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A Case-Control study of polymorphisms in xenobiotic and arsenic metabolism genes and arsenic-related bladder cancer in New Hampshire

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Abstract

Arsenic is associated with bladder cancer risk even at low exposure levels. Genetic variation in enzymes involved in xenobiotic and arsenic metabolism may modulate individual susceptibility to arsenic-related bladder cancer. Through a population-based case-control study in NH (832 cases and 1191 controls), we investigated gene-environment interactions between arsenic metabolic gene polymorphisms and arsenic exposure in relation to bladder cancer risk. Toenail arsenic concentrations were used to classify subjects into low and high exposure groups. Single nucleotide polymorphisms (SNPs) in *GSTP1, GSTO2, GSTZ1, AQP3, AS3MT* and the deletion status of *GSTM1* and *GSTT1* were determined. We found evidence of genotype-arsenic interactions in the high exposure group; *GSTP1* Ile105Val homozygous individuals had an odds ratio (OR) of 5.4 [95% confidence interval (CI): 1.5-20.2; *P* for interaction = 0.03] and *AQP3* Phe130Phe carriers had an OR=2.2 (95% CI: 0.8-6.1; *P* for interaction = 0.10). Bladder cancer risk overall was associated with *GSTO2* Asn142Asp (homozygous; OR=1.4; 95% CI: 1.0-1.9; *P* for trend=0.06) and *GSTZ1* Glu32Lys (homozygous; OR=1.3; 95%CI: 0.9-1.8; *P* for trend=0.06). Our findings suggest that susceptibility to bladder cancer may relate to variation in genes involved in arsenic metabolism and oxidative stress response and potential gene-environment interactions requiring confirmation in other populations.

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Keywords

arsenic; genetic polymorphisms; bladder cancer; case-control study; gene-environment interaction

1. INTRODUCTION

In the United States, bladder cancer is a considerable public health problem; an estimated 69,250 new cases occurred in 2011 (Siegel et al. 2011). Among recognized environmental risk factors for bladder cancer are cigarette smoke and arsenic exposure through drinking water [International Agency for Research on Cancer (IARC) 2004; European Food Safety Authority (EFSA) 2009]. Evidence includes an increased risk of bladder cancer in relation to arsenic exposure in US populations (Bates et al. 1995; Steinmaus et al. 2003; Karagas et al. 2004) with relatively low level exposures. The incidence rate of this malignancy is higher in New Hampshire compared to the rest of the country as indicated by data from The National Program of Cancer Registries Cancer Surveillance System and The Surveillance, Epidemiology and End Results program (NPCR-CSS/SEER 2010). Ground well water contamination occurs naturally in New Hampshire because of high arsenic content in rock formations (Peters et al. 1999). As a result over 10% of the state's unregulated private wells have arsenic levels beyond U.S. Maximum Contaminant Level (MCL) of 10 micrograms per liter (μg/l) (Karagas et al. 1998).

In drinking water, arsenic can be found in different subspecies: arsenite (AsIII) and arsenate (AsV). Arsenate is reduced to arsenite by two enzymes from the glutathione-S-transferase (GST) family: GSTO1 (Zakharyan et al. 2001) and GSTO2 (Whitbread et al. 2003) that use glutathione (GSH) as a reducing agent (Buchet and Lauwerys 1987). The GSTs family of enzymes (i.e. GSTP1, GSTZ1, GSTM1, and GSTT1) is involved in xenobiotic metabolism and plays a role in the cellular response mechanism against oxidative stress. Such effect is produced by arsenic through generation of reactive oxygen species (ROS) (Yamanaka et al. 1989) and is one of the explanations for arsenic's carcinogenic properties. Arsenite is actively transported inside the cell by aquaporins (AQP) cell membrane proteins (Liu et al. 2002) and is then modified by methyltransferases (Marafante and Vahter 1984) using *S*-adenosyl-methionine (SAM) as a methyl donor (Buchet and Lauwerys 1985) to produce monomethylarsonous acid [MMA(III)] and dimethylarsinous acid [DMA(III)]. Both become further oxidized to monomethylarsonic acid [MMA (V)] and dimethylarsinic acid [DMA(V)]: predominant metabolites in urine (Aphosian H and Aphosian M 2006). In mammals, the key methylation enzyme involved in this process is arsenic 3-methyltransferase (AS3MT), formerly known as CYT19 (Lin et al. 2002).

Variation in arsenic metabolism is considered to be, at least in part, genetically driven, and polymorphisms in *GSTT1*, *GSTM1* (Chiou et al. 1997), *AS3MT* (Agusa et al. 2011a) and *GSTP1* (Agusa et al. 2011b) have been associated with the distribution of arsenic metabolites in an individual's urine. Variants in some of these genes, in particular *GSTP1, GSTM1* and *GSTT1*, had been related with increased risk of bladder cancer in epidemiological studies (reviewed by Kellen et al., 2007; Zhang 2010; Zeng et al. 2010). These polymorphisms have also been associated with urinary transitional cell carcinoma (TCC) and urothelial carcinoma (UC) in an arsenic-endemic area in Taiwan (Hsu et al. 2008, 2011).

To our knowledge, there are no prior studies investigating polymorphisms in genes that may be related to arsenic metabolism or transport in areas with lower arsenic exposure levels such as those encountered in the US. As genetic susceptibility could play an even more important role at these lower levels, we investigated gene-environment interactions between

polymorphisms in genes related to xenobiotic and arsenic metabolism, specifically *GSTP1, GSTO2, GSTZ1, GSTM1, GSTT1, AQP3* and *AS3MT*, arsenic exposure, and bladder cancer risk in a population-based case-control study in New Hampshire

2. METHODS

2.1 Study population

Incident bladder cancer cases, aged 25-74 years, diagnosed in New Hampshire from July 1, 1994 to June 30, 1998 (Phase 1), and from July 1, 1998 to December 31, 2001 (Phase 2) were identified through the State Department of Health and Human Services rapid reporting Cancer Registry. A total of 857 bladder cancer cases were identified and interviewed; of these, 832 had histopathologically confirmed bladder cancer based on a standardized histopathology review (Karagas et al. 2011).

