Arsenic Methylation and Bladder Cancer Risk in Case–Control Studies in Argentina and the United States

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Objective: *We sought to assess whether the metabolism of arsenic impacts a person's susceptibility to bladder cancer.* ***Methods:*** *Urinary methylation products were measured in subjects from Argentina (114 cases and 114 controls) and the United States (23 cases and 49 controls).* ***Results:*** *In Argentina, the adjusted odds ratio (OR) for subjects with a high proportion of ingested arsenic excreted as monomethylarsonate (%MMA) was 2.17 (95% confidence interval [CI] = 1.02–4.63) in smokers and 0.48 (95% CI = 0.17–1.33) in nonsmokers. In the United States, the adjusted ORs for high %MMA in subjects with arsenic intakes less than and greater than 100 μg/d were 1.20 (95% CI = 0.27–5.38) and 2.70 (95% CI = 0.39–18.6).* ***Conclusions:*** *Overall, these results are consistent with data from Taiwan suggesting that some individuals who excrete a higher proportion of ingested arsenic as MMA are more susceptible to arsenic-related cancer.* (J Occup Environ Med. *2006;48: 478–488*)

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Inorganic arsenic (InAs) occurs naturally in the groundwater and surface water of many parts of the world, and millions of people worldwide are exposed to drinking water containing this known human carcinogen.[1–8] Ingested arsenic has been associated with cancer of the skin, bladder, lung, and possibly other organs, with the highest relative risks found for cancer of the bladder.[9] The excess risks associated with these exposures may be quite high.[9–13] The National Research Council has estimated that the excess cancer risks associated with lifetime exposures to arsenic at the new US standard of 10 μg/L may be approximately 1 in 300.[9] These risks may be even greater in susceptible subpopulations if they exist. The US drinking water standards for carcinogens other than arsenic have been set at levels associated with risks between 1 in 10,000 and 1 in 1,000,000.[14] Importantly, the new US standard of 10 μg/L only applies to public water systems. Approximately 15% of the US population obtain their water from private wells.[15] Although the number of private wells with high arsenic levels is unknown, arsenic concentrations appreciably greater than 10 μg/L have been documented in some private wells in many states in the United States.[16–18]

The primary metabolic pathway of ingested InAs in humans is methylation.[19–21] Once ingested, InAs is methylated to monomethylarsonic acid (MMA5), which is reduced to monomethylarsonous acid (MMA3).

MMA3 is then methylated to dimethylarsinic acid (DMA5), which is reduced to dimethylarsinous acid (DMA3). In humans, this process is not complete, and some arsenic remains as InAs and MMA. Urinary excretion is the primary pathway of elimination of arsenic and almost all ingested arsenic is excreted through the urine.[22] For this reason, the relative distribution of arsenic metabolites in urine commonly is used as a biomarker of arsenic methylation capacity.[11] Typically, ingested InAs is excreted as 10–20% InAs, 10–15% MMA, and 60–75% DMA.[23] However, large interindividual variations exist.[24]

Until recently, methylation of InAs was thought to be primarily a detoxification pathway because the methylated species most commonly found in human urine samples, MMA5 and DMA5, are more water soluble, more readily excreted, and less acutely toxic than InAs.[20,22,25–27] Thus, the focus of earlier studies of arsenic metabolism was on the portion of urinary arsenic that remained in the inorganic form. MMA3 and DMA3 are highly unstable in human urine and so have been measured in only a few human studies. However, there is increasing evidence that MMA3 is much more toxic in vitro than its pentavalent form and may be more toxic than InAs.[28–35] These findings have led to recent interest in the role of MMA in arsenic-caused human health effects. In fact, several epidemiological studies have reported associations between individual methylation patterns, specifically the proportion of MMA, and the risks of arsenic-caused disease.[36–41] To date, all of the published studies of methylation of ingested arsenic and cancer risks have taken place in Taiwan, where large proportions of the population in some areas had been exposed to arsenic concentrations well greater than 200 μg/L.[36,37,39,41] For example, the studies by Hsueh et al[39] and Yu et al[41] took place in the highly-exposed Blackfoot Disease endemic region of Taiwan, where past arsenic exposures greater than 1000 μg/L have been reported.

In this report, we present the results of two independently conducted case–control studies on arsenic methylation and cancer, one from the Córdoba Province, Argentina, and a smaller study from the western United States. Both studies include the participants of previously published case–control investigations of bladder cancer and moderate arsenic drinking water exposures.[18,42] These are the first investigations of ingested arsenic to assess the effect of individual methylation patterns on cancer risks in populations outside of Taiwan. As in all previously published studies of methylation and cancer, the measurement of urinary methylation patterns was taken after cancer diagnosis and assumed to be representative of subject's past methylation patterns.[36,37,39,41] Evidence suggests that methylation patterns remain fairly stable over time so that recent measurements of urinary arsenic metabolites can be used to assess long term methylation patterns.[19,24,43–47]

Materials and Methods

The Argentina study area consisted of Union and Marcos Juarez, two contiguous counties in the eastern part of Córdoba Province. Patients with new-incident cases of transitional cell bladder cancer aged 20 to 80 living in Union from 1996 to 2000 or Marcos Juarez from 1998 to 2000 were identified through rapid case ascertainment involving all pathologists in the study area. Controls, which were individually matched to cases by county, gender, and their exact year of birth, were selected from computerized voter registration lists.

Subjects in the US study were a subset of participants from a case–control study of arsenic ingestion and bladder cancer that included 118 cases and 328 controls and was conducted among residents of seven counties in California and Nevada.[18] This subset included those subjects who were residents of the cities of Hanford, California, and Fallon, Nevada, or the nearby surrounding areas. These areas were chosen for this investigation because they contain the largest populations in the United States with historic exposure to moderate levels of ingested arsenic (approximately 50–100 μg/L). All new-incident cases of bladder cancer among residents of the study area were obtained from the Nevada Central Cancer Registry, the Cancer Registry of Central California, and local physicians. Cases were subjects between the ages of 40 to 85 with primary bladder cancer first diagnosed between 1994 and 2000 who lived in the study area at the time of diagnosis. Control subjects were residents of the study area selected through random digit dialing and from randomly selected lists provided by the Health Care Financing Administration, frequency matched to cases by 5-year age groups and gender.

