Science of the Total Environment 409 (2011) 465–470

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Gene polymorphisms of glutathione S-transferase omega 1 and 2, urinary arsenic methylation profile and urothelial carcinoma

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ARTICLE INFO

Article history:
Received 29 June 2010
Received in revised form 28 October 2010
Accepted 28 October 2010
Available online 21 November 2010

Keywords:
Arsenic methylation profile
GSTO1
GSTO2
Polymorphism
Urothelial carcinoma

ABSTRACT

Genetic polymorphisms in arsenic-metabolizing enzymes may be involved in the biotransformation of inorganic arsenic and may increase the risk of developing urothelial carcinoma (UC). The present study evaluated the roles of glutathione S-transferase omega 1 (GSTO1) and GSTO2 polymorphisms in UC carcinogenesis. A hospital-based case-control study was conducted. Questionnaire information and biological specimens were collected from 149 UC cases and 251 healthy controls in a non-obvious inorganic arsenic exposure area in Taipei, Taiwan. The urinary arsenic profile was determined using high-performance liquid chromatography and hydride generator-atomic absorption spectrometry. Genotyping for GSTO1 Ala140Asp and GSTO2 Asn142Asp was conducted using polymerase chain reaction–restriction fragment length polymerase. GSTO1 Glu208Lys genotyping was performed using high-throughput matrix-assisted laser desorption and ionization time-of-flight mass spectrometry. A significant positive association was found between total arsenic, inorganic arsenic percentage and monomethylarsonic acid percentage and UC, while dimethylarsinic acid percentage was significantly inversely associated with UC. The minor allele frequency of GSTO1 Ala140Asp, GSTO1 Glu208Lys and GSTO2 Asn142Asp was 18%, 1% and 26%, respectively. A significantly higher MMA% was found in people who carried the wild type of GSTO1 140 Ala/Ala compared to those who carried the GSTO1 140 Ala/Asp and Asp/Asp genotype ($p = 0.02$). The homogenous variant genotype of GSTO2 142 Asp/Asp was inversely associated with UC risk (OR = 0.17; 95% CI, 0.03 - 0.88; $p = 0.03$). Large-scale studies will be required to verify the association between the single nucleotide polymorphisms of arsenic-metabolism-related enzymes and UC risk.

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1. Introduction

Urothelial carcinoma (UC) includes malignances of the bladder, the renal pelvis, the ureter and the urethra. Occupational exposure to aromatic amines, cigarette smoking and inorganic arsenic in drinking water are well known risk factors for the development of UC (Negri and La, 2001). Epidemiological evidence has demonstrated that exposure to arsenic in drinking water is associated with skin cancer, liver cancer and bladder cancer (Hsueh et al., 1997; Chiou et al., 1995). The significant correlation between urinary arsenic profile and UC was demonstrated in our previous study (Pu et al., 2007), as well as other studies (Chen et al., 2003; Steinmaus et al., 2006). These studies showed that people with unfavorable urinary arsenic profiles, e.g., higher total arsenic, higher inorganic arsenic percentage (InAs%), higher monomethylarsonic acid percentage (MMA%), or lower dimethylarsinic acid percentage (DMA%), had higher rates of UC than those with more favorable arsenic profiles.

Arsenic-induced carcinogenesis in the human bladder may be due to the fact that the bladder is exposed to high levels of arsenic, as it is bioconcentrated in urine (Chen et al., 1988). Upon entering the human body, inorganic arsenic is enzymatically transformed to MMA, DMA, and, in some species, trimethyl arsenic (TMA) (Cohen et al., 2006). Among these species, the trivalent arsenicals, particularly the trivalent methylated arsenic metabolites, have been identified as the most toxic forms of arsenic (Styblo et al., 2000). The toxicological effects of arsenic on urothelium have been established through UROtsa cells, including inducing malignant transformation, mediating cell proliferation and gene expression by upregulation of activating protein-1, as well as disturbing other signal transduction pathways (Eblin et al., 2008; Sens et al., 2004; Simeonova et al., 2000; Su et al., 2006).

