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Research Article

Associations between Arsenic (+3 Oxidation State) Methyltransferase (*AS3MT*) and N-6 Adenine-specific DNA Methyltransferase 1 (*N6AMT1*) Polymorphisms, Arsenic Metabolism, and Cancer Risk in a Chilean Population

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Inter-individual differences in arsenic metabolism have been linked to arsenic-related disease risks. Arsenic (+3) methyltransferase (AS3MT) is the primary enzyme involved in arsenic metabolism, and we previously demonstrated *in vitro* that N-6 adenine-specific DNA methyltransferase 1 (N6AMT1) also methylates the toxic inorganic arsenic (iAs) metabolite, monomethylarsonous acid (MMA), to the less toxic dimethylarsonic acid (DMA). Here, we evaluated whether *AS3MT* and *N6AMT1* gene polymorphisms alter arsenic methylation and impact iAs-related cancer risks. We assessed *AS3MT* and *N6AMT1* polymorphisms and urinary arsenic metabolites (%iAs, %MMA, %DMA) in 722 subjects from an arsenic-cancer case-control study in a uniquely exposed area in northern Chile. Polymorphisms were genotyped using a custom designed multiplex, ligation-dependent probe amplification (MLPA) assay for 6 *AS3MT* SNPs and 14 tag SNPs in the *N6AMT1* gene. We found several *AS3MT* polymorphisms associated with both urinary arsenic metabolite profiles and cancer risk. For example, compared to wildtypes, individuals carrying minor alleles in *AS3MT* rs3740393 had lower %MMA (mean difference = −1.9%, 95% CI: −3.3, −0.4), higher %DMA (mean difference = 4.0%, 95% CI: 1.5, 6.5), and lower odds ratios for bladder (OR = 0.3; 95% CI: 0.1–0.6) and lung cancer (OR = 0.6; 95% CI: 0.2–1.1). Evidence of interaction was also observed for both lung and bladder cancer between these polymorphisms and elevated historical arsenic exposures. Clear associations were not seen for *N6AMT1*. These results are the first to demonstrate a direct association between *AS3MT* polymorphisms and arsenic-related internal cancer risk. This research could help identify subpopulations that are particularly vulnerable to arsenic-related disease. Environ. Mol. Mutagen. 58:411–422, 2017. © 2017 Wiley Periodicals, Inc.

Key words: arsenic metabolism; N6AMT1; AS3MT; polymorphism; cancer

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INTRODUCTION

Inorganic arsenic (iAs) is a toxic metalloid and known human carcinogen [IARC (International Agency for Research on Cancer), 2012]. It is estimated that over 200 million individuals worldwide consume iAs contaminated drinking water at concentrations that exceed the World Health Organization's recommended standard of 10 $\mu g\ L^{-1}$ [Naujokas et al., 2013]. Chronic iAs ingestion is associated with increased risk of skin, lung, bladder, and kidney cancers, making iAs exposure a global health concern [Smith et al., 1992; Steinmaus et al., 2000].

Humans metabolize ingested iAs through methylation pathways mainly in the liver [Gebel, 2002; Vahter, 2002; Tseng, 2007]. Once ingested, iAs undergoes oxidative methylation to monomethylarsonic acid (MMA^V) which is then reduced to monomethylarsonous acid (MMA^{III}). MMA^{III} is methylated to dimethylarsinic acid (DMA^V) and a small amount is further reduced to dimethylarsinous acid (DMA^{III}) [Drobna et al., 2009b]. This metabolism process is incomplete in humans; therefore, all three forms (iAs, MMA, and DMA) are excreted in urine. Since MMA^{III} is rather unstable, most epidemiology studies report total MMA ($MMA^{III} + MMA^V$) present in urine [Kalman et al., 2013]. Traditionally, iAs methylation was considered a detoxification pathway; however *in vitro* evidence supports that MMA^{III} is more toxic to human cells than iAs or any other metabolite [Petrick et al., 2000; Stýblo et al., 2002]. In fact, a number of human studies have identified associations between increased MMA and decreased DMA percentages in urine and higher risk of skin, bladder and lung cancers and other arsenic related disease [Steinmaus et al., 2010; Smith and Steinmaus, 2009]. This suggests that individuals with less efficient arsenic metabolism may be particularly susceptible to arsenic toxicity.

There is considerable inter-individual variation in arsenic metabolism [Vahter, 1999]. The efficiency of arsenic metabolism is evaluated by the relative distribution of urinary arsenic metabolites [Buchet et al., 1981]. Average proportions of urinary iAs, MMA, and DMA across human population studies are ~10–20%, 10–15%, and 60–75%, respectively [Hopenhaynrich et al., 1993]. Multiple factors contribute to inter-individual variability in urinary arsenic metabolites. For example, sex, age, smoking status, levels of exposure, and folate intake all affect the proportion of arsenic metabolites excreted in urine [Gamble et al., 2006; Kile et al., 2009]. Genetic polymorphism may also influence inter-individual variability [Vahter, 2000; Engström et al., 2007]. However, the factors that determine most of the variation in arsenic metabolism, and the factors that make some people more susceptible to arsenic related disease than others, remain mostly unexplained. Understanding the role of the factors that impact arsenic metabolism might help identify individuals who are particularly susceptible to arsenic toxicity.

The activity of enzymes involved in converting MMA^{III} to DMA^V may influence toxicity resulting from arsenic bioactivation. Arsenic (+3 oxidation state) methyltransferase (AS3MT) is the primary enzyme involved in catalyzing arsenic methylation [Thomas et al., 2007]. However, *As3mt* knockout mice did not exhibit abolished arsenic metabolism, suggesting that alternative enzymes facilitate this methylation process [Drobna et al., 2009a]. Our previous *in vitro* study identified the novel role of N-6 adenine-specific DNA methyltransferase 1 (*N6AMT1*), a putative methyltransferase, in converting MMA^{III} to DMA^V [Ren et al., 2011]. A more recent *in vitro* investigation confirmed that N6AMT1 is involved in MMA^{III} methylation, but its effects were secondary to AS3MT [Zhang et al., 2015]. Although several epidemiology studies have found associations between *AS3MT* polymorphisms and the proportion of MMA in urine [Engström et al., 2011, 2007; Pierce et al., 2012; Drobná et al., 2016], few have examined cancer risk in the same study population [Chung et al., 2009; Engström et al., 2015], and only two have assessed the role of *N6AMT1* polymorphisms in arsenic metabolism [Harari et al., 2013; Chen et al., 2017].

