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Interindividual Variation in Arsenic Metabolism in a Vietnamese Population: Association with 17 Single Nucleotide Polymorphisms in *AS3MT*

Tetsuro AGUSA[1,2], Hisato IWATA[1], Junko FUJIHARA[2], Takashi KUNITO[3], Haruo TAKESHITA[2], Tu Binh MINH[1,4], Pham Thi Kim TRANG[4], Pham Hung VIET[4] and Shinsuke TANABE[1]

[1]*Center for Marine Environmental Studies (CMES), Ehime University, Bunkyo-cho 2-5, Matsuyama 790-8577, Japan*
[2]*Department of Legal Medicine, Shimane University Faculty of Medicine, Enya 89-1, Izumo 693-8501, Japan*
[3]*Department of Environmental Sciences, Faculty of Science, Shinshu University, 3-1-1 Asahi, Matsumoto 390-8621, Japan*
[4]*Center for Environmental Technology and Sustainable Development (CETASD), Hanoi University of Science, Vietnam National University, T3 Building, 334 Nguyen Trai Street, Thanh Xuan District, Hanoi, Vietnam*

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Abstract—To elucidate the role of genetic factors in arsenic metabolism, we investigated associations of 17 single nucleotide polymorphisms (SNPs) in arsenic (+3 oxidation state) methyltransferase (*AS3MT*) with urinary arsenic profile in a Vietnamese population. The homozygotes for SNPs 4602GG, 35991AA, and 37853AA, which showed strong linkage disequilibrium (LD), had lower percentage (%) of dimethylarsinic acid (DMA) in the urine. SNPs 4740 and 12590 had strong LD and were associated with urinary %DMA. Although SNPs 3963, 6144, 12390, 14215, 35587, and 37950 comprised LD cluster, only homozygotes in SNPs 12390GG and 35587TT exhibited lower ratios of DMA/monomethylarsonic acid (MMA) in the urine, implying that these SNPs might be related to the low methylation capacity from MMA to DMA. The LD group including SNPs 5913, 9749, and 27215 was correlated with %MMA. SNP 8979AA showed low %DMA compared with other genotypes. The heterozygote for SNP 14458TC had a higher MMA/inorganic arsenic (IA) ratio in the urine than the TT homozygote, indicating that the heterozygote may have the stronger methylation ability of IA. The present study suggests that these SNPs are associated with arsenic metabolism in this Vietnamese population.

Keywords: arsenic, genetic polymorphism, methylation capacity, Vietnam

INTRODUCTION

It is known that inorganic arsenic is one of chemicals with high carcinogenicity.

Large variations in the susceptibility to inorganic arsenic toxicity among individuals and populations have been shown, relating to genetic factors in the metabolism of inorganic arsenic (Vahter, 2002). It has been accepted in general that inorganic arsenic is oxidatively methylated in the human body (Challenger, 1945; Cullen and Reimer, 1989). On the other hand, reductive methylation of inorganic arsenic has recently been proposed (Hayakawa *et al.*, 2005; Naranmandura *et al.*, 2006). In either case, arsenic (+3 oxidation state) methyltransferase (AS3MT) catalyzes the methylation of trivalent arsenicals (Lin *et al.*, 2002; Wood *et al.*, 2006). Because the toxicity of organic arsenic is generally lower than that of inorganic forms and the methylated arsenic is readily excreted into the urine, methylation of inorganic arsenic by AS3MT is considered to be an important process to account for sensitivity to the toxicity of inorganic arsenic.

Wood *et al.* (2006) have reported that there are some single nucleotide polymorphisms (SNPs) in human *AS3MT* and several SNPs in exon regions of *AS3MT* are associated with the *in vitro* catalytic activities and the protein expression levels. Other studies have suggested that some of the SNPs including introns might be linked with arsenic metabolism in the Central European (Lindberg *et al.*, 2007), Chilean (Hernandez *et al.*, 2008), Mexican (Meza *et al.*, 2005), and Argentina (Schläwicke Engström *et al.*, 2007) populations. On the contrary, data on distribution of *AS3MT* polymorphisms and their relation to arsenic methylation ability are limited, especially in Asian populations.