To date, arsenic (+3 oxidation state)-methyltransferase (AS3MT), purine nucleoside phosphorylase (PNP), glutathione S-transferase omega

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doi:10.1016/j.scitotenv.2010.10.053

1 (GSTO1) and GSTO2 have been proposed to be involved in the arsenic metabolism pathway (Aposhian et al., 2004; Aposhian and Aposhian, 2006; Del Razo et al., 2001; Schmuck et al., 2005). AS3MT likely catalyzes the conversion of arsenite (iAs^{3+}) methylate to MMA^{5+} and of MMA^{3+} methylate to DMA^{5+} using S-adenosyl-methionine (SAM) as the methyl donor (Drobna et al., 2004, 2005, 2006; Lin et al., 2002; Thomas et al., 2007). Furthermore, PNP, GSTO1 and GSTO2 may be involved in reducing the pentavalent arsenic species, including arsenate (iAs^{5+}), MMA^{5+}, and DMA^{5+}, to trivalent arsenicals (Chowdhury et al., 2006; Radabaugh et al., 2002).

Individual genetic susceptibility may affect inorganic arsenic metabolic capability, changing the profile of urinary arsenic species and, thus, the potential to develop UC. Several single nucleotide polymorphisms (SNPs) have been reported in GSTO1 and GSTO2 (Kolsch et al., 2007; Leite et al., 2007). A genetic association study evaluated six polymorphic sites in GSTO1 and GSTO2 in 100 Vietnam people, and found that people with GSTO1 Glu155del heterozygous genotype had higher urinary iAs^{5+} than those with the wild homozygous genotype (Agusa et al., 2010). In addition, other studies found no associations between GSTO1 exon4 Ala140Asp (or GSTO2 Asn142Asp) gene polymorphism and urinary arsenic profile, including Chinese populations chronically exposed to arsenic in drinking water and copper mine workers occupationally exposed to arsenic (Paiva et al., 2008; Xu et al., 2009). Previous studies found conflicting results about the polymorphism of GSTO1 Ala140Asp related to the disease risks. For example, the polymorphism of GSTO1 Ala140Asp increased risks for the development of cerebrovascular atherosclerosis, hepatocellular carcinoma, cholangiocarcinoma, breast cancer (Kolsch et al., 2007; Marahatta et al., 2006) and a reduced risk for Parkinson's disease (Wahner et al., 2007). There were few studies exploring the relationship among the polymorphisms of arsenic-metabolized enzymes, urinary arsenic profile and UC risk. De Chaudhuri et al. attempted to analyze the association between these factors and arsenic-related skin lesions and found that the polymorphisms of GSTO1 Ala140Asp and GSTO2 Asn142Asp were not associated with arsenic-induced skin lesions and skin cancer in West Bengal individuals (De Chaudhuri et al., 2008).

Therefore, this study explored whether the arsenic-metabolizing genes of GSTO1 Ala140Asp, GSTO1 Glu208Lys and GSTO2 Asn142Asp affected the urinary arsenic profile and evaluated a possible association between these SNPs and the development of UC.

2. Materials and methods

2.1. Study area and participants

We conducted a hospital-based case-control study according to the protocol and recruitment strategy described previously (Pu et al., 2007). Briefly, we recruited 223 UC cases and 607 healthy controls from the Medical Center, which includes National Taiwan University Hospital and Taipei Municipal Wan Fang Hospital, from September 2002 to August 2007. Informed consent forms were provided to all participants prior to questionnaire interviews and collection of biological specimens. The Research Ethics Committee of the National Taiwan University Hospital, Taipei, Taiwan, approved the study and it was consistent with the World Medical Association Declaration of Helsinki. All UC cases were diagnosed by histological confirmation. Healthy controls with no prior history of cancer were matched to UC cases in terms of age (±5 years) and gender. A total of 185 UC cases and 412 healthy controls were matched.

2.2. Questionnaire interview and biological specimen collection

Well-trained interviewers collected detailed information through a face-to-face interview. The context of the structured questionnaire included demographics and socioeconomic characteristics, lifestyle habits such as cigarette smoking and alcohol consumption, residential and occupational history, and personal and family histories of UC. Spot urine samples were collected at the time of recruitment and immediately transferred to a −20 °C freezer until the analysis of urinary arsenic profiles. Simultaneously, blood samples were collected and frozen at −80 °C for DNA extraction. Among study participants, the questionnaire data or biological specimens of 36 cases and 161 controls were unavailable for collection. Finally a total of 149 UC cases and 251 healthy controls were included in the present study.