We previously examined the association between arsenic methylation and cancer in an arsenic exposed population from Chile, and found higher lung and bladder cancer risks associated with increased %MMA in urine [Melak et al., 2014]. In this study, we extend these analyses to investigate the role of *AS3MT* and *N6AMT1* polymorphisms in arsenic metabolism as well as internal cancer risk.

MATERIALS AND METHODS

Study Populations

The study uses data from participants that were recruited in northern Chile as part of a case-control study of arsenic and cancer. Details on subject recruitment and participation rates are described in Steinmaus et al. [2013]. Briefly, the study area comprised two contiguous regions (Regions I, II) in northern Chile. All incident cases of primary lung and bladder cancer newly diagnosed from October 2007 to December 2010 were ascertained from all pathologists, hospitals, and radiologists in the study area. Controls, frequency matched to cases by sex and five-year age groups, were randomly selected from computerized voter registration lists for Regions I and II, which include >95% of the population over age 50 in these regions. The appropriate review boards in the United States and Chile approved this study, and informed consent was obtained from all participants.

For this study, subjects had to be alive at the time of the interview and able to provide a urine sample and either blood or saliva for genotyping. Of the 937 living subjects in the original Chile case-control study, we genotyped 722 participants using 557 clots and 165 saliva samples that were collected during the study. We did not limit samples to matched pairs with genotyping information, thus the subset of study participants comprised different numbers of cases and controls. The response rate of participants did not differ between cases (75.1%) and controls (77.8%). Urinary arsenic metabolites were measured in the first 558 subjects recruited in the original case-control study, which included 494 of the genotyped subjects. The subset of individuals with genotype and metabolite data was comparable to the original case-control study

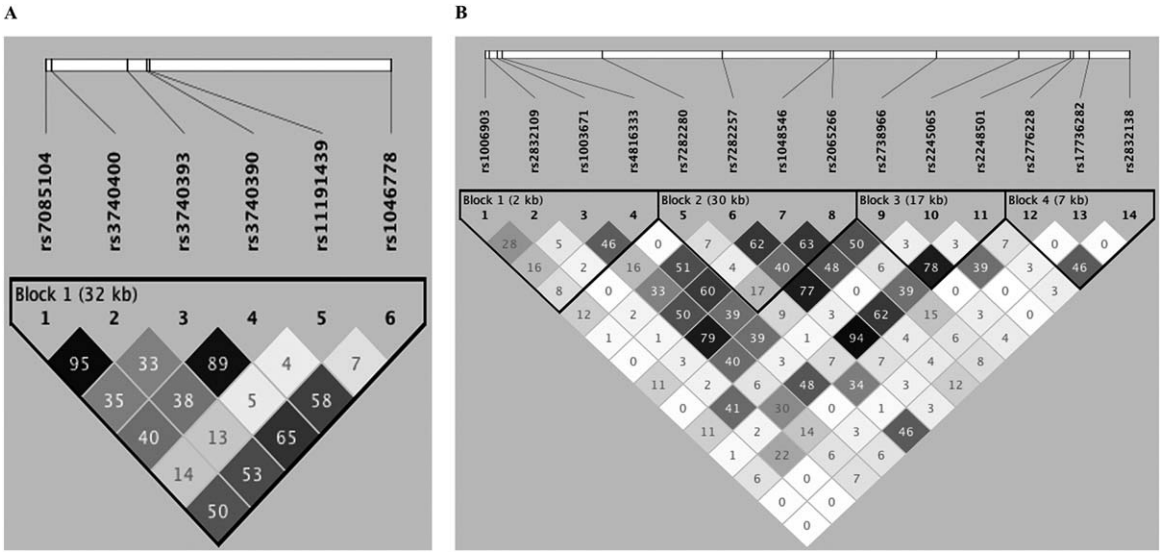

Fig. 1. Linkage disequilibrium values (R^2) for *AS3MT* (A) and *N6AMT1* (B) polymorphisms.

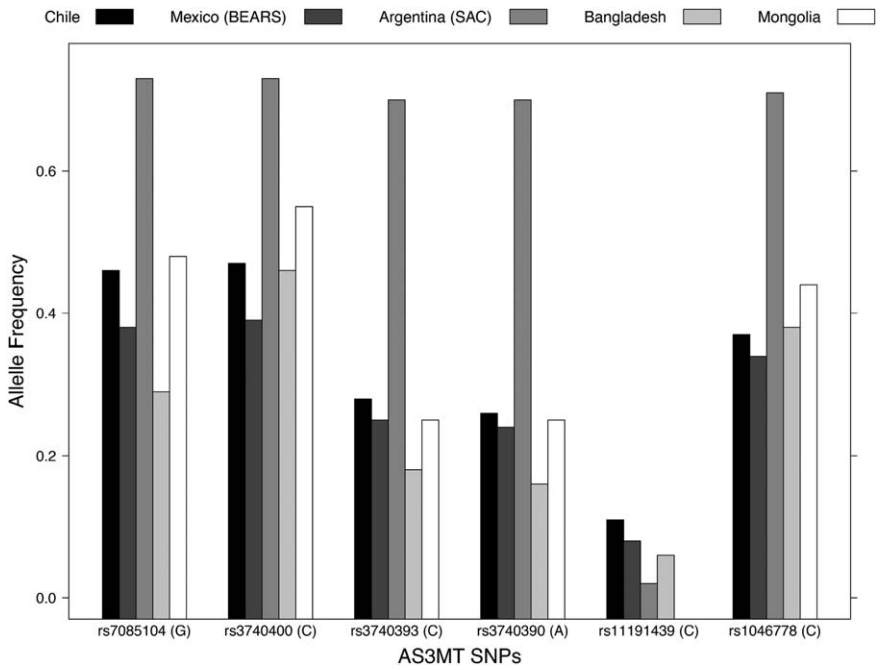

Fig. 2. Minor allele frequencies of *AS3MT* polymorphisms in Chile compared to populations from Gómez Palacio, Mexico (BEAR cohort), San Antonio de los Cobres (SAC), Argentina, Matlab, Bangladesh, and Wuyuan, Inner Mongolia.

population in terms of mean age, sex, smoking, and cancer status (Supporting Information Table I).

