

# Arsenic Methylation Capability, Myeloperoxidase and Sulfotransferase Genetic Polymorphisms, and the Stage and Grade of Urothelial Carcinoma

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## Key Words

Arsenic exposure · Bladder cancer · Urinary arsenic species · Arsenic methylation · Myeloperoxidase · Sulfotransferase

## Abstract

Arsenic exposure is associated with an increased risk of bladder cancer. To explore the distribution of the arsenic methylation capability and myeloperoxidase (*MPO*) and sulfotransferase (*SULT*) *1A1* genotypes in patients at different stages and grades of urothelial carcinoma (UC), 112 UC cases were recruited between September 2002 and May 2004 for this study. Urinary arsenic species, including inorganic arsenic ( $As^{III} + As^V$ ), monomethylarsonic acid, and dimethylarsinic acid, were determined with a high-performance liquid chromatography-linked hydride generator and atomic absorption spectrometry. The *MPO* and *SULT1A1* genotypes were examined with polymerase chain reaction and restriction fragment length polymorphism. Differential effects of the arsenic methylation capability were found among pa-

tients with different stages of UC; however, urinary arsenic concentrations were borderline significantly increased with the progress of UC patients regardless of whether or not they had been exposed to arsenic from drinking water. The *MPO* and *SULT* genetic polymorphisms might modify the arsenic methylation profile and UC progression, and thus are worthy of further investigation.

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## Introduction

Urothelial carcinoma (UC) arises exclusively from the urothelium including the renal pelvis, ureter, bladder, and urethra, with bladder cancer being the most common form. In most developed countries, it is among the top 10 leading cancers. In Taiwan, bladder UC was ranked as the 7th and 10th most common cancers for males and females, respectively, in 2000. The incidence rates of bladder UC have been progressively increasing in the past decades in Taiwan, with the age-specific rates for males and

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females in 2000 of 10.2 and 4.4 per 100,000, respectively [1]. These tumors exhibit a high frequency of recurrence, and some of them may progress to muscle-invasive and metastatic tumors and thus pose a serious threat to patient survival [2]. The pathological staging and grading system are currently the most significant factors for determining therapeutic interventions and clinical outcomes. In Taiwan, most cases of bladder cancer are transitional cell carcinoma, and epidemiological studies indicated that the incidence of this type of cancer is unusually high on the southwestern coast of Taiwan, and it was related to arsenic-contaminated artesian well water [3]. A study showed that chlorinated water supply was the main water source of patients affected by Ta-T1 transitional cell carcinoma of the bladder [4], and another study reported alcohol drinking interacted with N-acetyltransferase 2 genotype-induced bladder cancer [5]. However, the mechanism of bladder cancer is still unclear.

In drinking water, arsenic is usually found in the form of arsenate ( $\text{As}^{\text{V}}$ ) or arsenite ( $\text{As}^{\text{III}}$ ) [6]. Inorganic arsenic is biotransformed in humans to monomethylarsonic acid ( $\text{MMA}^{\text{V}}$ ) and dimethylarsinic acid ( $\text{DMA}^{\text{V}}$ ), and methylation of inorganic arsenic is considered a detoxification mechanism because DMA has a relatively low toxicity and is rapidly excreted in urine [7]. Our previous studies evaluating the association of the inorganic arsenic methylation capability were related to skin cancer [8] and bladder cancer risk [9]. Furthermore, an individual's capability of arsenic methylation might interact with cigarette smoking and modify the risk of bladder cancer [10]. The allowed arsenic concentration in public water supplies in Taiwan was 50  $\mu\text{g}/\text{l}$  until 2000, when a new standard of 10  $\mu\text{g}/\text{l}$  was announced. According to the Taipei Water Department of the Taipei City Government, the average arsenic concentration of tap water in Taipei is 0.7  $\mu\text{g}/\text{l}$ , ranging from the undetectable to 4.0  $\mu\text{g}/\text{l}$ . However, if it was shown that the arsenic metabolic capability affects cancer risks in subjects exposed to low levels (50  $\mu\text{g}/\text{l}$ ) of arsenic, would such low levels still be carcinogenic for some genetically predisposed individuals? Whether or not the arsenic methylation capability influences the prognosis of UC requires further investigation.

Arsenic is metabolized through reduction and oxidation processes after chronic exposure which is believed to produce reactive oxygen species (ROS) including superoxide anions, hydroxyl radicals, and hydrogen peroxide [11]. In vivo oxidative stress might be modulated by the enzyme, myeloperoxidase (MPO). MPO is a phase I metabolic enzyme located in neutrophils and monocytes which produces the strong oxidant, hypochlorous acid,

for microbicidal activity [12]. MPO also activates procarcinogens in tobacco smoke, such as benzo[a]pyrene through the release of ROS [13]. The -463(G  $\rightarrow$  A) transition variant of the *MPO* gene located in the chromosome 17q23.1 region has been associated with a lower cancer risk [14]. The *MPO* -463AA/AG genotype is associated with reduced MPO activity and DNA adduct levels in bronchoalveolar lavage fluid [15], while the *MPO* G-463A homozygous variant was associated with a reduced risk of bladder cancer in smokers [16]. However, determining whether or not the UC prognosis is susceptible to *MPO* genetic polymorphism requires closer examination.