Recently, we reported association of 13 SNPs in *AS3MT* with arsenic methylation in a population from northern Vietnam (Agusa *et al.*, 2009). In the present study, we additionally analyzed more SNPs in *AS3MT* and evaluated the effects of genetic factors on arsenic metabolism in this population.

MATERIALS AND METHODS

Human urine (n = 100) and blood (n = 100) were collected from Hoa Hau (HH) and Liem Thuan (LT) Communes in Ha Nam Province located in the Red River Delta, Vietnam in March, 2006. Informed consent was obtained from all the subjects and this study was approved by the Ethical Committee in Ehime University. More detailed information on donors was presented in our previous study (Agusa *et al.*, 2009). All samples were preserved in the *es*-BANK (Tanabe, 2006), Center for Marine Environmental Studies (CMES), Ehime University at –25°C until chemical analyses and genotyping.

Filtered (0.20 μm mixed cellulose ether) urine sample was diluted by Milli-Q water. Five arsenicals (dimethylarsinic acid (DMA^V), monomethylarsonic acid (MMA^V), arsenite (As^{III}), arsenate (As^V), and arsenobetaine (AB)) in the urine samples were determined with a high performance liquid chromatograph connected with an inductively coupled plasma-mass spectrometry using an anion exchange column (Agusa *et al.*, 2009). Sum of As^{III} and As^V detected by this method is represented as IA. Urinary creatinine was measured by SRL Inc. (Tokyo, Japan). Concentrations of arsenic compounds in the urine are expressed on creatinine basis.

Genomic DNA was extracted from blood samples by QIAamp DNA mini kit (Qiagen, Hilden, Germany). 17 SNPs (3963T > C (T to C substitution at nucleotide base 3963) (rs7098825), 4602A > G (rs7085104), 4740T > C (rs12416687), 5913T > C (rs4917986), 6144A > T (rs17878846), 7395G > A (rs12767543), 8979T > A (rs7904113), 9749A > G (rs17881367), 12390G > C (rs3740393), 12590T > C (rs3740392), 14215C > T (rs3740390), 14458T > C (rs11191439), 27215A > G (rs11191446), 35587T > C (rs11191453), 35991G > A (rs10748835), 37853G > A (rs11191459), and 37950C > T (rs17879819)) in *AS3MT* were genotyped using a polymerase chain reaction and restriction fragment length polymorphism method (Fujihara *et al*., 2007, 2009).

All statistical analyses were performed with StatView (version 5.0, SAS® Institute, Cary, NC, USA) and PASW Statistics (version 18, SPSS Inc., an IBM Company Headquarters, Chicago, IL, USA). Linkage disequilibrium of SNPs in *AS3MT* was assessed by Haploview (version 4.0, Day Lab at the Broad Institute Cambridge, MA, 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. Differences in urinary arsenic profiles depending on genetic polymorphisms were checked by Tukey–Kramer method, along with one-factor ANOVA. The p value of less than 0.05 was considered to indicate statistical significance.

RESULTS AND DISCUSSION

Numbers of subjects for each genotype of *AS3MT* are shown in Table 1. SNPs 5913CC, 9749GG, and 27215GG were found only in one donor and there was no SNP 14458CC in this population. All the genotypes except for SNPs 7395 and 35587 followed the Hardy–Weinberg Principle.

Distinct linkage disequilibrium (LD) ($R^2 > 0.50$) was found for SNPs in *AS3MT*; 3963–4740 ($R^2 = 0.64$), 3963–12390 ($R^2 = 0.70$), 3963–14215 ($R^2 = 0.71$), 3963–35587 ($R^2 = 0.57$), 4602–35991 ($R^2 = 0.71$), 4602–37853 ($R^2 = 0.53$), 4740–12590 ($R^2 = 0.61$), 5913–27215 ($R^2 = 0.93$), 6144–12390 ($R^2 = 0.82$), 6144–14215 ($R^2 = 0.72$), 6144–35587 ($R^2 = 0.62$), 9749–27215 ($R^2 = 0.53$), 12390–14215 ($R^2 = 0.84$), 12390–35587 ($R^2 = 0.74$), 12390–37950 ($R^2 = 0.54$), 14215–35587 ($R^2 = 0.78$), 14215–37950 ($R^2 = 0.52$), and 35991–37853 ($R^2 = 0.55$). Among these LDs, we could categorize four LD clusters; SNPs 3963, 6144, 12390, 14215, 35587, and 37950 as cluster 1, 4602, 35991, and 37853 as cluster 2, 4740 and 12590 as cluster 3, and 5913, 9749, and 27215 as cluster 4.