2.3. Urinary arsenic profiles assessment

Urinary arsenic profiles of iAs^{3+}, iAs^{5+}, MMA^{5+} and DMA^{5+} were analyzed by high-performance liquid chromatography, equipped with a hydride generator and atomic absorption spectrometer. Detailed methods for the analysis of these species have been reported in our previous study (Hsueh et al., 1998). As a quality control, freeze-dried SRM 2670 urine obtained from the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA) containing 480 ± 100 μg/L arsenic were analyzed along with the urine specimens of study subjects. The arsenic value in SRM 2670 was determined to be 507 ± 17 μg/L (n = 4). Recovery rates of the four arsenic species were calculated using the following formula: ([(sample spiked standard solution concentration) − sample concentration] /standard solution concentration × 100). The recovery rates of iAs^{3+}, DMA^{5+}, MMA^{5+}, and iAs^{5+} were from 93.8 to 102.2%, with detection limits of 0.02, 0.08, 0.05, and 0.07 μg/L, respectively. In consideration of the stability of urinary arsenic profiles, the assay of arsenic species was performed within 6 months of the collection (Chen et al., 2002). In addition, previous studies have demonstrated that seafood may affect the levels of urinary arsenic species (Ma and Le, 1998; Francesconi and Kuehnelt, 2004); however, our previous study found that the levels of $iAs^{3\pm}$, $iAs^{5\pm}$, MMA, DMA, total arsenic, InAs%, MMA% and DMA% were similar before and after 3 days of seafood restriction (Hsueh et al., 2002). We also found that the frequencies of fish, shellfish and seaweed intake do not significantly correlate with urinary arsenic species (Hsueh et al., 2002). These results were consistent with the findings of Lin (Lin, 1986). Therefore, it is unlikely that urinary arsenic species are confound by the consumption of seafood within 3 days in this study.

2.4. Genotyping

Genomic DNA was extracted from blood specimens using proteinase K digestion following phenol and chloroform extraction. Genotyping for SNPs in GSTO1 Glu208Lys (rs11509438) was performed using high-throughput matrix-assisted laser desorption and ionization time-of-flight (MALDI-TOF) mass spectrometry (SEQUENOM MassARRAY system; Sequenom, San Diego, CA, USA). The PCR primers for GSTO1 Glu208Lys were designed using Spectro-Designer software (SEQUENOM, Inc.). The necessary sequence information for primer design was based on the GenePipe database (http://genepipe.ngc.sinica.edu.tw/seqtool/pages/getSeq.jsp). Information on the primers is available from the authors upon request. Briefly, uniplex polymerase chain reaction (PCR) was carried out by the forward and reverse primers. After primer extension, the purified DNA fragments were spotted onto a 384-element silicon chip and analyzed in the Bruker Biflex III MALDI-TOF SpectroREADER mass spectrometer. The resulting spectra were processed with SpectroTYPER (Sequenom). To ensure the specificity and reliability of observed polymorphisms, the results were confirmed by repeating 10% of the assays. In addition, genotyping for GSTO1 Ala140Asp (rs4925) and GSTO2 Asn142Asp (rs156697) was carried out by using the polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) technique (Marahatta et al, 2006). In brief, the primers 5′-GAA CTT GAT GCA CCC TTG GT-3′ (forward) and 5′-TGA TAG CTA GGA GAA ATA ATT AC-3′ (backward) for GSTO1 Ala140Asp polymorphism, 5′-AGG CAG AAC AGG AAC TGG AA-3′ and 5′-GAG GGA CCC CTT TTT GTA CC-3′ for GSTO2 Asn142Asp polymorphism were used to amplify 254 bp and 185 bp PCR products,

respectively. PCR products were obtained in a total volume of 30 μL, containing an 80 ng sample DNA, 10× PCR buffer, 2.5 mM dNTP, 2 μM of each primer and 2 U Taq polymerase. After initial denaturation for 5 min at 94 °C, 30 cycles were performed at 94 °C for 1 min (denaturation), followed by 60 °C for 1 min (annealing) and finally 72 °C for 1 min (extension) forGSTO1, and for GSTO2 an additional final step at 72 °C for 5 min was included in each cycle. The amplified products were visualized by electrophoresis in a 2% agarose gel. PCR products were digested with *Cac8* I (18 h, at 37 °C) for GSTO1 and *Mbo* I (18 h, at 37 °C) for GSTO2. Genotypes were analyzed by electrophoresis on 3% agarose gels. For quality control, a random 5% of the samples were repeated with a concordance of 100%.