Sulfotransferases (SULTs) are important enzymes in sulfation that can modulate the toxicity of carcinogenic xenobiotics. *SULT1A1* (Arg213His) polymorphism influences SULT enzyme activity, and *SULT1A1* (213His) can reduce SULT enzyme activity [17]. Another study found that the *SULT1A1* (213Arg/Arg) genotype presented a higher risk for highly differentiated tumors among heavy smokers [18]. The *SULT1A1* (213His) allele was associated with statistically significantly increased risks of esophageal cancer in Taiwan [19]. For the moment, it is important to note the association between UC prognosis and *SULT1A1* genetic susceptibility.

## Materials and Methods

### *Study Subjects and Questionnaire Interview*

One hundred and twelve patients with pathologically proven UC (age range, 24–93 years, average age 65.97, SD 10.21) were recruited from the Department of Urology, Chi-Mei Medical Center, Tainan, between September 2002 and May 2004. Almost all UC patients came from Tainan City or places near the arsenic-contaminated areas of southwestern Taiwan. A tap water supply system was implemented in the arsenic-contaminated areas of southwestern Taiwan in the early 1960s, but its coverage remained low until the early 1970s. Artesian well water was no longer used for drinking and cooking after the mid-1970s. Bladder cancer was staged into three groups: non-muscle invasive (Ta, T1, and Tis), locally advanced (T2–4N0M0), and metastatic (N+ or M+) [20]. These stages were determined by pathological detection in the radical cystectomy specimen and image studies including CT scan and bone scan. The T4 and T3b were determined by image studies, but the difference between T2 and T3a was measured by pathological result from the radical cystectomy specimen. Tumor grading was based on the WHO 1999 classification system [21].

Well-trained personnel carried out standardized personal interviews based on a structured questionnaire. Information collected included demographic and socioeconomic characteristics, general potential risk factors for malignancies such as lifestyle, alcohol consumption, cigarette smoking in quantified details, exposure to potential occupational and environmental carcinogens

such as hair dyes and pesticides, chronic medication history, consumption of conventional and alternative medicines, and personal and family histories of urological diseases. The Research Ethics Committee of Taipei Medical University, Taipei, Taiwan, approved the study. All patients provided informed consent forms before sample and data collection. The study was consistent with the World Medical Association Declaration of Helsinki. Study subjects were administered the questionnaire interview; urine and blood samples were then collected on site and urine samples were stored at  $-20^{\circ}\text{C}$ , while blood samples were separated into plasma and buffy coat fractions and then stored at  $-80^{\circ}\text{C}$  until further use for urinary arsenic speciation, and the gene polymorphism assay, respectively.

#### Determination of Urinary Arsenic Species

It has been shown that urinary arsenic species are stable for at least 6 months when preserved at  $-20^{\circ}\text{C}$  [22]; thus, the urine assay was performed within 6 months after collection. Frozen urine samples were thawed at room temperature, dispersed by ultrasonic waves, filtered through a Sep-Pak C18 column (Mallinckrodt Baker, Phillipsburg, N.J., USA) and levels of  $\text{As}^{\text{III}}$ ,  $\text{As}^{\text{V}}$ ,  $\text{MMA}^{\text{V}}$ , and  $\text{DMA}^{\text{V}}$  were determined. A urine aliquot of 200  $\mu\text{l}$  was used for the determination of arsenic species by high-performance liquid chromatography (Waters 501, Waters Associates, Milford, Mass., USA) with columns obtained from Phenomenex (Nucleosil, Torrance, Calif., USA). Contents of inorganic arsenic and its metabolites were quantified by hydride generator-atomic absorption spectrometry [23]. Recovery rates for  $\text{As}^{\text{III}}$ ,  $\text{DMA}^{\text{V}}$ ,  $\text{MMA}^{\text{V}}$ , and  $\text{As}^{\text{V}}$  ranged between 93.8 and 102.2% with respective detection limits of 0.02, 0.06, 0.07, and 0.10  $\mu\text{g/l}$ . The total urinary arsenic concentration was normalized against urinary creatinine levels ( $\mu\text{g/g}$  creatinine). The standard reference material, SRM 2670, contains  $480 \pm 100 \mu\text{g/l}$  inorganic arsenic and was obtained from the National Institute of Standards and Technology (NIST, Gaithersburg, Md., USA). SRM 2670 was used as a quality standard and analyzed along with the urine samples. The mean value of SRM 2670 determined by our system was  $507 \pm 17$  (SD)  $\mu\text{g/l}$  ( $n = 4$ ). The arsenic methylation capability was assessed by percentages of the various urinary arsenic species of the total arsenic amount. The primary methylation index (PMI) was defined as the ratio between MMA and inorganic arsenic ( $\text{As}^{\text{III}} + \text{As}^{\text{V}}$ ) levels, while the secondary methylation index (SMI) was defined as the ratio between  $\text{DMA}^{\text{V}}$  and  $\text{MMA}^{\text{V}}$  [24].