Statistical results on the relationships between the urinary arsenic profile and 17 SNPs in *AS3MT* are shown in Table 1. There was no significant association of SNPs 3963, 6144, 7395, 14215, and 37950 with arsenic metabolism. Although SNPs 4602, 4640, 8979, 9749, and 37853 were related with urinary %AB, which is unlikely to contribute to the arsenic methylation, these results might partially be due to the relationships between these SNPs and %DMA as shown by a strong negative correlation between %DMA and %AB ($r = -0.865$, $p < 0.001$). Although SNPs 3963, 6144, 12390, 14215, 35587, and 37950 comprised LD cluster (cluster

Table 1. Composition of As compounds and concentration ratios of DMA/MMA and MMA/IA (arithmetic mean) in urine for SNPs in *AS3MT*.

SNP ID*	*n*	%AB	%DMA	%MMA	%IA	DMA/MMA	MMA/IA
3963							
TT	61	22.1	56.2	10.5	11.2	5.9	1.1
TC	34	20.3	58.4	10.2	11.1	6.3	1.0
CC	5	16.7	65.2	8.8	9.3	8.0	1.0
4602							
AA	22	16.2 y	61 x	10.8	11.9	5.9	1.0
AG	40	19.6 xy	59.8 x	10.0	10.6	6.5	1.1
GG	38	25.8 x	52.7 y	10.3	11.2	5.9	1.0
4740							
TT	52	17.1 y	61.2 y	10.3	11.5	6.5	1.0
TC	40	24.9 x	54.9 x	10.1	10.1	6.0	1.2
CC	8	29.4 x	45.6 x	11.3	13.7	4.6	0.9
5913							
TT	85	22.1	57.0	9.9 y	11.0	6.4	1.0
TC	14	15.1	60.6	12.7 x	11.6	5.1	1.3
CC	1	31.2	43.6	13.7	11.5	3.2	1.2
6144							
AA	57	20.3	57.6	10.7	11.4	5.8	1.1
TA	36	21.4	58.1	9.6	10.8	6.7	1.0
TT	7	27.5	52.5	10.0	9.9	6.1	1.0
7395							
GG	38	20.4	57.4	10.8	11.3	5.7	1.1
GA	57	20.8	58.2	10.0	11.0	6.5	1.0
AA	5	31.9	48.0	10.2	10.0	5.1	1.1
8979							
TT	36	18.9 y	59.7 x	10.3	11.0	6.3	1.1
TA	46	19.0 y	58.6 x	10.9	11.5	5.9	1.1
AA	18	31.4 x	49.6 y	8.7	10.3	6.5	0.9
9749							
AA	84	22.5 x	56.7	9.9 y	11.0	6.3	1.0
AG	15	13.4 y	62.5	12.3 x	11.8	5.7	1.2
GG	1	31.2	43.6	13.7	11.5	3.2	1.2
12390							
GG	59	20.5	56.9	11.0 x	11.6	5.6 y	1.1
GC	37	21.8	58.2	9.5 xy	10.6	6.8 x	1.0
CC	4	25.6	58.5	7.1 y	8.9	8.2 xy	0.8
12590							
TT	43	18.2	60.9 x	9.8	11.1	6.7 x	1.0
TC	42	22.5	56.8 xy	10.3	10.4	6.2 xy	1.1
CC	15	26.3	48.9 y	11.7	13.0	4.6 y	1.0

Table 1. (continued).