All cases and controls were administered standardized questionnaires either in their homes (Argentina) or over the telephone (United States). Information sought included residential history, water sources at each residence, typical amount of drinking water consumed (at time of interview, and 20 and 40 years ago), smoking, occupational, and medical history. These studies were approved by the appropriate institutional review boards in the United States and Argentina, and informed consent was obtained from all participants.

In both studies, all subjects were visited at their homes and first morning urine samples were collected by study personnel. A previous study has shown that a moderately strong correlation exists between arsenic excretion in single first morning samples and samples collected over the course of 24 hours.[48] In Argentina, a single urine sample was collected from each subject. The US study was designed to assess methylation variability over time; therefore, two or three morning urine samples were collected from each

subject during a period of 1 year. Because the exact mechanism of arsenic carcinogenesis and the relative importance of short periods of high exposure compared with longer periods of lower exposure are unknown, we calculated ORs for both the average %MMA and the highest recorded %MMA in the US subjects.

In both studies, urine samples were kept frozen in the field laboratories at –20°C and then transported on dry ice to the University of Washington, Seattle, for analysis. The urinary concentrations were measured using hydride generation atomic absorption spectroscopy.[49] The details of the laboratory methods are described in Chung et al.[45] Detection limits for InAs, MMA, and DMA were 0.5, 1.0, and 2.0 μg/L, respectively. The corresponding replicate precisions were 15%, 17%, and 11%. Concentrations below the detection limit were set at one half the detection limit. The MMA and DMA measured in this study are the sums of the trivalent and pentavalent forms. The trivalent forms, MMA3 and DMA3, are rapidly oxidized during storage and at the time of this study could not be reliably measured in field studies.[50] Most samples in this study were stored frozen for 1 to 4 months before analysis.

The relative proportion of arsenic in each species (%InAs, %MMA, and %DMA) was calculated by dividing the concentration of arsenic in each species by the concentration of arsenic in InAs, MMA, and DMA combined. Unconditional logistic regression was used to calculate bladder cancer ORs comparing subjects with high and low proportions of all three species; however, given the results of the earlier studies on methylation and cancer, the focus of our results is on %MMA. The Cochran-Mantel-Haenszel statistic was used to calculate confidence intervals (CIs) for crude ORs.

For most analyses, the category cutoff point for %MMA was set at the upper tertile of the distribution of cases and controls combined, calculated separately for US and Argentina subjects. This cutoff value is a rough average of the cutoff points used in other studies of methylation and arsenic-related health effects.[36–39,41] Because there were differences in the study populations and study designs and because the studies were done at different points in time, results are presented separately for the US and Argentina subjects. Odds ratios were adjusted for age (<65, 65–75, >75 years), gender, and smoking (current smokers, ex-smokers, and never smokers). Other smoking variables, such as pack-years of smoking and average cigarettes smoked per day, also were assessed but had no impact on the results. The Argentina analysis also was adjusted for the consumption of *mate con bombilla* (a beverage made from the herb *Ilex paraguariensis*) (ever or never).[42]

For investigation of possible interaction between smoking and arsenic methylation in causing bladder cancer, separate analyses were carried out for ever- and never-smokers. Starting ages for smoking were similar among current and former smokers. In addition, all of the former smokers had quit within 5 years of the study. Thus, smoking histories of the former and current smokers were similar; therefore, this analysis was performed with former and current smokers combined. In the analyses of ever-smokers, adjustment for smoking was performed using the average number of cigarettes smoked per day. A separate analysis is presented where smokers from the Argentina and US studies are combined. In this analysis, subjects are divided into high and low levels of %MMA based on the upper tertile cutoff point of all subjects from their respective country.

Because the latency of arsenic-caused cancer is probably more than 20 years,[9] absolute levels of InAs, MMA, and DMA in urine samples collected after cancer diagnosis would only represent the period of critical exposure if subjects remained on the same single water source for at least several decades. Becuse this was not the case for almost all of our study subjects, we did not calculate bladder cancer ORs for absolute levels of arsenic species. Most of the previous Taiwanese studies on methylation and cancer present risk estimates stratified by some estimate of historic arsenic exposure.[36,37,39,41] To examine the combined effect of high levels of past arsenic exposure and high levels of %MMA and to facilitate comparison of our results to those from the Taiwan studies, we also present results stratified by an estimate of past arsenic exposure. The method presented by Bland and Altman was used to compare relative risk estimates from this analysis.[51] To determine past arsenic intake for each subject, each residence that a subject had lived at for 6 months or longer within the study area was linked to a water arsenic measurement for that residence. Historical records of drinking water arsenic content were obtained from government agencies for community water supplies and for some private wells. Where historical records were not available, water samples were collected from all current and past residences that could be located. A study by the US Geological Survey (USGS) of drinking water supplies throughout the US provides some evidence that arsenic levels in ground water remain relatively stable over time, although this evidence is somewhat limited.[1] In a separate USGS analysis of 29 wells in the Fallon, Nevada area, little temporal change was observed in the concentration of arsenic of most wells during a period from 1989 to 2001.[52] Previous studies have shown that some inhibition of arsenic methylation may occur at very high exposures.[9] However, these effects usually are seen at exposures much higher than found in our study areas and the effects are small compared to the wide interindividual variation seen in urinary arsenic methylation patterns.

By linking subjects' residential histories to drinking water arsenic measurements, an arsenic drinking water concentration was assigned to each year of each subject's life within the

TABLE 1
Sociodemographic Characteristics of Bladder Cancer Cases and Controls

	Córdoba, Argentina				Western United States			
	Cases		Controls		Cases		Controls	
	No.	%	No.	%	No.	%	No.	%
Total	114		114		23		49	
Age								
<65	35	31	35	31	4	17	9	18
65–75	41	36	41	36	9	39	22	45
>75	38	33	38	33	10	44	18	37
Gender								
Female	20	18	20	18	3	13	11	23
Male	94	82	94	82	20	87	38	77
Smoking								
Current	29	25	23	20	3	13	5	10
Former	56	49	40	35	18	78	27	55
Never	29	25	51	45	2	9	17	35
Mean drinking water arsenic concentration*								
<10 μg/L	67	59	67	59	12	52	20	41
10–200 μg/L	41	36	35	31	11	48	28	57
>200 μg/L	6	5	12	11	0	0	1	2
Mean drinking water intake (L/d)†	2.50	1.04	2.28	1.02	2.48	1.26	2.44	1.23

*Highest contiguous five-year average arsenic concentration in drinking water sources over a subject's lifetime.
†Mean and standard deviation.

study area. The daily average arsenic intake was then calculated by multiplying the arsenic drinking water concentration by the typical daily amount of drinking water consumed. Analyses were stratified on the subject's highest average contiguous 5-year arsenic intake. A category cutoff point of 100 μg/d was chosen because this corresponds to consuming two liters of water per day containing arsenic at 50 μg/L, the former US drinking water standard. Additional details on study methods are provided in Bates et al. and Steinmaus et al.[18,42] All data analyses were conducted using the SAS statistical program package (Version 8.0e, SAS Institute, Cary, NC).