Urine Sample Collection and Analysis

A single first morning urine sample was collected from subjects. A previous study found a correlation between arsenic excretion in single first morning samples and samples collected over 24 h [Calderon et al., 1999]. 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. Urinary arsenic metabolites were measured using high performance liquid chromatography and inductively coupled mass spectrometry (HPLC-ICP/MS). Methodological details are provided elsewhere [Melak et al., 2014]. Quantitation limits were: MMA3, 0.5 μg L^{-1}; InAs3, 1 μg L^{-1}; DMA5, 5 μg L^{-1}; MMA5, 1 μg L^{-1}; InAs5, 2.5 μg L^{-1}; total arsenic, 1 μg L^{-1}; and arsenobetaine, 1 μg L^{-1}. MMA and DMA were measured as the sums of the trivalent and pentavalent forms because of the rapid oxidation of MMA^{III} and DMA^{III}. All samples were stored frozen at −80°C for 1 to 4 months before analysis. The proportion of arsenic in each species in urine (%iAs, %MMA, and %DMA) was calculated by dividing the concentration of arsenic in each species by the sum of the concentrations of iAs, MMA, and DMA.

TABLE I. Mean Proportions of Urinary Arsenic Species (Standard Deviations)

Variable	*N* (%)	%iAs	%MMA	%DMA
All[a]	722 (100)			
Urine sample	494 (68.4)	9.9 (6.3)	11.1 (4.8)	79.0 (6.3)
Missing urine	228 (31.6)			
Cancer status				
Control	306 (61.9)	10.7 (6.7)	10.5 (4.3)	78.8 (8.5)
Lung	80 (16.2)	10.3 (5.5)	12.9 (5.5)*	76.8 (8.1)*
Bladder	108 (21.9)	7.2 (4.9)*	11.6 (5.2)*	81.2 (7.4)*
Sex				
Male	353 (71.5)	10.1 (6.5)	11.5 (4.8)	78.4 (8.4)
Female	141 (28.5)	8.5 (5.9)	10.0 (4.4)*	80.5 (7.8)*
Age[b]				
<60	152 (30.8)	10.9 (5.9)	10.7 (4.5)	78.4 (7.9)
60–69	162 (32.8)	9.5 (6.6)	11.4 (5.4)	79.1 (9.1)
70+	180 (36.4)	9.4 (6.3)	11.2 (4.4)	79.5 (7.8)
		$r_S = -0.14$*	$r_S = 0.07$	$r_S = 0.06$
Tobacco smoking				
Never	148 (30.0)	10.6 (6.9)	10.4 (4.6)	78.9 (8.6)
Former	217 (43.9)	9.3 (6.5)*	11.0 (4.3)	79.7 (8.2)
Current	129 (26.1)	10.0 (5.2)	12.0 (5.4)*	78.0 (8.1)
0–20 Pack-years	215 (43.5)	10.4 (6.6)	10.8 (4.5)	78.8 (8.4)
>20 Pack-years	279 (56.5)	9.5 (6.0)	11.4 (5.0)	79.1 (8.3)
Obesity[c]				
No	413 (83.6)	9.8 (6.1)	11.3 (4.9)	78.9 (8.2)
Yes	81 (16.4)	10.3 (7.1)	9.9 (4.1)*	79.8 (8.7)
Race				
European	388 (78.5)	10.3 (6.6)	10.7 (4.8)	79.0 (8.53)
Other	106 (21.5)	9.8 (6.2)	11.2 (4.8)	79.0 (8.23)

[a]All genotyped Chile samples with known case status.
[b]Spearman correlation coefficients (r_S).
[c]Body mass index $\geq$30 kg m^{-2}.
*Statistically significant ($P < 0.05$) r_S or metabolite difference compared to reference group calculated by the Wilcoxon rank-sum test.

Genomic DNA Purification and Quantification

DNA was isolated from blood clots using the Gentra Puregene Blood Kit combined with Clotspin Baskets (Qiagen, Hilden, Germany) or saliva using Oragene™ saliva collection kits according to manufacturer's instructions (DNA Genotek Inc., Ontario, Canada). All DNA samples were quantified using PicoGreen dsDNA quantitation kits (Molecular Probes, Eugene, OR).

Genotyping AS3MT and N6AMT1 Polymorphisms

We selected six *AS3MT* SNPs (rs7085104, rs3740400, rs3740393, rs3740390, rs11191439, rs1046778) based on previously reported associations with arsenic metabolism [Engström et al., 2011]. Except for rs11191439 (Met287Thr), all genotyped *AS3MT* SNPs were intronic polymorphisms. We aimed to survey all common genetic variants in the *N6AMT1* gene to precisely map the association between SNPs and arsenic metabolism. All 108 polymorphisms in the gene region, including its 50 flanking region, with call rate >90% and minor allele frequency (MAF) $\geq$5% in Europeans (CEU) from the HapMap Project (release 28) were included. Fourteen tag SNPs were selected using Tagger within Haploview at $r^2 > 0.8$ [Barrett et al., 2005; de Bakker et al., 2005] and captured 100% of the common variability in *N6AMT1*.

AS3MT and *N6AMT1* polymorphisms were detected using a novel custom-designed assay based on the multiplex, ligation-dependent probe amplification (MLPA) method developed by MRC-Holland [Schouten et al., 2002] (www.mlpa.com). Protocol details are described in Akers et al. [2011]. Mixtures, concentrations, and sequences for each probe are provided in Supporting Information Table II. The PCR program was adapted to: 2 min at 98°C; 32 cycles of 5 sec at 98°C and 15 sec at 65°C; 1 min 72°C. Two probe pairs at non-variable sites were included in each reaction as positive controls for DNA quality. Blanks and control DNA samples were included on every plate for quality control. We verified our assay using 15 control DNA samples with known sequences acquired from the 1000 genomes project [The 1000 Genomes Project Consortium, 2012]. Additionally, we compared genotyping results for *N6AMT1* tag SNP rs1048546 from our method against the Taqman® SNP genotyping assay (Applied Biosystems, Carlsbad, CA) using 150 randomly selected Chile samples. Agreement was 100% between both methods.