#### MPO Genotyping

The *MPO* -463G  $\rightarrow$  A polymorphism was detected by the use of restriction fragment length polymorphism (RFLP) after a polymerase chain reaction (PCR). A 350-bp DNA fragment was amplified using the forward primer, MPOF (5'-CGG TAT AGG CAC ACA ATG GTG AG), and reverse primer, MPOR (5'-GCA ATG GTT CAA GCG ATT CTT C). The reactions were heated to  $94^{\circ}\text{C}$  for 5 min followed by 35 cycles at  $94^{\circ}\text{C}$  for 30 s,  $62^{\circ}\text{C}$  for 45 s, and  $72^{\circ}\text{C}$  for 1 min, with a final extension of 4 min at  $72^{\circ}\text{C}$ . PCR was performed, and 10  $\mu\text{l}$  of the PCR product was digested with the restriction enzyme *Aci*I. After electrophoresis, the digested products resulted in banding patterns indicative of the genotypes: 169-, 120-, and 61-bp fragments for the homozygous major type (-463GG); 289-, 169-, 120-, and 61-bp fragments for the heterozygous type (-463AG), and 289- and 61-bp fragments for the homozygous minor type (-463AA) [15].

#### SULT1A1 Genotyping

*SULT1A1* genotypes were examined using a PCR-RFLP-based assay. Two primers (forward primer, 5'-GGGTCTCTAGGAGAGGTGGC, and reverse primer, 5'-GCTGTGGTCCATGAACTCCT) were designed to amplify a 270-bp fragment of exon 7 that included the polymorphic site (codon 213, *His/CAC* to *Arg/CGC*) of the gene. The PCR reactions were carried out in 50  $\mu\text{l}$  of 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.5 mM  $\text{MgCl}_2$ , 0.2 mM deoxynucleotide triphosphate, and 1 unit of Taq polymerase. The reactions were heated to  $94^{\circ}\text{C}$  for 1 min followed by 35 cycles of  $94^{\circ}\text{C}$  for 30 s,  $62^{\circ}\text{C}$  for 30 s, and  $72^{\circ}\text{C}$  for 30 s, with a final extension of 7 min at  $72^{\circ}\text{C}$ . The PCR products (270 bp) were digested with *Hha*I and analyzed by 3% agarose gel electrophoresis. Digestion of each PCR product with *Hha*I gave rise to 155- and 115-bp fragments for the *Arg/CGC* allele and a single 270-bp fragment for the *His/CAC* allele [25].

#### Statistical Analysis

Continuous variables are expressed as the mean  $\pm$  standard deviation. ANOVA and Dunnett's test for multiple comparison corrections were applied to compare urinary arsenic profiles between the various tumor grades and stages. Linear regression was used to test the association between arsenic species and staging or grading of UC. The  $\chi^2$  test was used for associations of tumor grades and stages with genotype and demographic characteristics. SAS version 8.2 (SAS, Cary, N.C., USA) was used for all statistical analyses, and the level of significance was set at 5%.

## Results

Gender distribution, educational level, smoking habit, marital status, and MPO and *SULT* genotype of UC patients by stage and grade is shown in table 1. The UC patients were identified at various stages: 64 at the Ta/T1, 23 at the T2, and 23 at the T3/T4 stage, while 2 were not available for staging; 12 were at grade I, 55 were at grade II, and 43 were at grade III/IV, while 2 were not available for grading. We found that different stages and grades had similar distributions by gender, marital status, educational level, and smoking habit. *MPO* genotype was borderline significantly related to stage, and *SULT1A1* genotype was borderline significantly related to grade (table 1). Stage and grade of 2 patients were not determined; however their unavailability did not influence the distribution of stage or grade by gender, marital status, educational level and smoking habit. Table 2 compares the urinary arsenic profiles between genders and *MPO* and *SULT* genotypes. The mean and standard deviation of total arsenic, inorganic arsenic,  $\text{MMA}^{\text{V}}$ , and  $\text{DMA}^{\text{V}}$  were  $25.58 \pm 37.60$ ,  $0.94 \pm 0.98$ ,  $1.62 \pm 1.72$ , and  $23.01 \pm 36.58 \mu\text{g/l}$  in the 112 UC patients, respectively. Among UC cases, male subjects had an insignificantly higher total arsenic level, lower inorganic arsenic, higher  $\text{MMA}^{\text{V}}$

**Table 1.** Distribution of gender, marital status, educational level, and MPO and SULT genotype in UC patients by stage and grade

	Stage				p	Grade				p
	Ta/T1	T2	T3/T4	NA		I	II	III/IV	NA	
Total	64	23	23	2		12	55	43	2	
Gender					0.40					0.61
Male	37	11	15	2		7	30	26	2	
Female	27	12	8	0		5	25	17	0	
Marital status					0.49					0.86
Single	0	0	1	0		0	1	0	0	
Married	47	18	18	1		9	40	33	2	
Divorced or widowed	16	5	3	0		3	14	7	0	
NA	1	0	0	2		0	0	2	1	
Educational level					0.68					0.50
Illiterate	26	7	6	0		3	22	14	0	
Elementary and junior high school	31	13	13	2		8	24	25	2	
Above high school	7	3	4	0		1	9	4	0	
Smoking habit				51	0.23				51	0.83
Yes	23	8	11	2		6	35	26	1	
No	41	15	12	0		6	20	17	1	
MPO genotype					0.09					0.77
GG	45	18	18	0		9	38	33	1	
GA	19	5	4	2		3	17	9	1	
AA	0	0	1	0		0	0	1	0	
SULT genotype					0.92					0.09
AA	57	22	22	2		11	49	41	2	
AG	6	1	1	0		0	6	2	0	
GG	1	0	0	0		1	0	0	0	

Differences were calculated using Fisher's exact test; NA = not available.