Results

In the Argentina study, the participation rate was 93% among cases and 75% among controls. Further details of participation rates in the Argentina study are provided in Bates et al.[42] In the US study, 38 bladder cancer cases and 73 control subjects from the original bladder cancer case–control study had lived in the Hanford or Fallon areas and were eligible for the methylation study. Of these, 23 cases (61%) and 49 controls (67%) agreed to provide multiple urine samples during the 1-year period. Ten cases (26%) and 12 controls (16%) were contacted and declined participation. We were unable to locate five cases (13%) and 12 controls (16%). Nonparticipants were similar to participants in terms of age, gender, and smoking history.

Table 1 shows descriptive characteristics and arsenic exposure information for the Argentina and US subjects. In both studies, cases were more likely to be current or former smokers (Argentina: crude OR = 2.37; 95% CI = 1.36–4.13. US: crude OR = 5.58; 95% CI = 1.32–23.6). As shown in Table 1, most cases had mean drinking water concentrations less than 10 μg/L. In Argentina, cases were more likely to have consumed *mate con bombilla* (crude OR = 2.33; 95% CI = 0.71–8.8; data not shown).

Table 2 shows the mean relative proportions of each arsenic species stratified by case status, gender, smoking, age, and urinary arsenic. %InAs and %MMA were higher ($P < 0.01$ and $P = 0.08$, respectively), and %DMA was lower ($P < 0.01$) in the Argentina subjects than in the US subjects. In both study areas, women had lower %MMA and %InAs and higher %DMA than men. No associations were found between %MMA and the other variables presented in this Table 2.

Table 3 displays the adjusted ORs for the association between bladder cancer and urinary %MMA, dichotomized at the upper tertile, in the Argentina study. In the analysis of all subjects (ever-smokers and never-smokers combined) and the analysis confined to never-smokers, no association was observed between bladder cancer risk and %MMA. However, in analyses confined to ever-smokers, subjects with %MMA in the upper tertile had a twofold elevated risk of bladder cancer compared with subjects with a %MMA in the lower two tertiles (adjusted OR = 2.17; 95% CI = 1.02–4.63). In analyses confined to ever-smokers with arsenic intakes ≥100 μg/d, the bladder cancer adjusted OR comparing subjects with a %MMA in the upper tertile to those with lower %MMA levels was 4.10 (95% CI = 0.91–18.5). The corre-

TABLE 2
Univariate Analyses of the Proportions of Each Arsenic Species (Standard Deviations)

	Córdoba, Argentina					Western United States				
Variable	**N**	**%**	**%InAs**	**%MMA**	**%DMA**	**N**	**%**	**%InAs**	**%MMA**	**%DMA**
All	228	100	16.1 (10.0)	14.6 (9.7)	69.3 (16.3)	72	100	11.9 (4.9)	13.2 (4.1)	74.9 (6.9)
Bladder cancer										
Cases	114	50	14.9 (7.8)	14.9 (10.7)	70.2 (16.3)	23	32	11.9 (5.0)	13.4 (4.7)	74.6 (8.0)
Controls	114	50	17.3 (11.7)	14.3 (8.7)	68.3 (16.2)	49	68	11.8 (4.4)	13.1 (3.8)	75.1 (6.5)
Gender										
Women	40	18	13.7 (8.8)	13.4 (10.2)	72.9 (17.3)	14	19	9.6 (2.5)	10.4 (2.6)	80.0 (3.0)
Men	188	82	16.6 (10.2)	14.8 (9.7)	68.5 (16.0)	58	81	12.4 (4.8)	13.9 (4.1)	73.7 (7.1)
Smoking										
Current	52	23	17.0 (8.7)	14.5 (7.4)	68.5 (12.2)	8	11	13.7 (7.1)	13.3 (6.2)	73.0 (11.9)
Ex-smokers	96	42	15.5 (7.8)	14.4 (10.0)	70.1 (15.4)	45	63	11.5 (4.1)	13.5 (3.9)	75.0 (6.0)
Never	80	35	16.2 (12.8)	14.9 (10.8)	68.9 (19.4)	19	26	11.8 (4.5)	12.6 (3.6)	75.6 (6.7)
Age										
<65	70	31	17.7 (8.1)	14.3 (8.7)	68.0 (14.4)	13	18	12.4 (2.2)	12.1 (2.9)	75.5 (4.7)
65–75	82	36	15.1 (7.9)	13.9 (8.6)	71.0 (15.1)	31	43	13.2 (5.2)	13.6 (4.6)	73.3 (8.3)
>75	76	33	15.8 (13.1)	15.6 (11.7)	68.6 (19.0)	28	39	10.2 (4.3)	13.3 (3.9)	76.5 (5.9)
Urinary arsenic*										
Low tertile	76	33	17.5 (14.3)	16.1 (14.3)	66.4 (23.6)	24	33	13.0 (5.9)	13.2 (4.5)	73.8 (8.9)
Medium tertile	76	33	15.8 (6.8)	12.9 (6.8)	71.3 (11.3)	24	33	11.2 (3.6)	13.0 (4.5)	75.8 (6.1)
High tertile	76	33	15.0 (7.1)	14.7 (5.7)	70.2 (10.3)	24	33	11.4 (3.9)	13.4 (3.0)	75.2 (5.5)

*This is the sum of arsenic concentrations in the form of InAs, MMA, and DMA. Tertile cutoff points for this value for the Argentina study were 7.3 and 17.8 μg/L. Tertile cutoff points for the US study were 10.6 and 22.0 μg/L.

sponding adjusted OR for smokers with arsenic exposures less than 100 μg/d was lower than this (adjusted OR = 2.06; 95% CI = 0.81–5.22), although the difference between these OR was not statistically significant ($P = 0.45$). The median arsenic drinking water concentrations for subjects in the <100 μg/d and ≥100 μg/d categories were 1.1 μg/L (standard deviation [SD] = 19.4 μg/L) and 145.7 μg/L [SD = 170.7 μg/L], respectively.