Controls were randomly selected from the New Hampshire Department of Transportation Registry (<65 years) and from the Health Care Financing Administration's Medicare Program database (>65 years) during the diagnostic reference period of July 1, 1993 to June 30, 1995 and July 1, 1997 to March 31, 2000. This control group also was used for a study of non-melanoma skin cancer (Karagas et al. 1998). Added controls were selected for the intervening diagnostic period and frequency matched to cases according to age and gender. The study protocols were approved by The Committee for the Protection of Human Subjects of Dartmouth College and participants provided informed consent. All participants were required to speak English and have an identifiable telephone number.

Personal interviews (~90% at home) were performed using a standardized questionnaire by trained interviewers masked to the case-control status of study participants. Detailed socio-demographic as well as medical personal and family history information was gathered. Individuals were asked to report medical history prior to a reference date: the diagnosis date for the cases and a similar assigned date for the controls. Overall, 85% of the cases and 70% of the controls were successful interviewed from the total eligible participants (Karagas et al. 2011).

2.2 Exposure assessment

A toenail clipping sample was requested at the time of the interview and analyzed for arsenic by Instrumental Neutron Activation Analysis (INAA) at the University of Missouri Research Reactor (Columbia, MO) (Nichols et al. 1998). To ensure accuracy, each assay included quality control samples, blanks and known standards, and the assays were performed blinded to case-control status (Karagas et al. 2001). Arsenic nail content is a reliable indicator of low levels (1μg/l) of arsenic exposure in drinking water and reflects a longer exposure period (6-12 months) than urine or blood (Karagas et al. 2000). Additionally, we collected data on the household water supply including: type of water (private versus public well) use and duration of current water source, surficial or deep/ artisan wells usage and use of at home water filters. Moreover, the study population was fairly stable with subjects reporting an average of 15 years using the same water source (Karagas et al. 2002, 2011).

2.3 Genotyping

Peripheral venous blood samples were collected at the time of the interview and promptly transported to the laboratory for processing. DNA was isolated from buffy coat using Qiagen® DNA extraction kits (Valencia, CA). Genomic DNA was whole genome amplified with Qiagen® Repli-G Midi kit. All genotyping assays were performed by laboratory personnel blinded to case-control status. We designed a custom Illumina Golden Gate 96

SNP panel array (San Diego, CA) and genotyped 670 cases and 974 controls with this platform. Samples from three individuals were repeated across plates and their genotype calls were 100% concordant within each of these quality controls (Karagas et al. 2011). From this panel thirteen SNPs (Supplemental Table 2) were selected from five arsenic-related genes to include in this study: *GSTP1* (Ile105Val/rs1695); *GSTO2* (Asn142Asp/rs156697); *GSTZ1* (Met27Thr/rs1046428 and Glu32Lys/rs3177427); *AQP3* (Phe130Phe/rs2228332) and *AS3MT* (rs1046778, rs3740391, rs3740392, rs3740393, Met287Thr/rs11191439, rs7085854, rs10748835 and rs11191454). Aditionally, for the determination of null genotypes of *GSTM1* and *GSTT1*, we used two methods: multiplex PCR reactions described previously (Cheng et al. 1995) for phase I study samples (Karagas et al. 2005) and phase II study samples were genotyped using whole genome amplified DNA by real time PCR Taqman® Copy Number Assays (*GSTT1* Hs02575461_cn and *GSTM1* Hs00010004_cn) from Applied Biosystems® (Foster City, CA). Quality controls for this assay consisted of three repeated control DNA samples in duplicate in each plate and the overall accuracy of genotype calls was >99%.

2.4 Statistical Analysis

In our prior analysis of the main effects of arsenic exposure, we observed a non-linear association with bladder cancer risk (Karagas et al. 2000). Thus, similar to our previous studies (Karagas et al. 2011); we divided the study population in groups according to the 90th percentile of toenail arsenic [0.205 microgram per gram (μg/g)]. Unconditional logistic regression analysis was used to compute odds ratios and their 95% confidence intervals for bladder cancer and arsenic related-bladder cancer in homozygous and heterozygous variants carriers compared to the most common allele homozygous individuals as the reference group. Statistical significance of multiplicative interactions was based on the likelihood ratio test (Breslow and Day 1980). The analysis were conducted in SAS 9.2 (SAS Institute Inc., Cary, NC) and adjusted for potential confounders such as age, sex and smoking.

We used Haploview software (Barret et al. 2005) to asses linkage disequilibrium (LD) within *GSTZ1* and *AS3MT* SNPs using genotypes from a similar CEPH population [Utah residents with Central-European ancestry (CEU)] obtained from HapMap website (release 28 Phase II and III) (The International HapMap Consortium 2003). Haplotype analyses assuming an additive model used by Haploview was performed within the *AS3MT* gene polymorphisms among cases and controls exposed to low and high arsenic levels.

3. RESULTS

The average age of the participants was 64 years, with more men than women overall. The majority of individuals (97%) reported Caucasian race. Current smokers were more commonly cases, never smokers were more commonly controls, and former smokers were equally distributed between cases and controls (Table 1). The mean toenail arsenic concentration was 0.12 μg/g (standard deviation (SD) 0.21 μg/g) and the categories obtained according to the 90th percentile cutoff were considered high (range 0.205–7.626 μg/g) and low (range 0.009– 0.204 μg/g) exposure groups.

From the GSTs variants analyzed (Table 2), we observed a gene-environment interaction (*P* for interaction=0.03) with *GSTP1* Ile105Val; homozygous variant individuals with high arsenic exposure had an odds ratio of 5.4 (95%CI: 1.5-20.2), whereas individuals with this genotype in the low arsenic group had an odds ratio closer to one (OR=0.8; 95%CI: 0.6-1.2). In addition, individuals with *GSTZ1* Met27Thr homozygous genotype had elevated risk (OR=2.2; 95%CI: 0.6-7.8) in the high arsenic group compared to the low arsenic group (OR=1.0; 95%CI: 0.6-1.8; *P* for interaction=0.35).