2.5. Statistical analysis

All data analyses were performed by using the SAS package (SAS, version 8.0, Cary, NC). The urinary total arsenic concentration was the sum of iAs^{3+}, iAs^{5+}, MMA^{5+}, and DMA^{5+} and was normalized against urinary creatinine levels (μg/g creatinine). The relative proportion of each arsenic species (InAs (iAs^{3+} + iAs^{5+})%, MMA% and DMA%) was estimated by dividing the concentration of each arsenic species by the total arsenic concentration. The frequency distributions of GSTO1 and GSTO2 polymorphisms were evaluated in controls to test the Hardy–Weinberg equilibrium. We calculated cumulative exposure of cigarette smoking (pack-years) using the information of smoking status (never, current or past) and daily numbers of cigarettes smoked. We used multivariate logistic regressions to estimate the odds ratios (OR) and 95% confidence intervals (CI) on UC risk associated with relevant variables, including urinary arsenic profiles and gene polymorphisms of GSTO1 and GSTO2. Finally, we tested the differences of urinary arsenic profiles among polymorphisms of GSTO1 and GSTO2 using the Wilcoxon rank-sum test or the Kruskal–Wallis test.

3. Results

The sociodemographic characteristics of cases and controls are shown in Table 1. Participants who had higher educational levels had a lower risk of UC than those with lower educational levels. Subjects who had paternal ethnicity of Mainland Chinese had a lower risk of UC than those with paternal ethnicity of Fukien Taiwanese. Occasional alcohol drinkers had a significantly lower UC risk than non-drinkers and frequent drinkers. Pesticide users or participants with cumulative cigarette smoking > 0 had a significantly higher 2.6-fold UC risk than non-users or those with cumulative cigarette smoking = 0. Age and gender, as well as other potential confounders including coffee drinking or hair dye use, did not affect the UC risk. The median of urinary total arsenic levels in 149 patients of UC was significantly higher than 251 controls (29.78 μg/g creatinine vs.16.47 μg/g creatinine; $p \leq 0.05$). A significantly elevated risk of UC was related with urinary total arsenic, or InAs%, or MMA% increment; however, a significantly reduced risk of UC was related with urinary DMA% increment after adjustments for age, gender, education, paternal ethnicity, cumulative cigarette smoking, alcohol drinking and pesticide usage ($p \leq 0.01$).

In Table 2, the distributions of GSTO1 Ala140Asp, GSTO1 Glu208Lys and GSTO2 Asn142Asp polymorphisms were fitted the Hardy–Weinberg equilibrium respectively in controls ($p \geq 0.05$). Participants who carried the homogeneous variant genotype of GSTO2 142 Asp/Asp had a significantly protective risk of UC (OR = 0.17; 95% CI, 0.03 - 0.88; $p = 0.03$) compared with those who carried the wild type of GSTO2 142 Asn/Asn after adjustment for age, gender and other potential confounders. However, any association was not observed between the polymorphisms of GSTO1 Ala140Asp or GSTO1 Glu208Lys and UC risk. In addition, no association was found between paternal ethnicity and genetic frequency of GSTO1 or GSTO2 (data not shown).

We compared the urinary arsenic profiles in different genotypes of GSTO1 or GSTO2 among control groups ($n = 251$) (in Table 3). The

Table 1
Sociodemographic characteristics and urinary arsenic profiles for UC cases and controls.