We performed linkage disequilibrium analysis and constructed haplotype blocks from the Chile genotyping data in Haploview using the solid spine method for *AS3MT* SNPs and the confidence intervals algorithm by Gabriel et al. [2002] for *N6AMT* SNPs (Fig. 1). Haplotypes were inferred by the PHASE software [Stephens and Donnelly, 2003].

Statistical Analysis

Associations between genotypes or haplotypes and each urinary arsenic species (%MMA, %DMA and %InAs) were analyzed using multivariate linear regression. The genotype with the largest number of subjects was used as the reference genotype. Minor allele and haplotype frequencies <5% were declared as rare and combined. Genotypes/haplotypes were modeled as categorical variables (zero, one, or two minor allelles/copies) and as zero copies versus at-least one minor allele or haplotype.

All metabolite models were adjusted for log transformed total urinary iAs concentrations, age (continuous), sex, current smoking status, and cancer case status.

Lung and bladder cancer odds ratios (ORs) were calculated for all SNPs and haplotypes using logistic regression. The relationship between genotypes/haplotypes, arsenic metabolism and cancer status were modeled in two ways: (1) Direct association between genotypes/haplotypes and cancer status; and, (2) Greater than additive biological interaction between historical arsenic water concentration exposures and genotypes/haplotypes on cancer ORs. Both approaches were adjusted for age, sex, and current smoking status. Further adjustments for pack-years or average cigarettes smoked had little impact on results. To examine synergy, we stratified subjects by genotypes and by having ever smoked or by highest average contiguous 5-year arsenic water concentrations, excluding the 5-years prior to cancer diagnosis or subject interview, above and below 200 μg L^{-1}. This cut-off divides the subjects into two approximately equal sized exposure groups. Details on calculating the highest average contiguous 5-year arsenic water concentrations can be found in Steinmaus et al. [2013]. Analyses were performed with R software, version 3.1.3 [R Core Team, 2015]. The *epiR* package was used to calculate Rothman synergy indices [Stevenson et al., 2015].

Mediation analyses were conducted to identify the indirect association between *AS3MT* genotypes and cancer risk attributed to differences in %MMA relative to the total association. We implemented the mediation package using nonparametric bootstrapping with 1000 iterations to quantify the proportion mediated and obtain 95% confidence intervals for these estimates [Tingley et al., 2014].

RESULTS

Of the 937 living subjects in the original Chile case-control study, 119 lung cancer cases, 147 bladder cancer cases and 456 controls were genotyped for *AS3MT* and *N6AMT1* SNPs. All polymorphisms were in Hardy–Weinberg equilibrium ($P < 0.001$) and had a MAF > 5%. Among the *AS3MT* SNPs, rs7085104 was in strong linkage disequilibrium (LD) with rs3740400 ($R^2 = 0.95$) and rs3740393 showed modest LD with rs3740390 ($R^2 = 0.89$) (Fig. 1). The *AS3MT* minor allele frequencies in this population were similar to previously published studies from Mexico [Drobná et al., 2016], Bangladesh [Engström et al., 2011], and Mongolia [Chen et al., 2017] (Fig. 2). Table I lists the mean proportions of each arsenic species stratified by case status, sex, age, current smoking status, and race for the 494 individuals with genotype and metabolite information in the study. All of these variables, except for race, were significantly associated with at least one arsenic metabolite and were adjusted for in all regression analyses.

We found statistically significant associations between *AS3MT* SNPs—rs3740393, rs3740390, rs11191439, rs1046778—and urinary arsenic metabolites, even after adjusting for total urinary arsenic, age, sex, current smoking status, and case status (Table II). There was a 3.0 (95% CI: 2.1, 4.0) percent increase in the proportion of MMA among carriers of the rs11191439 minor allele (Thr). A monotonic decrease in %MMA was also observed with each additional copy of the mutant allele for the other three SNPs. Associations between all *AS3MT* polymorphisms and %DMA were in the opposite direction and of similar magnitude compared to those associations identified with %MMA. These associations remained even after restricting the analysis to control subjects (Supporting Information Table III).

Associations between *AS3MT* polymorphisms and %MMA were in the same direction as the bladder and lung cancer odds ratios (Table II). For example, individuals carrying the rs11191439 minor allele (Thr), the polymorphism associated with the greatest increase in %MMA, had a statistically significant increase in lung cancer (OR = 1.7; 95% CI: 1.0, 2.7) and a modest increase in bladder cancer (OR = 1.3; 95% CI: 0.8, 2.1). Having at least one copy of the rs3740393 minor allele (C) was associated with both decreased lung and bladder cancer odds ratios (OR = 0.6; 95% CI: 0.4, 0.9 and OR = 0.5; 95% CI: 0.3, 0.8 for lung and bladder cancer, respectively). The minor alleles of *AS3MT* SNPs rs3740390 (A) and rs1046778 (C) were also associated with reduced bladder cancer odds ratios.

Mediation analysis revealed that 39.8% ($P = 0.02$) of the association between *AS3MT* SNP rs11191439 and lung cancer could be attributed to differences in %MMA. We also found that 33.5% ($P = 0.04$) of the association between rs3740393 and lung cancer was mediated by %MMA. The proportion of the association between *AS3MT* SNPs and bladder cancer mediated by %MMA was <5% and not statistically significant (data not shown).

Cancer ORs stratified by historical arsenic exposure and polymorphisms are shown in Table III. For rs11191439, compared to subjects with arsenic exposures <200 μg L^{-1} and genotype Met/Met, the lung cancer OR for exposures >200 μg L^{-1} was greater in those with at least one copy of the Thr allele (OR = 5.6; 95%CI: 3.0, 10.7), compared to wild-type (OR = 2.6; 95% CI: 1.6, 4.3; Rothman synergy index = 3.6, 95% CI: 1.2, 11.1). Evidence of interaction was also seen between arsenic and having at least one copy of the rs3740393 minor allele (C) for both lung and bladder cancer, although with Rothman synergy indices below 1.0 (i.e., antagonism).

We also examined the interaction between *AS3MT* SNPs and smoking in lung and bladder cancer. The minor allele of *AS3MT* SNPs rs3740393, rs3740390, and rs1046778 were protective against lung cancer among individuals who reported ever smoking (Supporting Information Table IV). Rothman synergy indices for each of these SNPs were 0.3 (95% CI: 0.1, 0.8), 0.3 (95% CI: 0.1, 0.8), and 0.4 (95% CI: 0.2, 0.9), respectively. Further adjustment for %MMA had little impact on these results (data not shown). There was no evidence of interaction between *AS3MT* SNPs and having ever smoked for bladder cancer (Supporting Information Table IV).