**Table 2.** Distribution of the urinary arsenic methylation profile in UC patients by gender, and the MPO and SULT 1A1 genotypes

	Patients	Total arsenic, $\mu\text{g/g}$ creatinine	Inorganic arsenic, %	MMA <sup>V</sup> , %	DMA <sup>V</sup> , %	PMI	SMI
Total	112	25.58 $\pm$ 37.60	6.39 $\pm$ 9.60	7.22 $\pm$ 5.87	86.38 $\pm$ 11.52	2.40 $\pm$ 4.88	24.91 $\pm$ 42.02
Gender							
Male	65	29.69 $\pm$ 47.54	6.33 $\pm$ 6.16	7.52 $\pm$ 6.13	86.15 $\pm$ 9.59	2.06 $\pm$ 2.50	29.12 $\pm$ 51.99
Female	46	19.89 $\pm$ 14.50	6.48 $\pm$ 13.09	6.81 $\pm$ 5.52	86.72 $\pm$ 13.9	2.98 $\pm$ 7.36	18.53 $\pm$ 17.68
p value <sup>a</sup>		0.12	0.94	0.53	0.81	0.47	0.15
MPO							
GG	81	28.10 $\pm$ 43.44	6.26 $\pm$ 10.56	6.75 $\pm$ 5.93	86.98 $\pm$ 11.81	2.49 $\pm$ 5.41	27.70 $\pm$ 45.72
GA/AA	31	19.00 $\pm$ 11.69	6.71 $\pm$ 6.62	8.45 $\pm$ 5.58	84.83 $\pm$ 10.74	2.19 $\pm$ 3.21	18.25 $\pm$ 31.22
p value <sup>a</sup>		0.09	0.79	0.17	0.37	0.75	0.24
SULT							
AA	103	26.37 $\pm$ 38.99	6.50 $\pm$ 9.92	6.88 $\pm$ 5.64	86.62 $\pm$ 11.64	1.98 $\pm$ 2.36	26.36 $\pm$ 43.82
AG/GG	9	16.60 $\pm$ 11.66	5.11 $\pm$ 4.62	11.22 $\pm$ 7.14	83.67 $\pm$ 10.23	7.85 $\pm$ 16.17	10.53 $\pm$ 5.89
p value <sup>a</sup>		0.08	0.46	0.03	0.46	0.37	0.01

Five patients were not available for PMI and 4 patients were not available for SMI.

<sup>a</sup> Student's t test.

**Table 3.** Distribution of the urinary arsenic methylation profiles of UC patients by stage

	Ta/T1 (n = 64)	T2 (n = 23)	T3/T4 (n = 23)	Not available (n = 2)	p value for ANOVA	p value for regression
Urinary arsenic species concentration, $\mu\text{g/l}$						
Inorganic arsenic	$0.91 \pm 0.73$	$0.62 \pm 0.63^a$	$1.42 \pm 1.60$	$0.26 \pm 0.36$	0.03	0.11
MMA <sup>V</sup>	$1.49 \pm 1.36$	$1.21 \pm 1.63$	$2.46 \pm 2.44$	$0.94 \pm 0.32$	0.06	0.05
DMA <sup>V</sup>	$19.99 \pm 15.44$	$17.10 \pm 15.29$	$37.92 \pm 74.36$	$16.42 \pm 11.66$	0.18	0.08
Total arsenic, $\mu\text{g/g}$ creatinine	$22.39 \pm 16.40$	$18.92 \pm 16.92$	$41.80 \pm 75.59$	$17.61 \pm 11.61$	0.13	0.07

<sup>a</sup> ANOVA and Dunnett's test, T<sub>2</sub> vs. T<sub>3</sub>/T<sub>4</sub>, p < 0.05.