Variables	UC cases (n = 149) no. (%)	Control (n = 251) no. (%)	Age and gender adjusted OR (95% CI)	*p*
Age (years) (Mean ± SE)	63.24 ± 1.04	62.69 ± 0.81	1.00 (0.99–1.02)	0.67
Gender				
Male	104 (69.80)	178 (70.92)	1.00	
Female	45 (30.20)	73 (29.08)	0.94 (0.60–1.46)	0.77
Highest educational level				
Elementary school or below	66 (44.30)	39 (15.66)	1.00[&]	
High school	56 (37.58)	90 (36.14)	0.31 (0.18–0.54)	≤0.01
College or above	27 (18.12)	120 (48.19)	0.10 (0.05–0.19)	≤0.01
Paternal ethnicity				
Fukien Taiwanese	108 (72.48)	131 (52.19)	1.00[&]	
Hakka Taiwanese	14 (9.40)	30 (11.94)	0.57 (0.29–1.12)	0.10
Mainland Chinese	27 (18.12)	90 (35.86)	0.34 (0.20–0.58)	≤0.01
Cumulative cigarette smoking (pack-years)	18.75 ± 2.34	9.44 ± 1.14	1.02 (1.01–1.03)	≤0.01
0	74 (51.39)	162 (66.94)	1.00	
>0[a]	70 (48.61)	80 (33.06)	2.62 (1.55–4.41)	≤0.01
Alcohol drinking				
Never	93 (62.42)	123 (49.00)	1.00	
Occasional	25 (16.78)	89 (35.46)	0.37 (0.22–0.63)	≤0.01
Regular	31 (20.81)	39 (15.54)	1.02 (0.57–1.84)	0.94
Pesticide usage				
No	130 (87.84)	238 (94.82)	1.00	
Yes	18 (12.16)	13 (5.18)	2.59 (1.23–5.48)	0.01
Urinary arsenic profile Median ± SE				
Total arsenic (μg/g creatinine)	29.78 ± 3.97	16.47 ± 0.93	1.03 (1.02–1.05)[b]	≤0.01
InAs%	6.47 ± 1.08	4.24 ± 0.63	1.03 (1.01–1.06)[b]	≤0.01
MMA%	9.05 ± 0.95	5.76 ± 0.46	1.03 (1.01–1.06)[b]	0.01
DMA%	83.68 ± 1.44	88.74 ± 0.75	0.97 (0.95–0.98)[b]	≤0.01

SE: standard error.
[a] Including people who were ever-smokers or who were smokers.
[b] Multivariate ORs were adjusted for the highest educational level, paternal ethnicity, cumulative cigarette smoking, alcohol drinking and pesticide usage.
[&] $p < 0.01$ for the trend test.

Table 2
Frequency of the GSTO1 and GSTO2 genotypes and the association between gene polymorphisms of arsenic-metabolism-related enzymes and UC risk.

	UC cases (n = 149) no. (%)	Control (n = 251) no. (%)	Age–gender adjusted OR (95% CI)	p	Multivariate adjusted OR (95% CI)	p
GSTO1 Ala140Asp[a]						
Ala/Ala	107(71.81)	166(66.14)	1.00		1.00	
Ala/Asp	41(27.52)	78(31.08)	0.82 (0.52–1.29)	0.39	0.70 (0.42–1.18)	0.18
Asp/Asp	1(0.67)	7(2.79)	0.22 (0.03–1.83)	0.16	0.21 (0.02–2.01)	0.18
Ala/Asp + Asp/Asp (vs. Ala/Ala)	42(28.19)	85(33.86)	0.77 (0.49–1.20)	0.25	0.66 (0.40–1.10)	0.11
Ala/Ala + Ala/Asp (vs. Asp/Asp)	148(99.33)	244(97.21)	0.24 (0.03–1.94)	0.18	0.23 (0.02–2.22)	0.20
GSTO1 Glu208Lys[b]						
Glu/Glu	145(97.32)	247(98.41)	1.00		1.00	
Glu/Lys	4(2.68)	4(1.59)	1.68 (0.41–6.85)	0.47	2.34 (0.49–11.11)	0.28
GSTO2 Asn142Asp[c]						
Asn/Asn	88(59.06)	134(53.39)	1.00		1.00	
Asn/Asp	59(39.60)	104(41.43)	0.88 (0.58–1.33)	0.53	0.85 (0.53–1.38)	0.52
Asp/Asp	2(1.34)	13(5.18)	0.24 (0.05–1.08)	0.06	0.17 (0.03–0.88)	0.03
Asn/Asp + Asp/Asp (vs. Asn/Asn)	61(40.94)	117(46.61)	0.80 (0.53–1.21)	0.30	0.76 (0.48–1.23)	0.26
Asn/Asn + Asn/Asp (vs. Asp/Asp)	147(98.66)	238(94.82)	0.25 (0.06–1.13)	0.07	0.19 (0.04–0.93)	0.04