Table 4 shows the logistic regression analysis for the US subjects. In the analysis involving all subjects, the bladder cancer adjusted OR for subjects in the upper tertile of average %MMA compared to those in the lower two tertiles was 1.19 (95% CI = 0.38–3.68). In analyses confined to subjects with arsenic intakes ≥100 μg/d, the adjusted OR for those in the upper tertile of average %MMA was 2.70 (95% CI = 0.39–18.6). The corresponding adjusted OR for subjects in the upper tertile of highest recorded %MMA was 6.24 (95% CI = 0.89–43.7). In never-smokers and ever-smokers, the adjusted OR for average %MMA in the upper tertile were 4.33 (95% CI = 0.21–90.8) and 0.85 (95% CI = 0.25–2.85), respectively. Too few subjects were available in the US study to calculate separate adjusted ORs for never-smokers and ever-smokers in subjects with arsenic intakes ≥100 μg/d. The median arsenic drinking water concentration in US subjects with arsenic intakes below and ≥100 μg/d were 4.2 μg/L (SD = 8.8 μg/L) and 100.0 μg/L (SD = 43.6 μg/L), respectively. When US and Argentina subjects were combined, in an analysis confined to smokers, the adjusted OR for subjects in the upper tertile of %MMA compared to those in the lower two tertiles was 1.79 (95% CI = 0.98–3.28).

Associations were not seen between %InAs or %DMA and bladder cancer risks in either the US or Argentina studies. For example, in analyses confined smokers, the adjusted ORs for Argentina subjects in the upper tertile of %InAs and %DMA compared with those in the two lower tertiles were 0.85 (95% CI = 0.28–2.59) and 1.19 (95% CI = 0.41–3.47), respectively.

Discussion

The direction and magnitude of the positive relative risk estimates for %MMA we identified in smokers from the Argentina study and in all subjects with arsenic intakes ≥100 μg/L from the US study are similar to the overall results reported in other studies of arsenic methylation and cancer. The results of the previously published studies on methylation of ingested arsenic in drinking water and cancer are presented in Table 5. As shown, all previous studies have taken place in Taiwan and all involved a relatively small number of cases. In several of the Taiwan studies, results are presented for the MMA/DMA ratio. Interindividual variability in MMA/DMA ratios is more dependent on %MMA than %DMA because the interindividual variability in %MMA generally is much greater than the interindividual variability in %DMA.[43,53,54] Thus, the relative risks identified for MMA/DMA ratio are more likely caused by differences in %MMA than differences in %DMA. Given this, the findings in all of the studies

TABLE 3
Bladder Cancer Odds Ratios (OR) for Low and High Levels of %MMA, Córdoba Province, Argentina

	All Subjects						Never-Smokers						Ever-Smokers					
	Case	Cont	OR*	95% CI*	OR†	95% CI†	Case	Cont	OR*	95% CI*	OR†	95% CI†	Case	Cont	OR*	95% CI*	OR†	95% CI†
All subjects																		
%MMA < 16.7%	73	79	Ref	—	Ref	—	22	31	Ref	—	Ref	—	51	48	Ref	—	Ref	—
%MMA ≥ 16.7%	41	35	1.27	0.73–2.20	1.33	0.74–2.39	7	20	0.49	0.18–1.37	0.48	0.17–1.33	34	15	2.13	1.03–4.40	2.17	1.02–4.63
Arsenic intake <100 μg/d‡																		
%MMA < 16.7%	54	54	Ref	—	Ref	—	16	22	Ref	—	Ref	—	38	32	Ref	—	Ref	—
%MMA ≥ 16.7%	28	26	1.07	0.56–2.07	1.15	0.58–2.29	5	15	0.46	0.14–1.52	0.42	0.12–1.44	23	11	1.76	0.75–4.16	2.06	0.81–5.22
Arsenic intake ≥100 μg/d‡																		
%MMA < 16.7%	19	25	Ref	—	Ref	—	6	9	Ref	—	Ref	—	13	16	Ref	—	Ref	—
%MMA ≥ 16.7%	13	9	1.90	0.67–5.37	2.78	0.79–9.82	2	5	0.60	0.09–4.17	0.61	0.09–4.26	11	4	3.38	0.87–13.2	4.10	0.91–18.5

*Unadjusted odds ratios and 95% confidence intervals.
†Odds ratios adjusted for age, gender, bombilla use (never vs ever), and smoking (current, former, and never). In the analysis of ever-smokers, adjustment for smoking is based on the average number of cigarettes smoked per day.
‡Arsenic intake refers to the highest contiguous five-year average arsenic intake in a subject's lifetime. The median arsenic drinking water concentrations for subjects in the <100 μg/d and ≥100 μg/d categories were 1.1 μg/L and 145.7 μg/L, respectively.
OR, odds ratio; CI, confidence interval; Ref, reference group; %MMA, the proportion of inorganic arsenic excreted in urine as MMA.

presented in Table 5 are consistent with the hypothesis that elevated proportions of MMA are associated with increased risks of cancer. Despite the small number of subjects and relatively wide confidence intervals in each investigation, the consistency of these findings, across different studies and different study populations, supports the hypothesis that individual differences in arsenic methylation patterns are associated with arsenic-related cancer susceptibility.

The results of Argentina investigation suggest that the impact of arsenic methylation in cancer causation is greatest in smokers, which is consistent with other epidemiologic evidence suggesting a strong synergistic relationship between smoking and arsenic. For example, in four bladder cancer studies involving low arsenic exposures, elevated risks associated with arsenic intake were identified only in smokers.[3,18,42,55] Synergistic relationships also have been identified between smoking and both ingested and inhaled arsenic in studies of arsenic-induced lung cancer.[56–58] Evidence of a synergistic relationship between smoking and %MMA was not observed in the US study, although this analysis involved very few study subjects, especially in the higher arsenic intake group. As a result, the ORs are highly unstable and confidence intervals are very wide.