Haplotypes were inferred from all six *AS3MT* polymorphisms (rs7085104, rs3740400, rs3740393, rs3740390, rs11191439, rs1046778) and analyzed in relation to urinary arsenic metabolites and cancer case status. The observed haplotypes were AAGGTT (51%), GCCATC

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TABLE II. Associations Between *AS3MT* Polymorphisms, Urinary Arsenic Metabolites, and Cancer Outcomes

SNP	Genotype	*N*	%iAs β[a]	%MMA β[a]	%DMA β[a]	N_C	N_L	Lung cancer OR[b]	N_B	Bladder Cancer OR[b]
rs7085104	AA	156				133	36		52	
	AG	222	0.3 (−1.0, 1.5)	0.4 (−0.5, 1.4)	−0.7 (−2.4, 1.0)	209	54	1.0 (0.6, 1.6)	69	0.8 (0.6, 1.3)
	GG	116	0.3 (−1.2, 1.8)	1.2 (0.1, 2.4)*	−1.5 (−3.5, 0.5)	114	29	1.0 (0.6, 1.8)	26	0.6 (0.4, 1.0)
	AG/GG	338	0.3 (−0.9, 1.4)	0.7 (−0.2, 1.6)	−1.0 (−2.5, 0.6)	323	83	1.0 (0.6, 1.6)	95	0.8 (0.5, 1.1)
rs3740400	AA	151				131	35		50	
	AC	220	0.1 (−1.2, 1.4)	0.5 (−0.5, 1.5)	−0.6 (−2.3, 1.1)	206	52	1.0 (0.6, 1.6)	69	0.9 (0.6, 1.4)
	CC	123	0.1 (−1.4, 1.5)	1.0 (−0.1, 2.1)	−1.1 (−3.0, 0.9)	119	32	1.1 (0.6, 1.9)	28	0.6 (0.4, 1.1)
	AC/CC	343	0.1 (−1.1, 1.3)	0.7 (−0.2, 1.6)	−0.8 (−2.3, 0.8)	325	84	1.0 (0.6, 1.6)	97	0.8 (0.5, 1.2)
rs3740393	GG	266				220	72		94	
	GC	180	−1.6 (−2.8, −0.4)*	−1.1 (−1.9, −0.2)*	2.7 (1.1, 4.2)*	182	38	0.6 (0.4, 1.0)*	47	0.6 (0.4, 0.9)*
	CC	48	−2.1 (−4.0, −0.2)*	−1.9 (−3.3, −0.4)*	4.0 (1.5, 6.5)*	54	9	0.6 (0.2, 1.1)	6	0.3 (0.1, 0.6)*
	GC/CC	228	−1.7 (−2.8, −0.6)*	−1.2 (−2.1, −0.4)*	2.9 (1.5, 4.4)*	236	47	0.6 (0.4, 0.9)*	53	0.5 (0.3, 0.8)*
rs3740390	GG	289				241	75		97	
	GA	160	−1.3 (−2.5, −0.2)*	−0.8 (−1.7, 0.1)	2.1 (0.6, 3.7)*	165	36	0.7 (0.4, 1.1)	44	0.7 (0.4, 1.0)*
	AA	44	−2.1 (−4.1, −0.2)*	−1.6 (−3.1, −0.1)*	3.7 (1.1, 6.3)*	49	8	0.6 (0.2, 1.2)	6	0.3 (0.1, 0.7)*
	GA/AA	204	−1.5 (−2.6, −0.4)*	−1.0 (−1.8, −0.1)*	2.5 (1.0, 3.9)*	214	44	0.7 (0.4, 1.0)	50	0.6 (0.4, 0.9)*
rs11191439	Met/Met	384				372	86		113	
	Met/Thr	110	2.2 (0.9, 3.5)*	3.0 (2.1, 4)*	−5.2 (−6.9, −3.5)*	84	33	1.7 (1.0, 2.7)*	34	1.3 (0.8, 2.1)
rs1046778	TT	210				173	53		71	
	TC	206	1.1 (−2.3, 0.1)	−0.5 (−1.4, 0.4)	1.6 (0, 3.2)*	200	51	0.8 (0.5, 1.3)	60	0.7 (0.5, 1.1)
	CC	75	−2.2 (−3.8, −0.5)*	−2.1 (−3.3, −0.8)*	4.2 (2.1, 6.4)*	82	14	0.6 (0.3, 1.1)	15	0.5 (0.2, 0.8)*
	TC/CC	281	−1.4 (−2.5, −0.3)*	−0.9 (−1.7, 0)*	2.3 (0.8, 3.7)*	282	65	0.8 (0.5, 1.1)	75	0.7 (0.4, 1.0)*

N_C = number of controls, N_L = number of lung cancer cases, N_B = number of bladder cancer cases.

95% confidence intervals are in parentheses.

[a]Mean metabolite difference compared to the wildtype genotype (β) adjusted for log(total urinary iAs), age, sex, current smoking status, and case status.

[b]Odds ratio (OR) compared to individuals homozygous for reference allele adjusted for age, sex and current smoking status.

*Statistically significant ($P < 0.05$) association.

TABLE III. Interaction Between *AS3MT* Polymorphisms and Historical Arsenic Exposure on Cancer Outcomes