Multivariate ORs were adjusted for the highest educational level, paternal ethnicity, cumulative cigarette smoking, alcohol drinking and pesticide usage.
[a] GSTO1 140Ala/Ala: the wild type; 140Ala/Asp: the heterozygote genotype; and 140Asp/Asp: homogeneous variant genotype.
[b] GSTO1 208Glu/Glu: the wild type; 208Glu/Lys: the heterozygote genotype; and 208Lys/Lys: homogeneous variant genotype.
[c] GSTO2 142 Asn/Asn: the wild type; 142 Asn/Asp: the heterozygote genotype; and 142Asp/Asp: homogeneous variant genotype.

results revealed a significantly higher MMA% in people who carried the wild type of GSTO1 140 Ala/Ala, as compared to those who carried the GSTO1 140 Ala/Asp and Asp/Asp genotype ($p = 0.02$). Urinary MMA% level was significantly different among three groups of people who carried GSTO2 Asn142Asp polymorphism ($p = 0.01$). However, an association was not observed between other genotypes of GSTO1 Ala140Asp, GSTO1 Glu208Lys or GSTO2 Asn142Asp and the levels of urinary total arsenic or InAs% or DMA%. In addition, while there is no homogeneous variant genotype of GSTO1 208 Lys/Lys; however, people with the heterozygous genotype of GSTO1 208 Glu/Lys had slightly higher iAs^{3+}% than those with the wild type of GSTO1 208 Glu/Glu ($0.05 < p < 0.1$; data not shown).

4. Discussion

To our knowledge, the present study is the first to evaluate the impact of GSTO1 and GSTO2 gene polymorphisms on UC susceptibility in a non-obvious inorganic arsenic exposure area in Taiwan. The GSTO2 142 Asp/Asp genotype was found to be significantly protective against UC risk. Furthermore, a significantly higher MMA% was observed in people who carried the wild type of GSTO1 140 Ala/Ala, as compared to those who carried the GSTO1 140 Ala/Asp and Asp/Asp genotype. Urinary MMA% levels were significantly different within three groups of GSTO2 Asn142Asp genotype ($p = 0.01$).

The standard concentration of arsenic in drinking water and the risk of low doses of arsenic have been extensively discussed. In 2000, the allowable arsenic level in drinking water was decreased from 50 to 10 μg/L in Taiwan, earlier than other most countries. According to the Taipei Water Department of the Taipei City Government, the average arsenic concentration in Taipei tap water is 0.7 μg/L (range from non-detectable to 4.0 μg/L). However, we randomly collected drinking water from 37 UC cases and measured the total arsenic level; the mean ± standard error was 17.14 ± 0.55 μg/L. The sum of urinary $iAs^{3\pm}$, $iAs^{5\pm}$, $MMA^{5\pm}$ and $DMA^{5\pm}$ of study participants in the present study was lower than those in our previous study (mean value of total arsenic 25 μg/ L vs. 70 μg/ L) (Hsueh et al., 1997). Nonetheless, we still observed a significantly increased risk of UC in those with unfavorable urinary arsenic profile, including higher total arsenic, or higher inorganic arsenic (%), or higher MMA (%) or lower DMA (%).

When mammals are exposed to inorganic arsenate it is reduced to arsenite. Then, arsenite is enzymatically methylated to monomethyl arsenic (MMA) and dimethylarsenic (DMA). Finally, PNP protein or MMA(V) reductase (GSTO1) protein catalyze the conversion of iAs^{5+} to MMA^{5+} or MMA^{5+} to DMA^{5+}, an arsenic species with an oxidation state

Table 3
Median ± standard error of urinary arsenic profiles for different genotypes in controls.