**Table 4.** Distribution of the urinary arsenic methylation profiles of UC patients by grade

	Grade				p value for ANOVA	p value for regression
	I (n = 12)	II (n = 55)	III and IV (n = 43)	not available (n = 2)		
Urinary arsenic species concentration, $\mu\text{g/l}$						
Inorganic arsenic	$0.76 \pm 0.83$	$0.84 \pm 0.72$	$1.16 \pm 1.28$	$0.41 \pm 0.58$	0.30	0.09
MMA <sup>V</sup>	$1.41 \pm 1.18$	$1.32 \pm 1.32$	$2.07 \pm 2.21$	$1.45 \pm 0.41$	0.19	0.06
DMA <sup>V</sup>	$26.33 \pm 22.19$	$19.00 \pm 14.66$	$27.26 \pm 55.57$	$22.13 \pm 3.57$	0.72	0.54
Total arsenic, $\mu\text{g/g}$ creatinine	$28.50 \pm 22.78$	$21.16 \pm 16.70$	$30.49 \pm 56.84$	$24.00 \pm 2.58$	0.67	0.47

percentage, and higher SMI than females. There were no significant differences in the urinary arsenic profiles among the different *MPO* genotypes. In contrast, patients with the *SULT* AG/GG genotype had lower total arsenic and inorganic arsenic percentages, a significantly higher MMA<sup>V</sup> percentage, and lower SMI than those with the *SULT* AA genotype (table 2). To examine if various cancer stages affected the arsenic methylation capability, we performed an analysis, which showed that the methylation capability differed between patients at various tumor stages in our case subjects (table 3). T3/T4 stage cases had significantly higher inorganic arsenic than T2 stage cases, while T3/T4 stage cases had insignificantly higher MMA<sup>V</sup> than T2 stage or Ta/T1 stage cases; however, the p value for ANOVA test was borderline significant. MMA<sup>V</sup>, DMA<sup>V</sup>, and total arsenic levels were borderline significantly increased with the stage progress. In contrast, the methylation capability did not differ among the various tumor grades in our case subjects, but inorganic arsenic and MMA<sup>V</sup> were also borderline significantly increased with the grade progress (table 4). Stage and grade of 2 patients were not determined; however, their unavailability did not influence the distribution of

stage or grade by urinary arsenic species. The distributions of urinary arsenic species profiles in UC patients by cigarette smoking status and *MPO* and *SULT* genetic polymorphism are presented in table 5. It was found that the SMI of subjects with the *MPO* GG genotype was higher than that with the GA/AA genotype in nonsmokers. On the other hand, the DMA<sup>V</sup> percentage of subjects with *SULT* AA was borderline significantly lower than that of subjects with the AG/GG genotype in nonsmokers (data not shown). Smokers with the *MPO* GG genotype had significantly higher DMA<sup>V</sup> than those with the GA/AA genotype. Similarly, smokers with the *SULT* AA genotype had significantly higher SMI than those with the AG/GG genotype.

## Discussion

In this study, urinary arsenic species were used to characterize the arsenic methylation capability of patients with UC who had drunk tap water with arsenic levels of <50  $\mu\text{g/l}$ . Grading is about the tumor behavior, staging is about the invasion area of the tumor. Generally grading

**Table 5.** Distribution of the urinary arsenic methylation profiles of UC patients by cigarette smoking status, and the *MPO* and *SULT 1A1* genotypes

	Cigarette smoking: No		Yes	
	<i>MPO</i> genotype:		<i>SULT</i> genotype:	
	GG	AG/AA	AA	AG/GG
Urinary arsenic species concentration, $\mu\text{g/l}$				
Inorganic arsenic	0.77 $\pm$ 0.64	1.08 $\pm$ 1.62	1.05 $\pm$ 0.85	1.04 $\pm$ 1.02
MMA <sup>V</sup>	1.31 $\pm$ 1.44	1.81 $\pm$ 2.21	1.84 $\pm$ 1.75	2.00 $\pm$ 1.55
DMA <sup>V</sup>	18.18 $\pm$ 13.59	17.16 $\pm$ 9.72	32.41 $\pm$ 58.21	16.92 $\pm$ 14.02
Total arsenic, $\mu\text{g/g}$ creatinine	20.26 $\pm$ 14.73	20.05 $\pm$ 12.07	35.30 $\pm$ 59.36	19.96 $\pm$ 16.23
Urinary methylation index				
PMI	2.81 $\pm$ 7.13	1.90 $\pm$ 1.99	2.31 $\pm$ 2.99	1.58 $\pm$ 0.96
SMI	20.22 $\pm$ 18.23 <sup>a, +</sup>	13.25 $\pm$ 9.85 <sup>a, +</sup>	37.95 $\pm$ 64.70 <sup>b, **</sup>	10.39 $\pm$ 6.37 <sup>b, **</sup>

<sup>a</sup> *MPO* genotype comparison, GG vs. AG/AA; <sup>b</sup> *SULT* genotype comparison, AA vs. AG/GG; \*\*  $p < 0.01$ ; \*  $p < 0.05$ ; <sup>+</sup>  $0.05 < p < 0.1$  tested by Student's t test.

could predict the possibility of further metastasis. Staging was used to predict the survival rate. Inorganic arsenic, MMA<sup>V</sup>, DMA<sup>V</sup>, and total arsenic levels were higher in T3/T4 patients than in T2 or Ta/T1 patients in this study. MMA<sup>V</sup>, DMA<sup>V</sup>, and total arsenic levels were borderline significantly increased with the progress of stage, and inorganic arsenic and MMA<sup>V</sup> were also borderline significantly increased with the progress of grade. These findings raise the possibility that a greater arsenic methylation capability increases the chance for a better tumor prognosis; however, we need further investigation.