We found a modest increase in risk associated with *GSTM1* null (OR=1.4; 95%CI: 0.7-2.9; *P* for interaction=0.41) and *GSTT1* null (OR=1.6; 95%CI: 0.7-4.1; *P* for interaction=0.26) genotypes amongst the high arsenic exposed individuals. In comparison, in the low arsenic group, individuals with the same genetic deletions had odds ratios very close to one (Table 2). Individuals with both GSTM1 null/GSTT1 null in the high arsenic group had about a 2-fold higher risk (OR=2.3; 95% CI: 0.7-7.4) compared to those with this genotype in the low exposure group (OR=0.9; 95% CI: 0.6-1.4; *P*=0.15) but with wide confidence intervals. In the high arsenic group, GSTM1present/GSTT1null carriers had a higher odds ratio (OR= 2.0; 95% CI: 0.4-9.9) than GSTM1null/GSTT1 present (OR=1.4; 95% CI: 0.6-2.9) individuals.

GSTO2 Asn142Asp (Table 4) was found to be associated (*P* for trend=0.06) with bladder cancer risk independently of arsenic exposure (heterozygous OR=1.2; 95% CI: 0.9-1.5 andhomozygous OR=1.4; 95%CI: 1.0-1.9). Additionally, a modestly elevated risk of bladder cancer was observed for *GSTZ1* Glu32Lys carriers (heterozygous OR=1.2; 95%CI: 1.0-1.5 and homozygous OR= 1.3; 95% CI: 0.9-1.8; *P* for trend=0.06).

For *AQP3* Phe130Phe (Table 2), we found over a two-fold odds ratio for bladder cancer in the high arsenic group (OR= 2.2; 95% CI: 0.8-6.1), whereas the odds ratio for this genotype in the low arsenic group was closer to one (OR=0.8; 95% CI: 0.6-1.1; *P* for interaction=0.1).

We evaluated several *AS3MT* polymorphisms (Table 3). While we did not find clear evidence of statistical interactions or main effects in either the high or low arsenic groups, many of these comparisons lacked statistical power. The *AS3MT* LD analysis (Supplemental Figure 1) shows linkage between two SNPs pairs: rs1046778/rs10748835 (r^2=0.60) and rs1046778/rs7085854 (r^2=0.71) (Supplemental Table 3). Among lower exposed cases and controls, eight haplotypes (frequency >1%) were found and grouped in two blocks: a diplotype between rs3740393 and rs3740392 and a second block extending over 23 kb formed by rs3740391, rs11191439, rs7085854, rs11191454, rs10748835 and rs1046778 (Supplemental Table 4). We did not find any haplotypes that appeared to modify arsenic-related bladder cancer risk (Supplemental Table 4).

4. DISCUSSION

Inter-individual variation in arsenic metabolism is a hypothesized susceptibility factor for arsenic-related disease. In a US population-based study, we found evidence of potential gene-environment interactions with *GSTP1* and *AQP3* polymorphisms, and weakly if at all with variants in other *GSTs* or *AS3MT*. We also found evidence of main effects with SNPs in *GSTO2* and *GSTZ1.*

Our finding with respect to *GSTP1* Ile105Val carriers is consistent with at least two previous Taiwanese studies reporting an increased TCC in high arsenic exposed individuals with at least one Val allele compared to wild type individuals. A case-control study (Hsu et al. 2008) with 221 cases and 223 controls, reported an odds ratio of 2.53 (95% CI: 0.61-7.75) and a cohort study (Hsu et al. 2011) with 764 individuals found a hazard ratio (HR) of 1.52 (95% CI: 0.66-3.48). However, like our study, both of these studies had limited statistical power. Our study has the advantage of using individual biomarker information (e.g., toenails) in order to more accurately assess lower levels of exposure. This *GSTP1* Ile105Val variant appears to be functionally relevant; in a Vietnamese population lower reduction capacity was observed for Val allele carriers reflected in a lower percentage of urinary AsIII compared to wild type individuals (Agusa et al. 2011b). Cell-culture studies indicate that *GSTP1* and GSH are required for arsenic transport by a multidrug resistance protein 1 (MRP1/ABCC1) (Leslie et al. 2004) and *GSTP1* Ile105Val variants have less

catalytic activity in erythrocytes (Zhong et al. 2006). Hence, while plausible, our findings need to be confirmed or refuted in other study populations.

Our results regarding *AQP3* Phe130Phe indicate a possible increase in bladder cancer susceptibility in homozygous carriers exposed to higher arsenic levels. AQP3, AQP9 and AQP7 are involved in arsenic transport (Bhattacharjee et al. 2009), and overexpression of these proteins make cells more sensitive to arsenic toxicity. Human lung adenocarcinoma cells with decreased levels of AQP3 are resistant to cellular arsenic toxicity (Lee et al. 2006). Yet, the role of aquaporins in arsenic transport has only recently been identified, and the functional significance of the polymorphism we examined is not fully understood.

GSTZ1 is another cytosolic GST essential for tyrosine and phenylalanine metabolism (Fernández-Cañón et al. 2002). In a case-control study from Spain, Met27Thr carriers were at increased risk of disinfection by-products-related bladder cancer (Cantor et al. 2010). We observed a modest increase in risk among high arsenic exposed homozygous variant individuals that did not appear in the less exposed group. But, this could be unrelated to disinfection by-products, as these individuals may be more likely to be using untreated well water. Perhaps, other unknown exposures or enzyme functions overlap in the GST family could account for our results. Another *GSTZ1* SNP (Glu32Lys) has been associated with increase blood lymphocyte micronuclei formation related to bromoform exposure (Kogevinas et al. 2010). We observed a slight main effect of this variant on bladder cancer risk. Our finding appeared largely in the low exposed individuals who may be using municipal disinfected water. Notably, these two *GSTZ1* SNPs were not in LD (r^2= 0.11).

Homozygous gene deletions or null genotypes of *GSTM1* and *GSTT1* are common variants associated with bladder cancer susceptibility in the epidemiologic literature (Zhang et al. 2010; Zeng et al 2010). We observed a somewhat greater risk of bladder cancer in the high arsenic exposure group associated with each of the *null* genotypes, but with a larger effect size for *GSTT1* null. This finding supports the extant arsenic-related literature. In Bangladesh, a cohort study (n=223) conducted in an arsenic-endemic area, found elevated arsenic concentrations in toenails from *GSTT1* null individuals compared to carriers of at least one allele (Kile et al. 2005). Similarly, in Taiwan two studies observed *GSTT1* null-arsenic interactions; a slight risk increase of TCC (OR=1.44; 95%CI: 0.51-3.84; *P* for interaction=0.45) was observed in a case-control study (Hsu et al. 2008) and a greater effect (HR=3.25; 95% CI: 1.20-8.80; *P*=0.02) in a more recent cohort study (Hsu et al. 2011). In contrast, *GSTM1* null-arsenic interactions obtained in the Taiwanese population showed mixed results in relation to TCC; an increase risk (OR= 3.69; 95% CI: 1.05-11.20; *P*=0.03) was observed in the case-control study (Hsu et al. 2008) that was not found in the cohort study (HR= 0.90; 95%CI: 0.40-2.03; *P*=0.79) (Hsu et al. 2011).