	n	Total arsenic	p [b]	InAs%	p [b]	MMA%	p [b]	DMA%	p [b]
GSTO1 Ala140Asp[c]									
Ala/Ala	166	15.94 ± 1.14	0.72 [a]	4.32 ± 0.62	0.81 [a]	6.23 ± 0.51	0.06 [a]	88.33 ± 0.84	0.64 [a]
Ala/Asp	78	17 ± 1.69		3.79 ± 1.48		4.03 ± 0.98		89.12 ± 1.63	
Asp/Asp	7	20.11 ± 4.90		3.48 ± 3.79		0 ± 2.32		84.04 ± 3.58	
Ala/Asp + Asp/Asp (vs. Ala/Ala)	85	17.25 ± 1.60	0.51	3.48 ± 1.39	0.53	3.73 ± 0.91	0.02	88.92 ± 1.52	0.48
Ala/Ala + Ala/Asp (vs. Asp/Asp)	244	16.38 ± 0.95	0.71	4.26 ± 0.64	0.55	5.77 ± 0.47	0.30	88.80 ± 0.77	0.65
GSTO1 Glu208Lys[d]									
Glu/Glu	247	16.47 ± 0.93	0.54	4.11 ± 0.64	0.07	5.76 ± 0.47	0.83	88.86 ± 0.77	0.54
Glu/Lys	4	22.85 ± 8.71		8.92 ± 1.77		5.00 ± 3.00		88.55 ± 3.77	
GSTO2 Asn142Asp[e]									
Asn/Asn	134	15.94 ± 1.30	0.75 [a]	4.36 ± 0.71	0.98 [a]	6.46 ± 0.67	0.01 [a]	87.52 ± 1.00	0.29 [a]
Asn/Asp	104	16.65 ± 1.41		3.49 ± 1.19		3.76 ± 0.62		89.47 ± 1.24	
Asp/Asp	13	17.25 ± 3.90		4.11 ± 2.12		6.52 ± 2.34		84.04 ± 2.72	
Asn/Asp + Asp/Asp (vs. Asn/Asn)	117	16.74 ± 1.32	0.46	3.52 ± 1.08	0.96	4.56 ± 0.61	0.30	89.01 ± 1.14	0.38
Asn/Asn + Asn/Asp (vs. Asp/Asp)	238	16.38 ± 0.96	0.99	4.29 ± 0.65	0.87	5.59 ± 0.47	0.62	88.87 ± 0.78	0.30

[a] Calculated by the Kruskal–Wallis test.
[b] Calculated by the Wilcoxon two-sample test.
[c] GSTO1 140Ala/Ala: the wild type; 140Ala/Asp: the heterozygote genotype; and 140Asp/Asp: homogeneous variant genotype.
[d] GSTO1 208Glu/Glu: the wild type; 208Glu/Lys: the heterozygote genotype; and 208Lys/Lys: homogeneous variant genotype.
[e] GSTO2 142 Asn/Asn: the wild type; 142 Asn/Asp: the heterozygote genotype; and 142Asp/Asp: homogeneous variant genotype.

of +5 to +3 (Aposhian et al., 2004). The MMA^{5+} reducing activity of GSTO1 was established from an *in vitro* study (Mukherjee et al., 2006). For GSTO1 gene knockout mice, the MMA^{5+} reducing activity of liver cytosol was estimated to be only 20% of that found in wild-type mice (Mukherjee et al., 2006), which may might point out the important function of the GSTO1 gene on arsenic metabolism. The major function of GSTO1 and GSTO2 could catalyze the conversion of the arsenic species with an oxidation state of +5 to +3; furthermore, the conversions of trivalent arsenicals to methylated pentavalent arsenic species were through the enzyme of AS3MT (Drobna et al., 2004, 2005, 2006; Chowdhury et al., 2006; Radabaugh et al., 2002). The trivalent arsenicals, particularly the trivalent methylated arsenic metabolites, have been identified as the most toxic forms of arsenic and the toxicological effects of arsenic on urothelium have been established (Eblin et al., 2008; Sens et al., 2004; Styblo et al., 2000). Although we did not measure the levels of urinary trivalent arsenicals, we supposed that the levels of urinary pentavalent arsenicals could reflect the certain extent of trivalent arsenicals (Del Razo et al., 2001; Francesconi and Kuehnelt, 2004). Therefore, we went on to further clarify whether the polymorphisms of arsenic-related metabolizing enzymes affect individual capacity of arsenic metabolism in human body.