In the US study, the OR in the analysis of highest recorded %MMA was greater than the OR in the analysis of average %MMA (6.26 vs. 2.70). This difference could indicate that peak %MMA is more strongly associated with cancer risks than average %MMA, or it may indicate that peak %MMA is a better predictor of past %MMA than average %MMA. Given the small number of cases and the limited number of samples taken per subject however, this difference is difficult to interpret and could be due to chance.

In the Taiwan studies presented in Table 5, consistent effects between methylation and cancer risks are

TABLE 4
Bladder Cancer Odds Ratios (OR) for Low and High Levels of %MMA, Western United States

	All Subjects						Never-Smokers						Ever-Smokers					
	Case	Cont	OR*	95% CI*	OR†	95% CI†	Case	Cont	OR*	95% CI*	OR†	95% CI†	Case	Cont	OR*	95% CI*	OR†	95% CI†
All subjects																		
Average %MMA <14.9%‡	14	34	Ref	—	Ref	—	1	14	Ref	—	Ref	—	13	20	Ref	—	Ref	—
Average %MMA ≥14.9%	9	15	1.46	0.52–4.10	1.19	0.38–3.68	1	3	4.67	0.22–97.5	4.33	0.21–90.8	8	12	1.03	0.33–3.19	0.85	0.25–2.85
Highest %MMA <17.7%‡	14	34	Ref	—	Ref	—	0	14	—	—	—	—	14	20	Ref	—	Ref	—
Highest %MMA ≥17.7%	9	15	1.46	0.52–4.10	1.30	0.42–4.00	2	3	—	—	—	—	7	12	0.83	0.26–2.65	0.68	0.20–2.32
Arsenic intake <100 μg/d§																		
Average %MMA <14.9%	10	21	Ref	—	Ref	—	0	7	—	—	—	—	10	14	Ref	—	Ref	—
Average %MMA ≥14.9%	4	10	0.84	0.21–3.35	0.78	0.17–3.61	0	2	—	—	—	—	4	8	0.70	0.16–2.98	0.63	0.13–3.08
Highest %MMA <17.7%	11	20	Ref	—	Ref	—	0	7	—	—	—	—	11	13	Ref	—	Ref	—
Highest %MMA ≥17.7%	3	11	0.50	0.11–2.16	0.43	0.09–2.12	0	2	—	—	—	—	3	9	0.39	0.08–1.83	0.33	0.32–1.73
Arsenic intake ≥100 μg/d§																		
Average %MMA <14.9%	4	13	Ref	—	Ref	—	1	7	Ref	—	—	—	3	6	Ref	—	—	—
Average %MMA ≥14.9%	5	5	3.25	0.61–17.3	2.70	0.39–18.6	1	1	7.00	0.22–226	—	—	4	4	2.00	0.28–14.2	—	—
Highest %MMA <17.7%	3	14	Ref	—	Ref	—	0	7	—	—	—	—	3	7	Ref	—	—	—
Highest %MMA ≥17.7%	6	4	7.00	1.18–41.3	6.24	0.89–43.7	2	1	—	—	—	—	4	3	3.11	0.41–23.4	—	—

*Unadjusted odds ratios and 95% confidence intervals.

†Odds ratios adjusted for age, gender, and smoking (current, former, and never). In the analysis of ever-smokers, adjustment for smoking is based on the average number of cigarettes smoked per day. Adjusted odds ratios could not be calculated in some analyses of ever-smokers and never-smokers due to the small number of study subjects.

‡In this study, two to three urine samples were collected from each subject. "Average %MMA" refers to the mean value of %MMA of all samples collected from each subject. 14.9% is the upper tertile cutoff point of this variable. "Highest %MMA" refers to the single highest proportion recorded among all samples collected from each subject. 17.7% is the upper tertile cutoff point of this variable.

§Arsenic intake refers to the highest contiguous five-year average arsenic intake in a subject's lifetime. The median arsenic drinking water concentration in US subjects with arsenic intakes below and ≥100 μg/d were 4.2 μg/L and 100.0 μg/L, respectively.

OR, odds ratio; CI, confidence interval; Ref, reference group; %MMA, the proportion of inorganic arsenic excreted in urine as MMA.

TABLE 5
Studies of Methylation Capacity and Arsenic-Associated Cancer

Study	Outcome	Key Findings		
Hsueh et al., 1997 (Taiwan)[39]	Skin cancer (16 cases)	CAE* %MMA	OR	95% CI
		≤20 ≤ 26.7	Ref	—
		≤20 > 26.7	3.00	0.32–27.83
		>20 ≤ 26.7	8.35	1.07–65.00
		>20 > 26.7	23.96	2.55–225.2
Yu et al., 2000 (Taiwan)[41]	Skin cancer (26 cases)	Species	OR†	95% CI
		%InAs > 12.3%	3.50	0.73–16.85
		%MMA > 15.5%	5.50	1.22–24.81
		%DMA < 72.2%	3.25	1.06–9.97
		MMA/DMA > 0.22	3.33	0.92–12.11
Chen et al., 2003 (Taiwan)[36]	Skin cancer (76 cases)	MMA/DMA < 0.20	OR	95% CI
		CAE ≤ 2*	Ref	—
		CAE > 2–15	1.68	0.52–5.39
		CAE > 15	1.89	0.60–6.01
		MMA/DMA > 0.20		
		CAE ≤ 2	Ref	—
		CAE > 2–15	2.53	0.54–11.85
		CAE > 15	7.48	1.65–33.99
Chen et al., 2003 (Taiwan)[37]	Bladder cancer (49 cases)	MMA/DMA <0.21	OR	95% CI
		CAE ≤ 2*	Ref	—
		CAE > 2–12	0.49	0.10–2.50
		CAE > 12	1.12	0.26–4.77
		MMA/DMA > 0.21		
		CAE ≤ 2	Ref	—
		CAE > 2–12	0.54	0.09–3.33
		CAE > 12	4.23	1.12–16.01
Argentina	Bladder cancer (114 cases)	Non-smokers	OR	95% CI
		%MMA <16.7	Ref	—
		%MMA ≥ 16.7	0.48	0.17–1.33
		Smokers		
		%MMA <16.7	Ref	—
		%MMA ≥ 16.7	2.17	1.02–4.63
United States	Bladder cancer (23 cases)	Arsenic intake >100 μg/d	OR	95% CI
		Ave. %MMA <14.9‡	Ref	—
		Ave. %MMA ≥ 14.9	2.70	0.39–18.6
		High. %MMA <17.7§	Ref	—
		High. %MMA ≥ 17.7	6.24	0.89–43.7