Interactions between AS3MT variants, arsenic exposure and bladder cancer risk have not, to our knowledge, been explored previously. On the other hand, *AS3MT* polymorphisms effects on urinary arsenic profiles have been the subject of much investigation (reviewed in Agusa et al. 2011a). Among a number of intronic and exonic variants explored across studies, two SNPs appear consistently associated with altered arsenic profiles in Mexican, Argentinian, Taiwanese, and Vietnamese populations. The C allele at *AS3MT* intronic variant 12390 G>C (rs3740393) is related to higher urine DMA/MMA ratios. However, we did not observe an association with this variant and arsenic-related bladder cancer. The other previously associated variant is Met287Thr (rs11191439); the Thr (C) allele has been related to higher urine concentrations of MMA% in Central Europe (Lindberg et al. 2007) and Northern Chile (Hernández et al. 2008). Increased risk of pre-malignant arsenic-skin lesions in carriers of at least one C allele (OR=4.28; 95% CI: 1.0–18.5; *P*=0.055) was observed in a case-control study conducted in a high-arsenic area in Mexico (Valenzuela et al. 2009). In

contrast, another case-control study (De Chaudhuri et al. 2008) conducted in West-Bengal found no association with arsenic-induced skin lesions. Additionally, the Met287Thr SNP was found to have increased methyltransferase activity in cultured human hepatocytes (Drobna et al. 2004). In our study, we found a somewhat higher odds ratio for bladder cancer in Met287Thr homozygous carriers exposed to high versus low arsenic, but the frequency of this allele in our population was too low to draw conclusions.

We observed a main effect of the *GSTO2* Asn142Asp variant on bladder cancer risk that was unrelated to arsenic exposure. Similarly, Hsu et al. 2011 found a borderline significant increase risk of UC (OR= 2.62; 95%CI: 0.88-7.83; P=0.08) in homozygous variant carriers in southwestern Taiwan. Another case-control study (Wang et al. 2009) conducted in Taipei found an odds ratio of 1.5 (95% CI: 0.93–2.5; P<0.1) in homozygote variant individuals. Additionally, in this study UC risk was higher in individuals with certain haplotypes that combined *GSTO2*, *GSTO1* and *CYP2E1* variants with exposure to environmental factors including cigarette smoking, alcohol consumption and residing in a high arsenic area. Contrary to our results, a case-control study (Chung et al. 2011) in the same non-endemic arsenic area (Taipei), found a decrease risk of UC in homozygous variant carriers of *GSTO2* Asn142Asp (OR=0.17; 95%CI: 0.03–0.88; P=0.03) along with higher levels of MMA% in urine in wild type individuals. In another study (Xu et al. 2009), no association was observed with urinary arsenic profiles and *GSTO2* Asn142Asp genotype in a Chinese population chronically exposed to arsenic. Thus, overall, the epidemiological evidence is unclear and will require further investigation.

Our study has a number of strengths including reliable individual exposure data, pathologic confirmation of bladder cancer cases, and a population-based design allowing for greater generalizability. Statistical power was a limitation of our study as lower arsenic exposure levels encountered in New Hampshire and in the US in general will require larger sample sizes to assess the effects of genetic polymorphisms on arsenic-associated bladder cancer. Future research also should consider other arsenic related genes (i.e. *GSTO1, AQP9*) that we were not able to evaluate. Identification of individuals with enhanced genetic susceptibility to arsenic-related disease including bladder cancer will help to ensure the appropriate prevention measures can be taken.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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List of abbreviations used

SNP	single nucleotide polymorphism
NH	New Hampshire
MCL	maximum contaminant level
GST	glutathione-*S*-transferase

AQP	aquaporin
AS3MT	arsenic methyltransferase
OR	odds ratio
CI	confidence interval
PCR	Polymerase chain reaction
RT-PCR	Real time-polymerase chain reaction

REFERENCES

Agusa T, Fujihara J, Takeshita H, Iwata H. Individual variations in inorganic arsenic metabolism associated with AS3MT genetic polymorphisms. International Journal of Molecular Sciences. 2011a; 12:2351–2382. [PubMed: 21731446]

Agusa T, Kunito T, Tue NM, Lan VT, Fujihara J, Takeshita H, Minh TB, Trang PT, Takahashi S, Viet PH, Tanabe S, Iwata H. Individual variations in arsenic metabolism in Vietnamese: the association with arsenic exposure and GSTP1 genetic polymorphism. Metallomics. 2011b doi: 10.1039/c1mt00133g.

Aposhian HV, Aposhian MM. Arsenic toxicology: five questions. Chem. Res. Toxicol. 2006; 19:1–15. [PubMed: 16411650]

Barrett J, Fry B, Maller J, Daly M. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics. 2005; 21:263–265. [PubMed: 15297300]

Bates MN, Smith AH, Cantor KP. Case-control study of bladder cancer and arsenic in drinking water. Am. J. Epidemiol. 1995; 141:523–530. [PubMed: 7900719]

Bhattacharjee H, Rosen BP, Mukhopadhyay R. Aquaglyceroporins and metalloid transport: implications in human diseases. Handb Exp Pharmacol. 2009:309–325. [PubMed: 19096785]

Breslow, NE.; Day, NE. Statistical Methods in Cancer Research. Iarc Lyon; 1980.