SNP	Genotype/arsenic	N_C	N_L	Lung cancer OR[a]	S^b_{Lung}	N_B	Bladder cancer OR[a]	$S^b_{Bladder}$
rs7085104	AA<200	72	11		1.4 (0.4, 4.3)	8		0.8 (0.4, 1.4)
	AG/GG<200	192	25	0.9 (0.4, 1.9)		21	1.0 (0.4, 2.5)	
	AA>200	61	25	2.7 (1.2, 6.2)		44	6.7 (3.0, 16.4)	
	AG/GG>200	131	58	3.2 (1.6, 6.8)		74	5.5 (2.6, 13.1)	
rs3740400	AA<200	70	10		1.3 (0.5, 3.6)	8		0.9 (0.5, 1.6)
	AC/CC<200	194	26	0.9 (0.4, 2.2)		21	1.0 (0.4, 2.4)	
	AA>200	61	25	2.9 (1.3, 6.8)		42	6.3 (2.8, 15.4)	
	AC/CC>200	131	58	3.4 (1.7, 7.6)		76	5.6 (2.6, 13.2)	
rs3740393	GG<200	128	18		0.4 (0.2, 1.0)*	19		0.5 (0.2, 1.0)*
	GC/CC<200	136	18	0.9 (0.5, 1.9)		10	0.5 (0.2, 1.0)	
	GG>200	92	54	4.2 (2.3, 7.9)		75	5.6 (3.2, 10.3)	
	GC/CC>200	100	29	2.2 (1.2, 4.4)		43	3.0 (1.7, 5.6)	
rs3740390	GG<200	139	19		0.5 (0.2, 1.1)	19		0.6 (0.3, 1.1)
	GA/AA<200	124	17	1.0 (0.5, 2.0)		10	0.6 (0.2, 1.3)	
	GG>200	102	56	4.1 (2.3, 7.6)		78	5.8 (3.3, 10.4)	
	GA/AA>200	90	27	2.4 (1.3, 4.7)		40	3.4 (1.9, 6.5)	
rs11191439	Met/Met<200	216	31		3.6 (1.2, 11.1)*	21		1.1 (0.5, 2.1)
	Met/Thr<200	48	5	0.7 (0.2, 1.8)		8	1.8 (0.7, 4.2)	
	Met/Met>200	156	55	2.6 (1.6, 4.3)		92	6.5 (3.9, 11.2)	
	Met/Thr>200	36	28	5.6 (3.0, 10.7)		26	7.8 (4.0, 15.7)	
rs1046778	TT<200	96	13		0.6 (0.3, 1.4)	14		0.7 (0.4, 1.5)
	TC/CC<200	167	23	1.0 (0.5, 2.1)		15	0.6 (0.3, 1.3)	
	TT>200	77	40	3.9 (2.0, 8.2)		57	5.1 (2.7, 10.2)	
	TC/CC>200	115	42	2.9 (1.5, 5.9)		60	3.8 (2.0, 7.4)	

N_C = number of controls, N_L = number of lung cancer cases, N_B = number of bladder cancer cases.
95% Confidence intervals are in parentheses.
[a]Odds ratio (OR) compared to individuals homozygous for reference allele and arsenic exposure <200 adjusted for age, sex and current smoking status.
[b]Rothman synergy index (S).
*Statistically significant ($P < 0.05$) association.

(25%), GCGGCT (11%), and GCGGTC (8%), with their frequencies listed in parentheses. The results from the haplotype analyses were similar to those obtained with individual SNPs (Supporting Information Table V). We observed a monotonic decrease in both %MMA and the bladder cancer odds ratio with each additional copy of the GCCATC haplotype. Furthermore, there was a 3.0 (95% CI: 2.1, 4.0) percent increase in MMA and a lung cancer OR of 1.7 (95% CI: 1.0, 2.7) among individuals with one copy of the GCGGCT haplotype.

Unlike *AS3MT*, there were no associations between *N6AMT1* tag SNPs and urine arsenic metabolite proportions that corresponded to differences in cancer risk (Table IV). For example, rs7282280 was the only tag SNP associated with a statistically significant decrease in %MMA and an increase in %DMA, but was not associated with cancer ORs. The haplotype analysis of *N6AMT1* tag SNPs did not provide any additional information beyond the individual SNPs (data not shown).

DISCUSSION

This study provides strong evidence that multiple *AS3MT* polymorphisms impact arsenic metabolism capacity and lung and bladder cancer risks in a Chilean population exposed to arsenic in drinking water. We identified several *AS3MT* polymorphisms associated with lung and/or bladder cancer risks, and all of these were associated with arsenic metabolic patterns that were consistent with these risks. For example, in vitro research has shown that MMA is a highly toxic metabolite of ingested arsenic, and the polymorphisms we found linked to decreases in %MMA were also linked to decreases in cancer risk. This consistency not only supports our findings linking these polymorphism to cancer, they also support our hypothesis that MMA may be the primary arsenic species responsible for these effects.

We observed that the minor allele of *AS3MT* SNPs—rs3740393 (C), rs3740390 (A), and rs1046778 (C)—were associated with decreased %MMA and increased %DMA, suggesting that carriers of these alleles metabolize arsenic more efficiently compared to the majority of individuals in our study who had the reference allele. The direction of these associations are consistent with those seen in other population studies [Chung et al., 2009; Agusa et al., 2009; Engström et al., 2011, 2007; Drobná et al., 2016]. We also confirmed previous findings that the Thr allele of rs111191439 increases %MMA and lowers %DMA [Lindberg et al., 2007; Hernández et al., 2008; Agusa et al., 2009; Valenzuela et al., 2009; Engström et al., 2011]. A review of

TABLE IV. Association Between *N6AMT1* Polymorphisms, Urinary Metabolites, and Cancer Outcomes