*Cumulative arsenic exposure in mg/L-yr
†Odds ratios for high vs low proportion of each arsenic species. For example, the odds ratio in subjects with %InAs > 12.3% compared to subjects with %InAs ≤ 12.3% was 3.50.
‡Odds ratios are for the average %MMA recorded for each subject.
§Odds ratios are for the highest %MMA recorded for each subject.
OR, odds ratio; CI, confidence interval; Ref, reference group.

found in analyses involving highly exposed subjects, but little information is available on the effects of methylation in subjects with moderate arsenic exposures. In two of the Taiwan studies, risk estimates are presented for subjects in moderate categories of cumulative arsenic exposure (CAE).[36,37] However, the middle category of CAE includes a small number of subjects. For example, in the bladder cancer study by Chen et al, the middle category of CAE includes only four cases. Subjects in these two studies were recruited from hospitals near the highly exposed Blackfoot Disease region of Taiwan, although the average historic water arsenic concentrations in the study subjects were not presented. As noted by the authors of both investigations, [36,37] these studies "may be generalizable to populations with high and low levels of CAE, but not to those with medium CAE, because sample size limitations."[36,37] One of the primary differences between our studies and the Taiwanese studies is that we identified associations between %MMA and arsenic-related cancer in areas where only a few subjects were exposed to arsenic concentrations greater than 200 μg/L.

In all of the studies presented in Table 5, the assessment of methylation patterns and disease status was

cross-sectional in nature, that is, urinary methylation products were measured after disease status was determined. Because the latency of arsenic-caused cancer may be several decades or more,[18,42] this raises questions about the validity of using a recent assessment of methylation to estimate past methylation patterns. Some evidence exists that stable genetic factors play a strong role in determining individual methylation patterns.[19,24,43–47] In the US study subjects, the intraclass correlation coefficients for %MMA in samples taken from the same subjects an average of 258 days apart was 0.46 (95% CI = 0.24–0.64), suggesting that individual methylation patterns remain fairly stable over time.[59] Intraindividual variability over the course of time, as well as imprecision in laboratory analyses, could lead to misclassification of past methylation patterns. Importantly though, because we collected data from cases and controls using the same protocols, imprecision and variability are likely be similar among cases and controls and therefore would likely bias relative risk estimates toward the null, not cause the positive associations summarized in Table 5.

The assessment of methylation after cancer diagnosis also raises concerns about the temporal relationship between disease and methylation capacity. The effects seen in these studies might not be due to the impact of methylation patterns on disease, but rather, due to the impact of disease or disease treatment on methylation patterns. However, three of the studies presented in Table 5 involved nonmelanoma skin cancer, which would not be expected to have systemic effects. Other studies have reported associations between %MMA and the development of arsenic-caused skin lesions and the presence of chromosomal aberrations in lymphocytes.[38,40] These conditions generally are benign and would not be expected to cause the major systemic metabolic changes that can be caused by more fatal diseases. Since associations were found in studies involving these relatively benign conditions, it seems unlikely that the results in Table 5 were due to the impact of disease on methylation capacity.

At the time of this study, it was not practicable to measure MMA3 and DMA3 because of the instability of these metabolites in urine. More recently, methods have been developed to measure these agents.[60,61] It is possible that MMA3 is the primary carcinogenic agent and that the relative proportion of this agent plays a role in susceptibility. If so, the pentavalent metabolites we measured may simply be markers of the degree to which the corresponding trivalent metabolites are formed. Currently, it is unknown whether the measurement of %MMA5 might serve as good surrogate for %MMA3. As discussed previously, several in vitro studies have shown that MMA3 is more acutely toxic than InAs. In a recent study in an arsenic-exposed region in Mexico, %MMA3 levels were higher in subjects with arsenic-caused skin lesions (mean %MMA3 = 7.7%, $n = 55$) than in exposed subjects without skin lesions (mean %MMA3 = 5.9%, $n = 21$, $P = 0.072$).[62] Whether similar effects occur in subjects with arsenic-caused cancer is unknown.

In conclusion, the results of the two investigations reported here provide additional evidence that methylation patterns are associated with the risks of arsenic-related bladder cancer. These results also provide some evidence that the relationship between methylation patterns and cancer causation may be greater in smokers than in nonsmokers and may occur in areas where arsenic exposures generally are less than 200 μg/L. Currently, little is known about the factors that control arsenic methylation in humans. The results of several studies suggest that inherited genetic traits play an important role in controlling this metabolic process,[19,24,43–46] although other data suggest environmental influences, such as diet and other lifestyle factors, also may play a role.[11,38,39,54,63–66] Future studies on the factors that regulate arsenic methylation and the health impacts of the trivalent methylated metabolites of inorganic arsenic could add insights into the carcinogenic mechanisms of arsenic and provide input into establishing a safe and effective drinking water standard for this common contaminant.

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References

1. Focazio MJ, Welch AH, Watkins SA, Helsel DR, and Horn MA. *A Retrospective Analysis on the Occurrence of Arsenic in Ground-water Resources in the United States and Limitations in Drinking-water-supply Characterizations*. Reston, VA: US Geological Survey; 2000.
2. Luo ZD, Zhang YM, Ma L, et al. Chronic arsenicism and cancer in Inner Mongolia—consequences of well-water arsenic levels greater than 50 ug/l. In: Abernathy CO, Calderon RL, Chappel WR, eds. *Arsenic: Exposure and Health Effects.* London: Chapman and Hall; 1997: 55–68.
3. Kurttio P, Pukkala E, Kahelin H, Auvinen A, Pekkanen J. Arsenic concentrations in well water and risk of bladder and kidney cancer in Finland. *Environ Health Perspect*. 1999;107:1–6.
4. Cebrian ME, Albores A, Aquilar M, Blakely E. Chronic arsenic poisoning in the north of Mexico. *Hum Toxicol*. 1983; 2:121–133.
5. Hopenhayn-Rich C, Biggs ML, Fuchs A, et al. Bladder cancer mortality associated with arsenic in drinking water in Argentina [published erratum appears in *Epidemiology* 1997;8:334]. *Epidemiology*. 1996;7:117–124.
6. Smith AH, Goycolea M, Haque R, Biggs ML. Marked increase in bladder and lung cancer mortality in a region of Northern Chile due to arsenic in drinking water. *Am J Epidemiol*. 1998;147:660–669.
7. Chen CJ, Kuo TL, Wu MM. Arsenic and cancer [letter]. *Lancet*. 1988;1:414–415.
8. Chowdhury UK, Biswas BK, Chowdhury