Buchet JP, Lauwerys R. Study of factors influencing the in vivo methylation of inorganic arsenic in rats. Toxicol. Appl. Pharmacol. 1987; 91:65–74. [PubMed: 3672518]

Buchet JP, Lauwerys R. Study of inorganic arsenic methylation by rat liver in vitro: relevance for the interpretation of observations in man. Arch. Toxicol. 1985; 57:125–129. [PubMed: 4026571]

Cantor KP, Villanueva CM, Silverman DT, Figueroa JD, Real FX, Garcia-Closas M, Malats N, Chanock S, Yeager M, Tardon A, Garcia-Closas R, Serra C, Carrato A, Castaño-Vinyals G, Samanic C, Rothman N, Kogevinas M. Polymorphisms in GSTT1, GSTZ1, and CYP2E1, disinfection by-products, and risk of bladder cancer in Spain. Environ. Health Perspect. 2010; 118:1545–1550. [PubMed: 20675267]

Cheng TJ, Christiani DC, Wiencke JK, Wain JC, Xu X, Kelsey KT. Comparison of sister chromatid exchange frequency in peripheral lymphocytes in lung cancer cases and controls. Mutat. Res. 1995; 348:75–82. [PubMed: 7477055]

Chiou H, Hsueh Y, Hsieh L, Hsu L, Hsu Y, Hsieh F, Wei M, Chen H, Yang H, Leu L, Chu T, Chen-Wu C, Yang M, Chen C. Arsenic methylation capacity, body retention, and null genotypes of glutathione S-transferase M1 and T1 among current arsenic-exposed residents in Taiwan. Mutation Research/Reviews in Mutation Research. 1997; 386:197–207.

Chung C, Pu Y, Su C, Huang C, Hsueh Y. Gene polymorphisms of glutathione S-transferase omega 1 and 2, urinary arsenic methylation profile and urothelial carcinoma. Sci. Total Environ. 2011; 409:465–470. [PubMed: 21094982]

De Chaudhuri S, Ghosh P, Sarma N, Majumdar P, Sau TJ, Basu S, Roychoudhury S, Ray K, Giri AK. Genetic variants associated with arsenic susceptibility: study of purine nucleoside phosphorylase, arsenic (+3) methyltransferase, and glutathione S-transferase omega genes. Environ. Health Perspect. 2008; 116:501–505. [PubMed: 18414634]

Drobná Z, Waters SB, Walton FS, LeCluyse EL, Thomas DJ, Stýblo M. Interindividual variation in the metabolism of arsenic in cultured primary human hepatocytes. Toxicol. Appl. Pharmacol. 2004; 201:166–177. [PubMed: 15541756]

EFSA Panel on Contaminants in the Food Chain (CONTAM). Scientific opinion on arsenic in food. EFSA J. 2009; 7:1351. doi: 10.2903/j.efsa.2009.1351.

Fernández-Cañón JM, Baetscher MW, Finegold M, Burlingame T, Gibson KM, Grompe M. Maleylacetoacetate isomerase (MAAI/GSTZ)-deficient mice reveal a glutathione-dependent nonenzymatic bypass in tyrosine catabolism. Mol. Cell. Biol. 2002; 22:4943–4951. [PubMed: 12052898]

Hernández A, Xamena N, Surrallés J, Sekaran C, Tokunaga H, Quinteros D, Creus A, Marcos R. Role of the Met287Thr polymorphism in the AS3MT gene on the metabolic arsenic profile. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis. 2008; 637:80–92.

Horner, M.; Ries, L.; Krapcho, M.; Neyman, N.; Aminou, R.; Howlader, N.; Altekruse, S.; Feuer, E.; Huang, L.; Mariotto, A. SEER Cancer Statistics Review, 1975-2006. National Cancer Institute; Bethesda, MD: 2009.

Hsu L, Chen WP, Yang TY, Chen YH, Lo WC, Wang YH, Liao YT, Hsueh YM, Chiou HY, Wu MM, Chen C. Genetic polymorphisms in glutathione S-transferase (GST) superfamily and risk of arsenic-induced urothelial carcinoma in residents of Southwestern Taiwan. J. Biomed. Sci. 2011; 18:51. [PubMed: 21798077]

Hsu L, Chiu AW, Huan SK, Chen C, Wang Y, Hsieh F, Chou W, Wang L, Chen C. SNPs of GSTM1, T1, P1, epoxide hydrolase and DNA repair enzyme XRCC1 and risk of urinary transitional cell carcinoma in southwestern Taiwan. Toxicol. Appl. Pharmacol. 2008; 228:144–155. [PubMed: 18199464]

IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Some drinking-water disinfectants and contaminants, including arsenic. Monographs on chloramine, chloral and chloral hydrate, dichloroacetic acid, trichloroacetic acid and 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone. IARC Monogr. Eval. Carcinog. Risks Hum. 2004; 84:269–477. [PubMed: 15645578]

Karagas MR, Tosteson TD, Blum J, Klaue B, Weiss JE, Stannard V, Spate V, Morris JS. Measurement of low levels of arsenic exposure: a comparison of water and toenail concentrations. Am. J. Epidemiol. 2000; 152:84–90. [PubMed: 10901333]

Karagas MR, Tosteson TD, Morris JS, Demidenko E, Mott LA, Heaney J, Schned A. Incidence of transitional cell carcinoma of the bladder and arsenic exposure in New Hampshire. Cancer Causes and Control. 2004; 15:465–472. [PubMed: 15286466]

Karagas MR, Andrew AS, Nelson HH, Li Z, Punshon T, Schned A, Marsit CJ, Morris JS, Moore JH, Tyler AL, Gilbert-Diamond D, Guerinot ML, Kelsey KT. SLC39A2 and FSIP1 polymorphisms as potential modifiers of arsenic-related bladder cancer. Hum. Genet. 2011 doi: 10.1007/s00439-011-1090-x.