Arsenic exposure is associated with increases in both the frequency and specific types of genetic alterations in bladder tumors [26]. Arsenic may cause increased genetic instability in bladder tumors, possibly by deregulating cell cycle control pathways via epigenetic mechanisms or by reducing the ability of cells to properly respond to or repair DNA damage. Both mechanisms may enhance the rates of bladder cancer development, chromosomal alterations, and tumor progression. This suggests that increasing arsenic exposure is also associated with tumor

stage and grade [26]. The arsenic methylation pathway ( $\text{As}^{\text{V}} \rightarrow \text{As}^{\text{III}} \rightarrow \text{MMA}^{\text{V}} \rightarrow \text{MMA}^{\text{III}} \rightarrow \text{DMA}^{\text{V}} \rightarrow \text{DMA}^{\text{III}}$ ) [27] was considered to be a detoxification process because the major methylated metabolites, MMA<sup>V</sup> and DMA<sup>V</sup>, are more readily excreted and less toxic than inorganic arsenic [28]. Nevertheless, the cytotoxicity [29], genotoxicity [30], and inhibition of enzymes with antioxidant functions [31] of the minor trivalent methylated arsenicals, MMA<sup>III</sup> and DMA<sup>III</sup>, are more potent than those of either As<sup>III</sup> or As<sup>V</sup>. An individual's methylation capacity plays an important role in determining his/her susceptibility to the adverse health effects of arsenic, especially MMA% or the MMA/DMA ratio and arsenic-related skin cancer and bladder cancer [8, 9]. Inherited genetic traits might play important roles in determining individual arsenic methylation capabilities [32]. A previous study proved that the *GSTM1* null genotype is related to the stage of bladder cancer [33]. This suggests that increased urinary excretion of unknown substances metabolized by *GSTM1* may promote cancer progression in patients with bladder cancer [34].

Arsenic exposure increases H<sub>2</sub>O<sub>2</sub> rather than O<sub>2</sub> through the mediator of nuclear Nrf2 accumulation [35]. If H<sub>2</sub>O<sub>2</sub> is not neutralized, it may react with chloride to generate hypochlorous acid, a potent oxidizing agent, by a reaction catalyzed by MPO. The MPO -463 A allele was presumed to be associated with lower levels of ROS and has been associated with decreased lung cancer risk [36]. In this study, subjects with the MPO GG genotype had borderline significantly higher total arsenic levels than those with the GA/AA genotype. These results suggest that the G allele increases total arsenic excretion, enhances ROS levels, and influences the UC prognosis. This is consistent with results which found that subjects carrying the MPO GG genotype with high arsenic exposure had a significantly higher hyperkeratosis risk than those with the MPO GA/AA genotype with lower arsenic exposure [37].

Exposure to polycyclic aromatic hydrocarbons and aromatic amines, mainly through smoking or one's occupation, has been shown to be associated with bladder carcinogenesis [38]. SULT1A1 involved in the metabolism of procarcinogens is polymorphic in humans. In this study, subjects with the SULT1A1 AG/GG genotype had lower total arsenic and significantly higher MMA<sup>V</sup> percentages and a significantly lower SMI than those with the AA genotype. On the other hand, T3/T4 stage subjects had higher total arsenic, MMA percentage and a lower DMA percentage than Ta/T1 subjects (data not shown). This possibly suggests that the SULT1A1 AG/GG genotype decreases total arsenic excretion, increases first-phase methylation (MMA%), and decreases second-phase

methylation (DMA%), thus enhancing UC progression. These results are consistent with the SULT 213His allele (G allele), which has been shown to be associated with lower enzyme activity and decreased mutagen activation [39], which might therefore result in a protective effect against bladder carcinogenesis [16]. Genetic polymorphism of SULT1A1 is a risk factor for urothelial cancer [40], and cigarette smoke toxicants act as substrates for human cytosolic SULTs [41]. Cigarette smoking was found to interact with urinary arsenic profile in affecting the UC risk [10]. Based on these findings, the SULT genotype might influence the arsenic methylation capability indirectly. Our preliminary finding requires careful consideration before making meaningful inferences because of the limited sample size and the lack of an appropriate healthy control group to calculate the cancer risk. In summary, urinary arsenic concentrations were borderline significantly increased with the progress of UC regardless of whether or not the patients had been exposed to arsenic from drinking water; determining whether SULT gene polymorphism modifies UC progression requires further investigations with larger samples.