SNP ID*	*n*	%AB	%DMA	%MMA	%IA	DMA/MMA	MMA/IA
14215							
CC	57	20.9	57.0	10.8	11.3	5.7	1.1
CT	37	21.3	57.8	9.8	11.1	6.6	1.0
TT	6	23.3	59.2	8.3	9.2	7.4	0.9
14458							
TT	96	21.1	57.5	10.2	11.1	6.2	1.0 y
TC	4	23.0	54.7	11.9	10.4	4.8	1.6 x
27215							
AA	86	22.0	57.2	9.9 y	11.0	6.4 x	1.0 y
AG	13	14.9	60.1	12.9 x	12.1	4.9 y	1.3 x
GG	1	31.2	43.6	13.7	11.5	3.2	1.2
35587							
TT	59	21.0	56.6	10.9	11.5	5.6 y	1.1
TC	28	18.4	60.1	9.9	11.5	6.9 x	0.9
CC	13	28.1	55.1	8.3	8.5	6.9 xy	1.0
35991							
GG	19	17.2	60.2 xy	10.9	11.7	5.8	1.0
GA	45	19.6	60.0 x	10.2	10.3	6.4	1.1
AA	36	25.3	52.7 y	10.2	11.8	6.0	0.9
37853							
GG	26	17.5 y	60.5 x	11.0 x	11.1	5.7	1.2
GA	49	18.2 y	59.6 x	10.7 xy	11.6	6.2	1.0
AA	25	31.0 x	50.0 y	8.8 y	10.2	6.5	0.9
37950							
CC	64	19.7	57.5	10.9	11.9	5.9	1.0
CT	32	23.5	57.0	9.6	9.9	6.4	1.1
TT	4	25.6	58.5	7.1	8.9	8.2	0.8

IA; sum of As^{III} + As^{V}.
Values with same letters are not significantly different at $p < 0.05$.
*SNP ID indicates the SNP identification number relative to the location in the consensus sequence, with the first base of the consensus numbered 1.

1), only homozygotes in SNPs 12390GG and 35587TT exhibited lower ratios of DMA/MMA in the urine ($p < 0.05$), implying that these SNPs might be related to the low methylation capacity from MMA to DMA. Lower DMA/MMA ratios in the urine from people with 12390GG were similarly reported in Mexican children (Meza *et al.*, 2005) and Argentina women (Schläwicke Engström *et al.*, 2007). Homozygotes for SNPs 4602GG, 35991AA, and 37853AA, which showed strong LD (cluster 2), had significantly lower %DMA in the urine compared with other

genotypes in each corresponding SNP ($p < 0.05$). On the contrary, SNP 35991GG had lower %DMA in Argentina women (Schläwicke Engström *et al.*, 2007). Also, there was no relationship between SNP 4602 and urinary arsenic in Mexicans (Meza *et al.*, 2005). For SNP 37853, %MMA in AA homozygote was lower than that in GG type ($p < 0.05$). SNPs 4740 and 12590 had strong LD (cluster 3) and were associated with urinary %DMA ($p < 0.05$). On the other hand, SNP 4740 in Mexicans had no significant correlation with urinary arsenic speciation (Meza *et al.*, 2005). For SNP 12590, the TT type had a higher DMA/MMA ratio in the urine than other genotypes ($p < 0.05$), suggesting the prompted 2nd methylation capacity in this carrier. The LD group including SNPs 5913, 9749, and 27215 (cluster 4) was correlated with %MMA ($p < 0.05$). Among this cluster, SNP 27215AA had higher DMA/MMA and lower MMA/IA ratios than the SNP27215AG. SNP 8979AA showed low %DMA compared with other genotypes ($p < 0.05$). The heterozygote for SNP 14458TC had a higher MMA/IA ratio in the urine than the TT homozygote ($p < 0.05$). This SNP lead to the amino acid substitution from Met to Thr at the position of 287th amino acid. Wood *et al.* (2006) have reported higher activities of *AS3MT* Met287Thr in the hetero type. Hence, our result indicates that the heterozygote may have the stronger methylation ability of IA.

In summary, we found that 12 SNPs (4602A > G, 4740T > C, 5913T > C, 8979T > A, 9749A > G, 12390G > C, 12590T > C, 14458T > C, 27215A > G, 35587T > C, 35991G > A, and 37853G > A) in *AS3MT* may affect arsenic metabolism in a Vietnamese population. Significant relationships between SNPs 5913, 8979, 9749, 27215, and 37853, and the urinary arsenic profile of this population were in particular observed for the first time. The reason why non-exonic SNPs in *AS3MT* are associated with arsenic metabolism remains unknown. Further study is required to understand the relationship between these SNPs and the expression pattern of *AS3MT*.

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H. Iwata (e-mail: iwatah@agr.ehime-u.ac.jp)