Genes encoding GSTO1/ GSTO2 mapped to chromosomes 10q24.3 (Wood et al., 2006). Recently, many studies have verified several SNPs and researchers attempted to explore the connection between these SNPs and urinary arsenic profiles in human studies (Fujihara et al., 2007; Lindberg et al., 2007; Marnell et al., 2003; Paiva et al., 2008; Yu et al., 2003). Until now, there were no candidate gene polymorphisms of GSTO1 or GSTO2 reported and few studies were carried out to elucidate the correlation of these genotypes and urinary arsenic profiles. Paiva et al. measured the urinary arsenic species of 205 Chilean males with occupational arsenic exposure and observed the wide range of total urinary arsenic concentration of 0–600 μg/L. Among different gene polymorphisms of GSTO1 Ala140Asp, Glu155Del and Ala236Val, the GSTO1 Ala236Val heterozygote type was found to be associated with decreased urinary DMA% ($p \leq 0.045$) (Paiva et al., 2008). However, 100 Vietnamese people with GSTO1 Glu155del heterozygote type had significantly higher As^{5+} level than those with the wild homogenous type (Agusa et al., 2010). Conflicting results of other studies existed between GSTO1 Ala236Val and urinary arsenic profiles (Meza et al., 2007; Yu et al., 2003; Marnell et al., 2003). Xu et al. recruited 204 subjects from Inner Mongolia, China who were chronically exposed to arsenic in drinking water and found that the variant allele frequency of GSTO1 Ala140Asp or GSTO2 Asn142Asp was 0.17 and 0.25, respectively. These were close to 0.18 and 0.26 of GSTO1 Ala140Asp or GSTO2 Asn142Asp in our study. However, Xu et al. further analyzed urinary arsenic profiles but showed no association between the polymorphisms of GSTO1 Ala140Asp or GSTO2 Asn142Asp and urinary arsenic profiles in their results (Xu et al., 2009). In our study, people who carried the wild type of GSTO1 140 Ala/Ala had significantly increased MMA% compared to those carrying the heterozygote or variant homogenous genotype. In addition, urinary MMA% levels were significantly different among the three groups of GSTO2 Asn142Asp genotype ($p = 0.01$). The urinary MMA% level was decreased in people who carried heterozygous genotype of GSTO2 142 Asn/Asp. However we did not observe the lower urinary MMA% in people who carried the homogenous variant genotype of GSTO2 142 Asp/Asp compared to those who carried the wild type of GSTO2 142 Asn/Asn. Additional subjects are needed for further investigation. In addition, De Chaudhuri et al. recruited 229 patients of skin cancer and 199 controls exposed to similar levels of arsenic in their drinking water and to elucidate the association between the polymorphisms of AS3MT, PNP, GSTO1 and GSTO2 and arsenic-associated skin lesions (De Chaudhuri et al., 2008). In their study, the polymorphisms of PNP His20Hsi, Gly51Ser and Pro57Pro were significantly associated with arsenic-associated skin lesions, but no association was found between gene polymorphisms of GSTO1 and GSTO2 and arsenic-associated skin lesions. Although the important roles of PNP other than GSTOs on urinary arsenic profiles have been addressed (De Chaudhuri et al., 2008), the functional role of PNP for reduction of arsenate to arsenite is still not clear (Nemeti et al., 2003; Radabaugh et al, 2002). In our study, we showed the homogenous variant genotype of GSTO2 142 Asp/Asp was inversely associated with UC risk. However we did not directly observe the association between the polymorphisms of GSTO2 Asn142Asp and UC risk through the change of urinary arsenic profile.

After exclusion of those of incomplete biological specimen collection or questionnaire information, there was a relatively small sample size in our study; however, there were no differences in other covariates, such as genotyping or urinary total arsenic levels (data not shown) between people included and excluded from analysis. While the enzyme activity of GSTO1 and GSTO2 was unknown in our studies; we still observed differences in urinary MMA% among varied genotypes of GSTO1 and GSTO2. Furthermore, our findings showed a significant protective effect of the polymorphism of GSTO2 Asn142Asp on UC risk. Large-scale studies may be required to verify the association between the single nucleotide polymorphisms of arsenic-metabolism-related enzymes and UC risk.

Acknowledgments

The study was supported by grants from the National Science Council of the ROC (NSC 86-2314-B-038-038, NSC 87-2314-B-038-029, NSC 88-2314-B-038-112, NSC 89-2314-B038-049, SC-89-2320-B038-013, NSC 90-2320-B-038-021, NSC 91-3112-B-038-0019, NSC 92-3112-B-038-001, NSC 93-3112-B-038-001, NSC 94-2314-B-038-023, NSC 95-2314-B-038-007, NSC 96-2314-B038-003, NSC 97-2314-B-038-015-MY3 (1-3), and NSC 97-2314-B-038-015-MY3 (2-3)).

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