SNP	Genotype	*N*	%iAs β[a]	%MMA β[a]	%DMA β[a]	N_C	N_L	Lung cancer OR[b]	N_B	Bladder cancer OR[b]
rs1006903	GG	356				331	90		104	
	GC/CC	137	−0.2 (−1.4, 1)	0.1 (−0.8, 1)	0.1 (−1.6, 1.7)	125	29	0.8 (0.5, 1.3)	42	1.1 (0.7, 1.6)
rs2832109	AA	441				409	112		128	
	AT/TT	50	1.3 (−0.5, 3.1)	0 (−1.4, 1.3)	−1.3 (−3.7, 1.1)	44	7	0.6 (0.2, 1.2)	19	1.4 (0.8, 2.4)
rs1003671	AA	127				114	32		47	
	AG	247	0.1 (−1.2, 1.4)	0.1 (−0.9, 1.1)	−0.2 (−2.0, 1.5)	223	63	1.0 (0.6, 1.7)	63	0.7 (0.4, 1.1)
	GG	120	1.0 (−0.6, 2.5)	0 (−1.1, 1.2)	−1.0 (−3.1, 1.0)	119	24	0.7 (0.4, 1.3)	37	0.7 (0.4, 1.2)
	AG/GG	367	0.4 (−0.8, 1.6)	0.1 (−0.8, 1.0)	−0.5 (−2.1, 1.2)	342	87	0.9 (0.6, 1.5)	100	0.7 (0.5, 1.1)
rs4816333	CC	232				217	50		63	
	CG	207	−0.2 (−1.4, 0.9)	−0.1 (−1.0, 0.8)	0.3 (−1.2, 1.9)	189	54	1.3 (0.8, 1.9)	66	1.2 (0.8, 1.8)
	GG	54	−0.6 (−2.4, 1.2)	0.1 (−1.3, 1.5)	0.5 (−1.9, 2.9)	50	14	1.2 (0.6, 2.3)	18	1.3 (0.7, 2.3)
	CG/GG	261	−0.3 (−1.4, 0.8)	−0.1 (−0.9, 0.8)	0.4 (−1.1, 1.8)	239	68	1.2 (0.8, 1.9)	84	1.2 (0.9, 1.8)
rs7282280	CC	350				327	85		103	
	CT/TT	142	−0.7 (−1.9, 0.6)	−0.9 (−1.8, 0)*	1.5 (0, 3.1)	128	33	1.0 (0.6, 1.6)	44	1.1 (0.7, 1.7)
rs7282257	AA	240				226	49		69	
	AT	210	−0.8 (−1.9, 0.4)	0.2 (−0.6, 1.1)	0.6 (−1.0, 2.1)	190	58	1.4 (0.9, 2.1)	66	1.1 (0.8, 1.7)
	TT	44	−1.0 (−3.0, 1.0)	1.0 (−0.5, 2.5)	0 (−2.6, 2.6)	40	12	1.4 (0.7, 2.8)	12	1.0 (0.5, 2.0)
	AT/TT	254	−0.8 (−1.9, 0.3)	0.4 (−0.5, 1.2)	0.5 (−1.0, 1.9)	230	70	1.4 (0.9, 2.1)	78	1.1 (0.8, 1.6)
rs1048546	GG	176				161	37		54	
	GT	241	−1.3 (−2.5, −0.1)*	−0.1 (−1.0, 0.8)	1.4 (−0.2, 3.0)	225	61	1.2 (0.7, 1.9)	69	0.9 (0.6, 1.4)
	TT	77	−0.6 (−2.3, 1.0)	0.1 (−1.2, 1.3)	0.6 (−1.6, 2.7)	70	21	1.3 (0.7, 2.4)	24	1.0 (0.6, 1.8)
	GT/TT	318	−1.1 (−2.3, 0)	−0.1 (−0.9, 0.8)	1.2 (−0.3, 2.7)	295	82	1.2 (0.8, 1.9)	93	1.0 (0.7, 1.4)
rs2065266	CC	126				111	33		47	
	CT	252	0 (−1.3, 1.4)	0.1 (−0.9, 1.1)	−0.1 (−1.9, 1.6)	239	61	0.9 (0.5, 1.4)	63	0.6 (0.4, 1.0)*
	TT	116	1.3 (−0.3, 2.8)	0.2 (−0.9, 1.4)	−1.5 (−3.6, 0.6)	106	25	0.8 (0.4, 1.5)	37	0.8 (0.5, 1.3)
	CT/TT	368	0.4 (−0.8, 1.7)	0.1 (−0.8, 1.1)	0.1 (−0.8, 1.1)	345	86	0.9 (0.5, 1.4)	100	0.7 (0.4, 1.0)
rs2738966	AA	206				194	45		59	
	AG	228	−0.4 (−1.5, 0.8)	0.3 (−0.6, 1.1)	0.1 (−1.5, 1.6)	209	60	1.2 (0.8, 1.9)	69	1.1 (0.7, 1.7)
	GG	60	−1.2 (−3.0, 0.5)	0.6 (−0.8, 1.9)	0.7 (−1.7, 3.0)	53	14	1.1 (0.5, 2.2)	19	1.2 (0.7, 2.2)
	AG/GG	288	−0.6 (−1.7, 0.6)	0.3 (−0.5, 1.2)	0.2 (−1.3, 1.7)	262	74	1.2 (0.8, 1.8)	88	1.1 (0.8, 1.7)
rs2245065	AA	425				391	108		126	
	AG/GG	67	0 (−1.6, 1.6)	−0.1 (−1.3, 1.1)	0 (−2.1, 2.1)	63	11	0.6 (0.3, 1.1)	21	1.1 (0.6, 1.8)
rs2248501	GG	240				225	50		69	
	GT	207	−0.7 (−1.9, 0.4)	0.2 (−0.7, 1.1)	0.5 (−1.0, 2.0)	188	57	1.3 (0.9, 2.1)	65	1.1 (0.8, 1.7)
	TT	45	−1.0 (−3.0, 1.0)	1.0 (−0.5, 2.4)	0.1 (−2.6, 2.7)	41	11	1.2 (0.6, 2.5)	12	1.0 (0.5, 1.9)
	GT/TT	252	−0.8 (−1.9, 0.3)	0.3 (−0.5, 1.2)	0.4 (−1.0, 1.9)	229	68	1.3 (0.9, 2.0)	77	1.1 (0.8, 1.6)
rs2776228	GG	341				311	93		100	
	GT/TT	142	0.5 (−0.7, 1.7)	−0.7 (−1.6, 0.2)	0.2 (−1.4, 1.8)	136	25	0.6 (0.4, 1.0)*	43	1.0 (0.7, 1.5)
rs17736282	AA	426				389	105		136	
	AT/TT	68	0.8 (−0.8, 2.4)	−0.1 (−1.3, 1.1)	−0.7 (−2.8, 1.4)	67	14	0.8 (0.4, 1.4)	11	0.5 (0.2, 0.9)*
rs2832138	GG	420				386	104		125	
	GA/AA	74	0.2 (−1.3, 1.8)	−1.1 (−2.2, 0)	0.9 (−1.2, 2.9)	70	15	0.8 (0.4, 1.5)	22	1.0 (0.6, 1.6)

N_C = number of controls, N_L = number of lung cancer cases, N_B = number of bladder cancer cases.
95% Confidence intervals are in parentheses.
[a]Mean metabolite difference compared to the wildtype genotype (β) adjusted for log(total urinary iAs), age, sex, current smoking status and case status.
[b]Odds Ratio (OR) adjusted for age, sex and current smoking status.
*Statistically significant ($P < 0.05$) association.