TR, et al. Groundwater arsenic contamination in Bangladesh and West Bengal, India. *Environ Health Perspect*. 2000; 108:393–397.
9. NRC. *Arsenic in Drinking Water 2001 Update*. Washington, DC: Subcommittee to Update the 1999 Arsenic in Drinking Water Report. National Research Council; 2001.
10. Smith AH, Hopenhayn-Rich C, Bates MN, et al. Cancer risks from arsenic in drinking water [see comments]. *Environ Health Perspect*. 1992;97:259–267.
11. NRC. *Arsenic in Drinking Water*. Washington, DC: Subcommittee on Arsenic in Drinking Water. National Research Council; 1999:229–250.
12. Morales K, Ryan L, Kuo T, Wu M, Chen C. Risk of internal cancers from arsenic in drinking water. *Environ Health Perspect*. 2000;108:655–661.
13. Chen CJ, Chen CW, Wu MM, Kuo TL. Cancer potential in liver, lung, bladder and kidney due to ingested inorganic arsenic in drinking water. *Br J Cancer*. 1992;66:888–892.
14. Smith A, Lopipero P, Bates M, Steinmaus C. Arsenic epidemiology and drinking water standards. *Science*. 2002; 296:2145–2146.
15. U.S. Geological Survey. Estimated Use of Water in the United States in 2000. 2004. Available at: http://water.usgs.gov/pubs/circ/2004/circ1268/. Accessed December 20, 2005.
16. Welch AH, Helsel DR, Focazio MJ, Watkins SA. Arsenic in ground water supplies of the United States. In: Chappell WR, Abernathy CO, Calderon RL, eds. *Arsenic Exposure and Health Effects: Proceedings of the Third International Conference on Arsenic Exposure and Health Effects, July 12–15, 1998, San Diego, California.* Amsterdam; New York: Elsevier Science Ltd; 1999:9–17.
17. Ayotte JD, Montgomery DL, Flanagan SM, Robinson KW. Arsenic in groundwater in eastern New England: occurrence, controls, and human health implications. *Environ Sci Technol*. 2003; 37:2075–2083.
18. Steinmaus C, Yuan Y, Bates MN, Smith AH. Case-control study of bladder cancer and drinking water arsenic in the western United States. *Am J Epidemiol*. 2003; 158:1193–1201.
19. Vahter M. Mechanisms of arsenic biotransformation. *Toxicology*. 2002;181: 211–217.
20. Gebel TW. Arsenic methylation is a process of detoxification through accelerated excretion. *Int J Hyg Environ Health*. 2002;205:505–508.
21. Styblo M, Drobna Z, Jaspers I, Lin S, Thomas DJ. The role of biomethylation in toxicity and carcinogenicity of arsenic: a research update. *Environ Health Perspect*. 2002;110(Suppl 5):S767–S771.
22. Buchet JP, Lauwerys R, Roels H. Comparison of the urinary excretion of arsenic metabolites after a single oral dose of sodium arsenite, monomethylarsonate, or dimethylarsinate in man. *Int Arch Occup Environ Health*. 1981;48:71–79.
23. Hopenhayn-Rich C, Smith AH, Goeden HM. Human studies do not support the methylation threshold hypothesis for the toxicity of inorganic arsenic. *Environ Res*. 1993;60:161–177.
24. Vahter M. Variation in human metabolism of arsenic. In: Chappell WR, Abernathy CO, Calderon RL, eds. *Arsenic: Exposure and Health Effects.* Amsterdam: Elsevier; 1999:267–279.
25. Hughes MF, Kenyon EM. Dose-dependent effects on the disposition of monomethylarsonic acid and dimethylarsinic acid in the mouse after intravenous administration. *J Toxicol Environ Health Part A*. 1998;53:95–112.
26. Buchet JP, Lauwerys R, Roels H. Urinary excretion of inorganic arsenic and its metabolites after repeated ingestion of sodium metaarsenite by volunteers. *Int Arch Occup Environ Health*. 1981;48: 111–118.
27. Moore MM, Harrington-Brock K, Doerr CL. Relative genotoxic potency of arsenic and its methylated metabolites. *Mutat Res*. 1997;386:279–290.
28. Mass MJ, Tennant A, Roop BC, et al. Methylated trivalent arsenic species are genotoxic. *Chem Res Toxicol*. 2001;14: 355–361.
29. Cullen WR, McBride BC, Manji H, Pickett AW, Reglinski J. The metabolism of methylarsine oxide and sulfide. *Appl Organometal Chem*. 1989;3:71–78.
30. Styblo M, Serves S, Cullen W, Thomas D. Comparative inhibition of yeast glutathione reductase by arsenicals and arsenothiols. *Chem Res Toxicol*. 1997;10: 27–33.
31. Styblo M, Vega L, Germolec DR, et al. Metabolism and toxicity of arsenicals in cultured cells. In: Chappell WR, Abernathy CO, and Calderon RL, eds. *Arsenic: Exposure and Health Effects.* Amsterdam: Elsevier Science Ltd; 999:311–324.
32. Styblo M, Del Razo LM, Vega L, et al. Comparative toxicity of trivalent and pentavalent inorganic and methylated arsenicals in rat and human cells. *Arch Toxicol*. 2000;74:289–299.
33. Lin S, Cullen WR, Thomas DJ. Methylarsenicals and arsinothiols are potent inhibitors of mouse liver thioredoxin reductase. *Chem Res Toxicol*. 1999;12: 924–930.
34. Lin S, Del Razo LM, Styblo M, Wang C, Cullen WR, Thomas DJ. Arsenicals inhibit thioredoxin reductase in cultured rat hepatocytes. *Chem Res Toxicol*. 2001;14: 305–311.
35. Petrick JS, Ayala-Fierro F, Cullen WR, Carter DE, Aposhian VH. Monomethylarsonous acid (MMA(III)) is more toxic than arsenite in Chang human hepatocytes. *Toxicol Appl Pharmacol*. 2000; 163:203–207.
36. Chen YC, Guo YL, Su HJ, et al. Arsenic methylation and skin cancer risk in southwest Taiwan. *J Occup Environ Med*. 2003;45:241–248.
37. Chen YC, Su HJ, Guo YL, et al. Arsenic methylation and bladder cancer risk in Taiwan. *Cancer Causes Control*. 2003; 14:303–310.
38. Del Razo LM, Garcia-Vargas GG, Vargas H, et al. Altered profile of urinary arsenic metabolites in adults with chronic arsenicism. A pilot study. *Arch Toxicol*. 1997;71:211–217.
39. Hsueh YM, Chiou HY, Huang YL, et al. Serum beta-carotene level, arsenic methylation capability, and incidence of skin cancer. *Cancer Epidemiol Biomarkers Prev*. 1997;6:589–596.
40. Maki-Paakkanen J, Kurttio P, Paldy A, Pekkanen J. Association between the clastogenic effect in peripheral lymphocytes and human exposure to arsenic through drinking water. *Environ Mol Mutagen*. 1998;32:301–313.
41. Yu RC, Hsu KH, Chen CJ, Froines JR. Arsenic methylation capacity and skin cancer. *Cancer Epidemiol Biomarkers Prev*. 2000;9:1259–1262.
42. Bates M, Rey O, Biggs M, et al. Case-control study of bladder cancer and exposure to arsenic in Argentina. *Am J Epidemiol*. 2004;159:381–389.
43. Vahter M. Methylation of inorganic arsenic in different mammalian species and population groups. *Sci Prog*. 1999;82: 69–88.
44. Vahter M. Genetic polymorphism in the biotransformation of inorganic arsenic and its role in toxicity. *Toxicol Lett.* 2000;112–113:209–217.
45. Chung JS, Kalman DA, Moore LE, et al. 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.
46. Chiou HY, Hsueh YM, Hsieh LL, et al. Arsenic methylation capacity, body retention, and null genotypes of glutathione S-transferase M1 and T1 among current