Karagas MR, Park S, Warren A, Hamilton J, Nelson HH, Mott LA, Kelsey KT. Gender, smoking, glutathione-S-transferase variants and bladder cancer incidence: a population-based study. Cancer Lett. 2005; 219:63–69. [PubMed: 15694665]

Karagas MR, Tosteson TD, Blum J, Morris JS, Baron JA, Klaue B. Design of an epidemiologic study of drinking water arsenic exposure and skin and bladder cancer risk in a U.S. population. Environ. Health Perspect. 1998; 106(Suppl 4):1047–1050. [PubMed: 9703491]

Karagas MR, Stukel TA, Morris JS, Tosteson TD, Weiss JE, Spencer SK, Greenberg ER. Skin Cancer Risk in Relation to Toenail Arsenic Concentrations in a US Population-based Case-Control Study. American Journal of Epidemiology. 2001; 153:559–565. [PubMed: 11257063]

Karagas MR, Stukel TA, Tosteson TD. Assessment of cancer risk and environmental levels of arsenic in New Hampshire. Int. J. Hyg. Environ. Health. 2002; 205:85–94. [PubMed: 12018020]

Kellen E, Hemelt M, Broberg K, Golka K, Kristensen VN, Hung RJ, Matullo G, Mittal RD, Porru S, Povey A, Schulz WA, Shen J, Buntinx F, Zeegers MP, Taioli E. Pooled Analysis and Meta-analysis of the Glutathione S-Transferase P1 Ile 105Val Polymorphism and Bladder Cancer: A HuGE-GSEC Review. American Journal of Epidemiology. 2007; 165:1221–1230. [PubMed: 17404387]

Kile ML, Houseman EA, Rodrigues E, Smith TJ, Quamruzzaman Q, Rahman M, Mahiuddin G, Su L, Christiani DC. Toenail arsenic concentrations, GSTT1 gene polymorphisms, and arsenic exposure from drinking water. Cancer Epidemiol. Biomarkers Prev. 2005; 14:2419–2426. [PubMed: 16214926]

Kogevinas M, Villanueva CM, Font-Ribera L, Liviac D, Bustamante M, Espinoza F, Nieuwenhuijsen MJ, Espinosa A, Fernandez P, DeMarini DM, Grimalt JO, Grummt T, Marcos R. Genotoxic effects in swimmers exposed to disinfection by-products in indoor swimming pools. Environ. Health Perspect. 2010; 118:1531–1537. [PubMed: 20833606]

Lee TC, Ho I, Huang J. Enhanced expression of multidrug resistance-associated protein 2 and reduced expression of aquaglyceroporin 3 in an arsenic-resistant human cell line. J. Biol. Chem. 2006; 281:18401–18407. [PubMed: 16672223]

Leslie EM, Haimeur A, Waalkes MP. Arsenic transport by the human multidrug resistance protein 1 (MRP1/ABCC1). J. Biol. Chem. 2004; 279:32700–32708. [PubMed: 15161912]

Lin S, Shi Q, Nix FB, Styblo M, Beck MA, Herbin-Davis KM, Hall LL, Simeonsson JB, Thomas DJ. A novel S-adenosyl-L-methionine:arsenic(III) methyltransferase from rat liver cytosol. J. Biol. Chem. 2002; 277:10795–10803. [PubMed: 11790780]

Lindberg AL, Kumar R, Goessler W, Thirumaran R, Gurzau E, Koppova K, Rudnai P, Leonardi G, Fletcher T, Vahter M. Metabolism of low-dose inorganic arsenic in a central European population: influence of sex and genetic polymorphisms. Environ. Health Perspect. 2007; 115:1081–1086. [PubMed: 17637926]

Liu Z, Shen J, Carbrey JM, Mukhopadhyay R, Agre P, Rosen BP. Arsenite transport by mammalian aquaglyceroporins AQP7 and AQP9. Proc. Natl. Acad. Sci. U. S. A. 2002; 99:6053–6058. [PubMed: 11972053]

Marafante E, Vahter M. The effect of methyltransferase inhibition on the metabolism of [74As]arsenite in mice and rabbits. Chem. Biol. Interact. 1984; 50:49–57. [PubMed: 6733804]

Nichols T, Morris J, Mason M, Spate V, Baskett C, Cheng T, Tharp C, Scott J, Horsman T, Colbert J. The study of human nails as an intake monitor for arsenic using neutron activation analysis. J. Radioanal. Nucl. 1998; 236:51–57.

Peters SC, Blum JD, Klaue B, Karagas MR. Arsenic occurrence in New Hampshire drinking water. Environ. Sci. Technol. 1999; 33:1328–1333.

Siegel R, Ward E, Brawley O, Jemal A. Cancer statistics, 2011: The impact of eliminating socioeconomic and racial disparities on premature cancer deaths. CA: a cancer journal for clinicians , caac. 2011:20121v1.

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. [PubMed: 14652304]

The International HapMap Consortium. The international HapMap project. Nature. 2003; 426:789–796. [PubMed: 14685227]

US Cancer Statistics Working Group, 2010. United States Cancer Statistics: 1999-2006 incidence and mortality web-based report, US Department of Health and Human Services. Centers for Disease Control and Prevention and National Cancer Institute; Atlanta: 2011.

Valenzuela OL, Drobná Z, Hernández-Castellanos E, Sánchez-Peña LC, García-Vargas GG, Borja-Aburto VH, Stýblo M, Del Razo LM. Association of AS3MT polymorphisms and the risk of premalignant arsenic skin lesions. Toxicol. Appl. Pharmacol. 2009; 239:200–207. [PubMed: 19538983]

Wang Y, Yeh S, Shen K, Shen C, Juang G, Hsu L, Chiou H, Chen C. A significantly joint effect between arsenic and occupational exposures and risk genotypes/diplotypes of CYP2E1, GSTO1 and GSTO2 on risk of urothelial carcinoma. Toxicol. Appl. Pharmacol. 2009; 241:111–118. [PubMed: 19686770]

Whitbread AK, Tetlow N, Eyre HJ, Sutherland GR, Board PG. Characterization of the human Omega class glutathione transferase genes and associated polymorphisms. Pharmacogenetics. 2003; 13:131–144. [PubMed: 12618591]

Xu Y, Li X, Zheng Q, Wang H, Wang Y, Sun G. Lack of association of glutathione-S-transferase omega 1(A140D) and omega 2 (N142D) gene polymorphisms with urinary arsenic profile and

oxidative stress status in arsenic-exposed population. Mutation Research/Genetic Toxicology and Environmental Mutagenesis. 2009; 679:44–49.