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### References

- 1 Department of Health ROC: Cancer Registry Annual Report Republic of China, 1999 (in Chinese). Taipei, Department of Health, 2002.
- 2 Nseyo UO, Lamm DL: Therapy of superficial bladder cancer. *Semin Oncol* 1996;23:598-604.
- 3 Chen CJ, Chuang YC, Lin TM, Wu HY: Malignant neoplasms among residents of a blackfoot disease-endemic area in Taiwan: high-arsenic artesian well water and cancers. *Cancer Res* 1985;45:5895-5899.
- 4 Serretta V, Morgia G, Altieri V, Pavone-Macaluso M, Scuto F, Allegro R, Di LA, Cindolo L, Melloni D: Preliminary report of a multicentric study on environmental risk factors in Ta-T1 transitional cell carcinoma of the bladder. A study from Gruppo Studi Tumori Urologici Foundation. *Urol Int* 2006;77:152-158.
- 5 Lu CM, Chung MC, Huang CH, Ko YC: Interaction effect in bladder cancer between N-acetyltransferase 2 genotype and alcohol drinking. *Urol Int* 2005;75:360-364.
- 6 Andrae MO: Determination of arsenic species in natural waters. *Anal Chem* 1977;49:820-823.
- 7 Vahter M, Marafante E, Dencker L: Tissue distribution and retention of 74As-dimethylarsinic acid in mice and rats. *Arch Environ Contam Toxicol* 1984;13:259-264.
- 8 Hsueh YM, Chiou HY, Huang YL, Wu WL, Huang CC, Yang MH, Lue LC, Chen GS, Chen CJ: Serum beta-carotene level, arsenic methylation capability, and incidence of skin cancer. *Cancer Epidemiol Biomarkers Prev* 1997;6:589-596.
- 9 Chen YC, Su HJ, Guo YL, Hsueh YM, Smith TJ, Ryan LM, Lee MS, Christiani DC: Arsenic methylation and bladder cancer risk in Taiwan. *Cancer Causes Control* 2003;14:303-310.
- 10 Pu YS, Yang SM, Huang YK, Chung CJ, Huang SK, Chiu AW, Yang MH, Chen CJ, Hsueh YM: Urinary arsenic profile affects the risk of urothelial carcinoma even at low arsenic exposure. *Toxicol Appl Pharmacol* 2007;218:99-106.
- 11 Kitchin KT, Ahmad S: Oxidative stress as a possible mode of action for arsenic carcinogenesis. *Toxicol Lett* 2003;137:3-13.
- 12 Hofstra AH, Uetrecht JP: Myeloperoxidase-mediated activation of xenobiotics by human leukocytes. *Toxicology* 1993;82:221-242.