arsenic-exposed residents in Taiwan. *Mutat Res*. 1997;386:197–207.
47. Concha G, Volger G, Nermell B, Vahter M. Intra-individual variation in the metabolism of inorganic arsenic. *Int Arch Occup Environ Health*. 2002;75:576–580.
48. Calderon R, Hudgens E, Le XC, Schreinmachers D, Thomas DJ. Excretion of arsenic in urine as a function of exposure to arsenic in drinking water. *Environ Health Perspect*. 1999;107:663–667.
49. Crecelius EA. Modification of the arsenic speciation technique using hydride generation. *Anal Chem*. 1978;50:826–827.
50. Del Razo LM, Styblo M, Cullen WR, Thomas DJ. Determination of trivalent methylated arsenicals in biological matrices. *Toxicol Appl Pharm*. 2001;174:282–293.
51. Altman D, Bland J. Interaction revisited: the difference between two estimates. *BMJ*. 2003;326:219.
52. Seiler RL. Temporal changes in water quality at a childhood leukemia cluster. *Ground Water*. 2004;42:446–455.
53. Buchet JP, Geubel A, Pauwels S, Mahieu P, Lauwerys R. The influence of liver disease on the methylation of arsenite in humans. *Arch Toxicol*. 1984;55:151–154.
54. Hopenhayn-Rich C, Biggs ML, Smith AH, Kalman DA, Moore LE. Methylation study of a population environmentally exposed to arsenic in drinking water. *Environ Health Perspect*. 1996;104:620–628.
55. Bates M, Smith A, Cantor K. Case-control study of bladder cancer and arsenic in drinking water. *Am J Epidemiol*. 1995;141:523–530.
56. Ferreccio C, Gonzalez C, Milosavjlevic V, Marshall G, Sancha AM, Smith AH. Lung cancer and arsenic concentrations in drinking water in Chile. *Epidemiology*. 2000;11:673–679.
57. Hertz-Picciotto I, Smith AH, Holtzman D, Lispett M, Alexeef G. Synergism between occupational arsenic exposure and smoking in the induction of lung cancer. *Epidemiology*. 1992;3:23–31.
58. Tsuda T, Babazono A, Yamamoto E, et al. Ingested arsenic and internal cancer: a historical cohort study followed for 33 years. *Am J Epidemiol*. 1995;141:198–209.
59. Steinmaus C, Yuan Y, Kalman D, Atallah R, Smith A. Intra-individual variability in arsenic methylation in a US Population. *Cancer Epidemiol Biomarkers Prev*. 2005;14:919–924.
60. Aposhian HV, Gurzau ES, Le XC, et al. Occurrence of monomethylarsonous acid in urine of humans exposed to inorganic arsenic. *Chem Res Toxicol*. 2000;13:693–697.
61. Le XC, Ma M, Lu X, Cullen WR, Aposhian HV, Zheng B. Determination of monomethylarsonous acid, a key arsenic methylation intermediate, in human urine. *Environ Health Perspect*. 2000;108:1015–1018.
62. Valenzuela O, Borja-Aburto V, Garcia-Vargas G, et al. Urinary trivalent methylated arsenic species in a population chronically exposed to inorganic arsenic. *Environ Health Perspect*. 2005;113:250–254.
63. Hsueh YM, Ko YF, Huang YK, et al. Determinants of inorganic arsenic methylation capacity among residents of the Lanyang Basin, Taiwan: arsenic and selenium exposure and alcohol consumption. *Toxicol Lett*. 2003;137:49–63.
64. Kurttio P, Komulainen H, Hakala E, Kahelin H, Pekkanen J. Urinary excretion of arsenic species after exposure to arsenic present in drinking water. *Arch Environ Contam Toxicol*. 1998;34:297–305.
65. Hsueh YM, Huang YL, Huang CC, et al. Urinary levels of inorganic and organic arsenic metabolites among residents in an arseniasis-hyperendemic area in Taiwan. *J Toxicol Environ Health*. 1998;54:431–444.
66. Chen CJ, Wu MW, Lee SS, Wang JD, Cheng SH, Wu HY. Atherogenicity and carcinogenicity of high-arsenic artesian well water: multiple risk factors and related malignant neoplasms of blackfoot disease. *Arteriosclerosis*. 1988;8:452–460.