Yamanaka K, Hasegawa A, Sawamura R, Okada S. Dimethylated arsenics induce DNA strand breaks in lung the production of active oxygen in mice. Biochem. Biophys. Res. Commun. 1989; 165:43–50. [PubMed: 2590239]

Zakharyan RA, Sampayo-Reyes A, Healy SM, Tsaprailis G, Board PG, Liebler DC, Aposhian HV. Human monomethylarsonic acid (MMA(V)) reductase is a member of the glutathione-S-transferase superfamily. Chem. Res. Toxicol. 2001; 14:1051–1057. [PubMed: 11511179]

Zeng FF, Liu SY, Wei W, Yao SP, Zhu S, Li KS, Wan G, Zhang HT, Zhong M, Wang BY. Genetic polymorphisms of glutathione S-transferase T1 and bladder cancer risk: a meta-analysis. Clin. Exp. Med. 2010; 10:59–68. [PubMed: 19779959]

Zhang R, Xu G, Chen W, Zhang W. Genetic polymorphisms of glutathione S-transferase M1 and bladder cancer risk: a meta-analysis of 26 studies. Mol. Biol. Rep. 2010 doi: 10.1007/s11033-010-0386-6.

Zhong S, Zhou S, Chen X, Chan SY, Chan E, Ng K, Duan W, Huang M. Relationship between genotype and enzyme activity of glutathione S-transferases M1 and P1 in Chinese. European Journal of Pharmaceutical Sciences. 2006; 28:77–85. [PubMed: 16488119]

Highlights

- We studied genetic modification of arsenic associated bladder cancer risk.
- *GSTP1* Ile105Val variant increases the risk of arsenic-related bladder cancer.
- *AQP3* Phe130Phe carriers may be at increased risk of arsenic-related bladder cancer.
- *GSTO2* Asn142Asp and *GSTZ1* Glu32Lys are associated with overall bladder cancer risk.

Table 1

Characteristics of the Study Participants

Characteristic	Controls	Bladder Cancer Cases
Total	1191	832
Subject Age (yrs) (SD)	64 (±10)	64 (±9)
Gender, n (%)		
Men	728 (61.1%)	632 (76.0)
Women	463 (38.9%)	200 (24.0)
Smoking History *		
Never	399 (33.7%)	141(17.2%)
ormer	578 (48.9%)	407 (49.8%)
Current	206 (17.4%)	270 (33.0%)

* smoking history was missing on 22 subjects (8 controls, 14 cases)

Table 2

Bladder cancer odds ratios (95% confidence interval) for GSTs and AQP3 genetic polymorphisms by toenail arsenic concentration

		Low (≤90th percentile) (n=1678)		High (>90th percentile) (n=184)		
SNP		**N case/controls§**	**OR (95% CI) †**	**N case/controls§**	**OR (95% CI) †**	**P for interaction^A^**
rs1695	AA	257/355	1.0 (ref)	23/40	1.0 (ref)	0.03**
GSTP1	AG	240/365	0.9 (0.7- 1.2)	26/41	1.0 (0.5- 2.1)	
Ile105Val	GG	59/99	0.8 (0.6- 1.2)	11/4	5.4 (1.5- 20.2)	
			P for trend=0.26		P for trend=0.05**	
rs156697	AA	206/355	1.0 (ref)	23/36	1.0 (ref)	0.81
GSTO2	AG	285/391	1.3 (1- 1.6)	23/34	1.5 (0.6- 3.3)	
Asn142Asp	GG	65/73	1.5 (1.02- 2.2)	14/15	1.4 (0.5- 3.4)	
			P for trend=0.02**		P for trend=0.45	
rs1046428	TT	357/503	1.00 (ref)	35/48	1.00 (ref)	0.35
GSTZ1	TC	170/281	0.8 (0.7- 1.1)	18/32	0.8 (0.4- 1.8)	
Met27Thr	CC	25/35	1.0 (0.6- 1.8)	7/5	2.2 (0.6- 7.8)	
			P for trend=0.33		P for trend=0.56	
rs3177427	GG	207/371	1.0 (ref)	27/43	1.0 (ref)	0.69
GSTZ1	GA	239/327	1.3 (1- 1.6)	21/28	1.1 (0.5- 2.3)	
Glu32Lys	AA	68/88	1.3 (0.9- 1.9)	6/9	1.0 (0.3- 3.5)	
			P for trend=0.05**		P for trend=0.90	
GSTM1	present	236/367	1.0 (ref)	22/41	1.0 (ref)	0.41
	null	317/448	1.0 (0.8-1.3)	38/46	1.4 (0.7-2.9)	
			P for trend=0.70		P for trend=0.32	
GSTT1	present	469/684	1.0 (ref)	49/74	1.0 (ref)	0.26
	null	88/126	0.9 (0.7-1.3)	13/13	1.6 (0.7-4.1)	
			P for trend=0.73		P for trend=0.30	
rs2228332	CC	189/270	1.0 (ref)	16/30	1.0 (ref)	0.1*

SNP		Low (≤90th percentile) (n=1678)		High (>90th percentile) (n=184)		
		N case/controls[§]	OR (95% CI) [†]	N case/controls[§]	OR (95% CI) [†]	P for interaction[A]
AQP3	CT	279/391	1.0 (0.8-1.3)	31/42	1.5 (0.7-3.4)	
Phe130Phe	TT	85/149	0.8 (0.6-1.1)	13/13	2.2 (0.8- 6.1)	
			P for trend=0.23		P for trend=0.14	

[§]79 and 82 subjects in control and case separately without toenail data, 25 subjects without bladder cancer status and subjects without SNP data were excluded from analysis

[†]Adjusted for age, gender, current and former smoking;

[A]treating SNP as continuous variable;

[**] $P \leq 0.05$;

[*] $P \leq 0.1$

Table 3

Bladder cancer odds ratios and (95% confidence intervals) for selected *AS3MT* SNPs by toenail arsenic concentration