- 13 Petruska JM, Mosebrook DR, Jakab GJ, Trush MA: Myeloperoxidase-enhanced formation of (+)-trans-7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene-DNA adducts in lung tissue in vitro: a role of pulmonary inflammation in the bioactivation of a procarcinogen. *Carcinogenesis* 1992;13:1075–1081.
- 14 Cascorbi I, Henning S, Brockmoller J, Gephart J, Meisel C, Muller JM, Loddenkemper R, Roots I: Substantially reduced risk of cancer of the aerodigestive tract in subjects with variant -463A of the myeloperoxidase gene. *Cancer Res* 2000;60:644–649.
- 15 Van Schooten FJ, Boots AW, Knaapen AM, Godschalk RW, Maas LM, Borm PJ, Drent M, Jacobs JA: Myeloperoxidase (MPO) -463G → A reduces MPO activity and DNA adduct levels in bronchoalveolar lavages of smokers. *Cancer Epidemiol Biomarkers Prev* 2004;13:828–833.
- 16 Hung RJ, Boffetta P, Brennan P, Malaveille C, Hautefeuille A, Donato F, Gelatti U, Spaliviero M, Placidi D, Carta A, Scotto di Carlo A, Porru S: GST, NAT, SULT1A1, CYP1B1 genetic polymorphisms, interactions with environmental exposures and bladder cancer risk in a high-risk population. *Int J Cancer* 2004;110:598–604.
- 17 Glatt H, Meinel W: Pharmacogenetics of soluble sulfotransferases (SULTs). *Naunyn Schmiedeberg Arch Pharmacol* 2004;369:55–68.
- 18 Tsukino H, Kuroda Y, Nakao H, Imai H, Inatomi H, Osada Y, Katoh T: Cytochrome P450 (CYP) 1A2, sulfotransferase (SULT) 1A1, and N-acetyltransferase (NAT) 2 polymorphisms and susceptibility to urothelial cancer. *J Cancer Res Clin Oncol* 2004;130:99–106.
- 19 Wu MT, Wang YT, Ho CK, Wu DC, Lee YC, Hsu HK, Kao EL, Lee JM: SULT1A1 polymorphism and esophageal cancer in males. *Int J Cancer* 2003;103:101–104.
- 20 Pashos CL, Botteman MF, Laskin BL, Redaelli A: Bladder cancer: epidemiology, diagnosis, and management. *Cancer Pract* 2002;10:311–322.
- 21 World Health Organization: *Histological Typing of Urinary Bladder Tumours; International Classification of Tumours*. Geneva, World Health Organization, 1999.
- 22 Chen YC, Amarasiriwardena CJ, Hsueh YM, Christiani DC: Stability of arsenic species and insoluble arsenic in human urine. *Cancer Epidemiol Biomarkers Prev* 2002;11:1427–1433.
- 23 Hsueh YM, Huang YL, Huang CC, Wu WL, Chen HM, Yang MH, Lue LC, Chen CJ: Urinary levels of inorganic and organic arsenic metabolites among residents in an arseniasis-hyperendemic area in Taiwan. *J Toxicol Environ Health A* 1998;54:431–444.
- 24 Tseng CH, Huang YK, Huang YL, Chung CJ, Yang MH, Chen CJ, Hsueh YM: Arsenic exposure, urinary arsenic speciation, and peripheral vascular disease in blackfoot disease-hyperendemic villages in Taiwan. *Toxicol Appl Pharmacol* 2005;206:299–308.
- 25 Zheng W, Xie D, Cerhan JR, Sellers TA, Wen W, Folsom AR: Sulfotransferase 1A1 polymorphism, endogenous estrogen exposure, well-done meat intake, and breast cancer risk. *Cancer Epidemiol Biomarkers Prev* 2001;10:89–94.
- 26 Moore LE, Smith AH, Eng C, Kalman D, DeVries S, Bhargava V, Chew K, Moore D, Ferreccio C, Rey OA, Waldman FM: Arsenic-related chromosomal alterations in bladder cancer. *J Natl Cancer Inst* 2002;94:1688–1696.
- 27 Kitchin KT: Recent advances in arsenic carcinogenesis: modes of action, animal model systems, and methylated arsenic metabolites. *Toxicol Appl Pharmacol* 2001;172:249–261.
- 28 Gebel TW: Arsenic methylation is a process of detoxification through accelerated excretion. *Int J Hyg Environ Health* 2002;205:505–508.
- 29 Styblo M, Del Razo LM, Vega L, Germolec DR, LeCluyse EL, Hamilton GA, Reed W, Wang C, Cullen WR, Thomas DJ: Comparative toxicity of trivalent and pentavalent inorganic and methylated arsenicals in rat and human cells. *Arch Toxicol* 2000;74:289–299.
- 30 Mass MJ, Tennant A, Roop BC, Cullen WR, Styblo M, Thomas DJ, Kligerman AD: Methylated trivalent arsenic species are genotoxic. *Chem Res Toxicol* 2001;14:355–361.
- 31 Lin S, Del Razo LM, Styblo M, Wang C, Cullen WR, Thomas DJ: Arsenicals inhibit thio-redoxin reductase in cultured rat hepatocytes. *Chem Res Toxicol* 2001;14:305–311.
- 32 Chung JS, Kalman DA, Moore LE, Kosnett MJ, Arroyo AP, Beeris M, Mazumder DN, Hernandez AL, Smith AH: Family correlations of arsenic methylation patterns in children and parents exposed to high concentrations of arsenic in drinking water. *Environ Health Perspect* 2002;110:729–733.
- 33 Georgiou I, Filiadis IF, Alamanos Y, Bouba I, Giannakopoulos X, Lolis D: Glutathione S-transferase null genotypes in transitional cell bladder cancer: a case-control study. *Eur Urol* 2000;37:660–664.
- 34 Kim EJ, Jeong P, Quan C, Kim J, Bae SC, Yoon SJ, Kang JW, Lee SC, Jun WJ, Kim WJ: Genotypes of TNF-alpha, VEGF, hOGG1, GSTM1, and GSTT1: useful determinants for clinical outcome of bladder cancer. *Urology* 2005;65:70–75.
- 35 Pi J, Qu W, Reece JM, Kumagai Y, Waalkes MP: Transcription factor Nrf2 activation by inorganic arsenic in cultured keratinocytes: involvement of hydrogen peroxide. *Exp Cell Res* 2003;290:234–245.
- 36 Feyler A, Voho A, Bouchardy C, Kuokkanen K, Dayer P, Hirvonen A, Benhamou S: Point: myeloperoxidase -463G → A polymorphism and lung cancer risk. *Cancer Epidemiol Biomarkers Prev* 2002;11:1550–1554.
- 37 Ahsan H, Chen Y, Kibriya MG, Islam MN, Slavkovich VN, Graziano JH, Santella RM: Susceptibility to arsenic-induced hyperkeratosis and oxidative stress genes myeloperoxidase and catalase. *Cancer Lett* 2003;201:57–65.
- 38 Kogevinas M, Trichopoulos D: Urinary bladder cancer; in Adami HO, Hunter D, Trichopoulos D (eds): *Textbook of Cancer Epidemiology*. New York, Oxford University Press, 2002, pp 446–466.
- 39 Glatt H, Engelke CE, Pabel U, Teubner W, Jones AL, Coughtrie MW, Andrae U, Falany CN, Meinel W: Sulfotransferases: genetics and role in toxicology. *Toxicol Lett* 2000;112–113:341–348.
- 40 Ozawa S, Katoh T, Inatomi H, Imai H, Kuroda Y, Ichiba M, Ohno Y: Association of genotypes of carcinogen-activating enzymes, phenol sulfotransferase SULT1A1 (ST1A3) and arylamine N-acetyltransferase NAT2, with urothelial cancer in a Japanese population. *Int J Cancer* 2002;102:418–421.
- 41 Yasuda S, Idell S, Fu J, Carter G, Snow R, Liu MC: Cigarette smoke toxicants as substrates and inhibitors for human cytosolic SULTs. *Toxicol Appl Pharmacol* 2007;221:13–20.

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