kolmogorov-Smirnov's one sample test. Outlier (As concentration of 2,120  $\mu\text{g/l}$  in groundwater) was checked by Thompson test. Student's *t*-test was used to determine the differences in As levels in water and human urine. Differences in urinary As profiles depending on genetic polymorphisms were checked by Tukey-Kramer method, along with one-factor ANOVA. A *p* value of less than 0.05 was considered to indicate statistical significance.

## RESULTS AND DISCUSSION

Arsenic was detected in all groundwater samples and the levels were 0.7–2,120  $\mu\text{g/l}$ . One water sample which showed the highest concentration of As (2,120  $\mu\text{g/l}$ ) was considered as an outlier ( $p < 0.05$ ) and was removed from further statistical analysis. Without this outlier, the range of As concentrations in groundwater was 0.7–502  $\mu\text{g/l}$ . Arsenic concentrations in groundwater from HH (geometric mean (GM), 368  $\mu\text{g/l}$ ) were significantly higher than those from LT (GM, 1.4  $\mu\text{g/l}$ ) ( $p < 0.001$ ). Furthermore, all samples from HH contained As concentrations exceeding WHO drinking water guideline of 10  $\mu\text{g/l}$  (WHO, 2004). These results suggest widespread contamination of groundwater by As in HH and, thus, a greater risk of the human health by As exposure.

On the contrary to the results of groundwater analysis, there was no significant difference in urinary SAs concentrations between HH (GM, 93  $\mu\text{g/g}$  creatinine) and LT (GM, 98  $\mu\text{g/g}$  creatinine). Human urinary As compositions in HH were also similar to those in LT, with DMA<sup>V</sup> being the dominant species. Arsenobetaine, which may be derived from consumption of seafood was also detected in the urine. No significant correlation between total As concentrations in groundwater and As species concentrations in human urine was observed. This indicates that residents of HH are not exposed to As through drinking groundwater.

To understand the association of As metabolism with gene polymorphisms, relationships between urinary As concentrations and profile in each genotype were statistically analyzed (Fig. 1). There is no significant relationship between

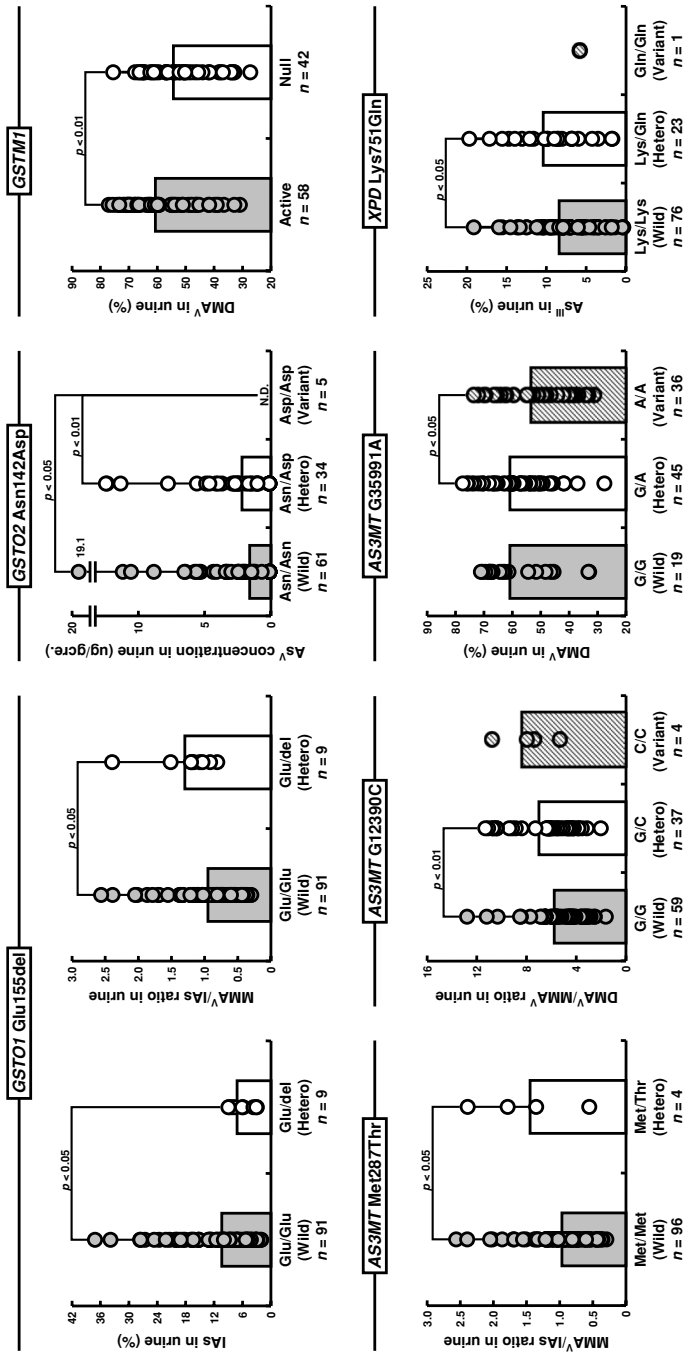


Fig. 1. Concentrations and composition of arsenicals in urine in the genotypes *GSTO1* Glu155del, *GSTO2* Asn142Asp, *GSTM1*, *AS3MT* Met287Thr, *AS3MT* G12390C, *AS3MT* G3591A, and *XPD* Lys751Gln in residents from Hoa Hau and Liem Thuan in the Red River Delta, Vietnam. Bars indicate geometric mean values of concentrations and mean values of composition, while plots show individual values.

As and genetic polymorphisms of *GSTT1* (active or null) and *MTHFR* (Ala222Val). *GSTM1* null was found to be associated with percentage of urinary DMA<sup>V</sup>. Higher inorganic As and MMA<sup>V</sup> percentages in urine were observed from the null type in Taiwanese (Chiou *et al.*, 1997) and Argentines (Schl awicke *et al.*, 2007; Stelmaus *et al.*, 2007), respectively, and our result was inconsistent with data from these previous studies. Although an *in vitro* study reported no difference in reductive activity between wild and hetero types of *GSTO2* Asn142Asp (Schmuck *et al.*, 2005), higher concentrations of As<sup>V</sup> in urine were observed in individuals with the hetero genotype. Subjects with gene polymorphisms of Glu155del in *GSTO1* and Met287Thr in *AS3MT* had higher ratios of urinary MMA<sup>V</sup>/IAs, indicating that they have higher capacity for primary As methylation. Wood *et al.* (2006) have reported higher activities of *AS3MT* Met287Thr in the hetero type. We found that intronic polymorphisms of G12390C wild-type homozygosity and G35991A variant-type homozygosity in *AS3MT* were associated with lower DMA<sup>V</sup>/MMA<sup>V</sup> ratio and DMA<sup>V</sup> percentage in urine, respectively. These results for Vietnamese were different from those for Argentines whose urinary DMA<sup>V</sup>/MMA<sup>V</sup> ratio and DMA<sup>V</sup> percentage in the hetero and variant types were higher than those in the wild type (Schl awicke *et al.*, 2007). Percentages of As<sup>III</sup> in the urine of subjects with Lys751Gln genotype in *XPD* were higher than those in wild type. In conclusion, these findings indicate that genetic polymorphisms in *GSTM1*, *GSTO1*, *GSTO2*, *AS3MT*, and *XPD* may be responsible for As metabolism and toxicity in Vietnamese.

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