		Low (≤90th percentile) (n=1678)		High (>90th percentile) (n=184)		
SNP		**N cases/controls** §	**OR (95% CI)** †	**N cases/controls** §	**OR (95% CI)** †	**P for interaction**A
rs1046778	TT	255/376	1.0 (ref)	25/37	1.0 (ref)	0.91
AS3MT	TC	218/344	0.9 (0.7-1.2)	22/34	0.9 (0.4-2.0)	
	CC	44/68	0.9 (0.6-1.4)	7/9	0.9 (0.3-2.9)	
			P for trend=0.47		P for trend=0.81	
rs3740391	AA	366/549	1.0 (ref)	41/59	1.0 (ref)	0.47
AS3MT	AC	138/218	1.0 (0.8-1.2)	9/18	1.0 (0.4-2.5)	
	CC	13/21	0.8 (0.4-1.7)	4/3	2.3 (0.4-12.2)	
			P for trend=0.71		P for trend=0.52	
rs3740392	TT	304/451	1.0 (ref)	29/37	1.0 (ref)	0.64
AS3MT	TC	218/289	1.1 (0.9-1.4)	26/36	0.9 (0.4- 1.9)	
	CC	35/46	1.2 (0.72-1.89)	5/6	1.0 (0.3- 4.0)	
			P for trend=0.33		P for trend=0.90	
rs3740393	GG	385/565	1.0 (ref)	38/57	1.0 (ref)	0.77
AS3MT	GC	119/205	0.8 (0.6- 1.1)	16/19	1.0 (0.4- 2.4)	
	CC	12/18	0.9 (0.4- 1.8)	0/4	0 (0- >999.99)	
			P for trend=0.22		P for trend=0.32	
rs11191439	TT	412/651	1.0 (ref)	44/61	1.0 (ref)	0.433
AS3MT	TC	99/123	1.3 (1.0-1.8)	8/17	0.8 (0.3- 2.0)	
Met287thr	CC	6/14	0.7 (0.3-1.9)	1/1	1.2 (0.1- 21.0)	
			P for trend=0.30		P for trend=0.69	
rs7085854	TT	326/495	1.0 (ref)	31/48	1.0 (ref)	0.75
AS3MT	TC	208/293	1.0 (0.9-1.4)	25/34	1.1 (0.6-2.4)	
	CC	22/31	1.1 (0.6-1.9)	4/3	1.5 (0.3-7.6)	
			P for trend=0.53		P for trend=0.56	

SNP		Low (≤90th percentile) (n=1678)		High (>90th percentile) (n=184)		
		N cases/controls §	OR (95% CI) †	N cases/controls §	OR (95% CI) †	P for interaction[A]
rs10748835	GG	148/269	1.0 (ref)	17/21	1.0 (ref)	0.44
AS3MT	GA	222/394	1.0 (0.8-1.4)	27/42	0.7 (0.3-1.5)	
	AA	68/113	1.1 (0.7-1.6)	10/15	0.7 (0.2-2.1)	
			P for trend=0.70		P for trend=0.47	
rs11191454	AA	475/678	1.0 (ref)	49/72	1.0 (ref)	0.43
AS3MT	AG	77/136	0.8 (0.6-1.1)	11/12	1.4 (0.5-3.5)	
	GG	4/5	0.9 (0.2-3.3)	0/1	0 (0- >999.99)	
			P for trend=0.11		P for trend=0.79	

§ 79 and 82 subjects in control and case separately without toenail data, 25 subjects without bladder cancer status and subjects without SNP data were excluded from analysis

† Adjusted for age, gender, current and former smoking;

A treating SNP as continuous variable.

Table 4

Bladder cancer odds ratios (95% confidence interval) for selected SNPs SNP

SNP	value	N case/Controls §	OR (95% CI) †
rs1695	AA	294/411	1.0 (ref)
GSTP1	AG	289/414	1.0 (0.8- 1.2)
Ile105Val	GG	75/103	1.0 (0.7- 1.4)
			P for trend=0.96
rs156697	AA	255/398	1.0 (ref)
GSTO2	AG	321/439	1.2 (0.9- 1.5)
Asn142Asp	GG	82/91	1.4 (1.0- 1.9)
			P for trend=0.06 *
rs1046428	TT	421/565	1.0 (ref)
GSTZ1	TC	198/320	0.8 (0.7- 1.0)
Met27Thr	CC	35/43	1.2 (0.7- 1.9)
			P for trend=0.48
rs3177427	GG	252/424	1.0 (ref)
GSTZ1	GA	276/366	1.2 (1.0- 1.5)
Glu32Lys	AA	80/99	1.3 (0.9- 1.8)
			P for trend=0.06 *
GSTM1	present	275/420	1.0 (ref)
	null	378/508	1.1 (0.9- 1.3)
			P for trend=0.46
GSTT1	present	556/780	1.0 (ref)
	null	106/143	1.0 (0.7- 1.3)
			P for trend=0.82
rs2228332	CC	223/306	1.0 (ref)
AQP3	CT	323/449	1.0 (0.8- 1.3)
Phe130Phe	TT	109/164	0.9 (0.6- 1.2)
			P for trend=0.43
rs1046778	TT	302/423	1.0 (ref)
AS3MT	TC	255/388	0.9 (0.7- 1.1)
	CC	54/80	0.9 (0.6- 1.3)
			P for trend=0.33
rs3740391	AA	433/627	1.0 (ref)
AS3MT	AC	158/240	1.0 (0.8- 1.3)
	CC	20/24	1.2 (0.6- 2.2)
			P for trend=0.74
rs3740392	TT	357/503	1.0 (ref)

SNP	value	N case/Controls [§]	OR (95% CI) [†]
AS3MT	TC	260/332	1.1 (0.9- 1.3)
	CC	42/53	1.1 (0.7- 1.8)
			P for trend=0.42
rs3740393	GG	457/636	1.0 (ref)
AS3MT	GC	140/233	0.8 (0.6- 1.0)
	CC	13/22	0.8 (0.4- 1.5)
			P for trend=0.08 [*]
rs11191439	TT	490/733	1.0 (ref)
AS3MT	TC	112/142	1.2 (0.9- 1.6)
Met287thr	CC	7/15	0.7 (0.3- 1.9)
			P for trend=0.46
rs7085854	TT	381/556	1.0 (ref)
AS3MT	TC	250/336	1.1 (0.9- 1.3)
	CC	27/36	1.0 (0.6- 1.8)
			P for trend=0.55
rs10748835	GG	182/299	1.0 (ref)
AS3MT	GA	266/445	1.0 (0.8- 1.3)
	AA	82/133	1.0 (0.7- 1.4)
			P for trend=0.87
rs11191454	AA	562/770	1.00 (ref)
AS3MT	AG	92/152	0.8 (0.6- 1.1)
	GG	4/6	0.7 (0.2- 2.7)
			P for trend=0.12

[§] 25 subjects without bladder cancer status and subjects without SNP data were excluded from analysis;

[†] Adjusted for age, gender, current and former smoking;

[*] $P \leq 0.1$