AS3MT SNPs highlights the global relationship between rs3740393 and rs11191439 with arsenic metabolism efficiency [Agusa et al., 2011]. In 2014, another review conducted a pooled analysis of all published studies and observed that rs3740390 and rs11191439 were associated with statistically significant changes in %MMA across multiple populations [Antonelli et al., 2014]. The reproducibility of these SNPs in our study confirms their importance in arsenic metabolism across several populations, including Chile.

To date, few studies have examined the relationship between *AS3MT* polymorphism and internal cancer risk. For instance, our study is the first to analyze the relationship between these polymorphisms and lung cancer. This is particularly important because lung cancer is the number one cause of long-term mortality from ingested arsenic [Smith et al., 2006; Marshall et al., 2007]. We demonstrate that a significant proportion of the association between *AS3MT* polymorphisms and lung cancer risk is mediated

by arsenic methylation, which provides additional evidence that the human lung is a major target site of arsenic. A previous case-control study by Lesseur *et* al. did not find associations between rs3740393 or rs11191439 and bladder cancer in their New Hampshire population [Lesseur et al., 2012]. A similar case-control study in Southeastern Michigan did not find any direct associations between *AS3MT* SNPs rs7085104, rs3740400, rs11191439, or rs1046778 and bladder cancer. However, possessing at least one copy of the rs11191439 Thr allele, in addition to higher average arsenic exposure, did increase bladder cancer risk [Beebe-Dimmer et al., 2012]. We observed a similar result where individuals with historical arsenic exposure >200 μg L^{-1} and the rs11191439 Thr allele had a higher lung cancer OR compared to those with two copies of the wild-type allele, suggesting a gene-environment interaction. In both previous case-control studies, arsenic water concentrations were an order of magnitude lower than those in our study. This may have limited the ability of these studies to identify true associations, and highlights the potential advantage of investigating associations, at least initially, in areas where exposures are high.

The reason we found evidence of greater mediation by %MMA for lung cancer than for bladder cancer is not entirely clear. Intra-individual variability in %MMA and the fact that we only assessed %MMA at a single point in time likely affected our mediation analysis although it is not clear that this would impact bladder cancer more than lung cancer. It is possible that inter-individual differences in %MMA have greater impacts on lung cancer. In a previous report using the same data we used here, associations with %MMA were seen for both cancer types but were two to three times greater for lung cancer. For each one percent increase in %MMA ORs were 1.11 (95% CI, 1.05–1.17) for lung cancer and 1.04 (95% CI, 1.00–1.09) for bladder cancer. It is also possible that other risk factors or other mechanisms have different roles in the two types of arsenic-related cancers but this is mostly speculative. Overall, further research is needed to explore this issue.

We did not observe clear associations between *N6AMT* tag SNPs, %MMA, and cancer risk. Harari et al. analyzed the *N6AMT1* SNP rs1048546 in the San Antonio de los Cobres (SAC) population of highly arsenic-exposed indigenous women and observed a significant association with %MMA. In our analysis, the association between rs1048546 genotypes and %MMA was in the same direction observed by Harari et al., despite not being statistically significant. We confirmed that our null findings were not a result of the genotyping method. The reason for this inconsistency is unclear, but our population was much larger than the SAC population, had much lower recent arsenic exposure, and consisted of mostly males, smokers and Europeans. Several studies have shown marked differences in arsenic methylation patterns and genotypes based on ethnicity, and it is possible these caused the differences we identified [Engström et al., 2010; Fu et al., 2014]. Furthermore, the distribution of the protective *AS3MT* haplotype in the SAC population is higher than most populations around the world, making them extremely efficient arsenic metabolizers [Schlebusch et al., 2013, 2015]. Therefore, the contribution of N6AMT1 to arsenic metabolism within this population may differ from Chile. Chen et al. also examined the relationship between several *N6AMT* tag SNPs included in our study and urinary metabolite patterns in an arsenic-exposed population from Wuyuan, Inner Mongolia [Chen et al., 2017]. Rs1003671 was the only N6AMT polymorphism associated with %MMA in the Mongolian population. This SNP did not influence arsenic metabolism or cancer risk in our study population. It is important to note that Chen *et* al. did not find a direct association between *AS3MT* polymorphisms and urinary metabolites, but did show interaction with between *AS3MT* and *N6AMT1* SNPs on arsenic metabolism. This indicates that the involvement of these two enzymes in the arsenic metabolism process may differ between the Chilean and Mongolian populations. Overall, the inconsistency we observed for N6AMT1 limits any conclusions we can make regarding this gene at this time and suggest additional research may be needed on this topic. Furthermore, although the selected 14 tag SNPs capture all the common genetic variation in *N6AMT* in HapMap-CEU (r28), these might not cover the whole *N6AMT* variant spectrum in Chileans due to its admix ancestry nature. Therefore, additional tag SNPs selected from appropriate reference panels that better capture the LD structure in Chilean populations should be included in future analyses to get a better understanding of the association between *N6AMT* SNPs with %MMA and cancer risk in this population.

For this analysis, we only considered the influence of two methyltransferases on arsenic metabolism and toxicity. However, additional methyltranserases (e.g., DNMT1a and DNMT3b) and other enyzymes involved in one-carbon metabolism and glutathione biosynthesis, such as methylenetetrahydrofolate reductase (MTHFR), cystathionine-beta-synthase (CBS), and glutathione-*S*-transferase omega 1 (GSTO1), have been shown to influence the metabolism process [Schläwicke Engström et al., 2009; Porter et al., 2010; Steinmaus et al., 2010; Engström et al., 2011]. Follow-up studies in this population should explore the contribution of these genes to the inter-individual variability in methylation patterns, as well as internal cancer risk.

Urinary methylation patterns were assessed after disease diagnosis and were assumed to be representative of subject's past methylation patterns. It is possible that using a cross-sectional urine collection at the time of cancer diagnosis influenced methylation status, either from the disease itself or through treatment and lifestyle changes adopted after diagnosis. However, we observed associations between genotypes and metabolites even after cancer cases were excluded. Studies assessing methylation patterns in the same individual over time suggests that patterns remain

fairly stable [Concha et al., 2002; Steinmaus et al., 2005]. Evidence also suggests that stable genetic factors play a more important role in determining inter-individual differences in methylation patterns than do factors that are likely to have greater day to day variability such as diet or smoking [Chung et al., 2002]. It should also be noted that although intra-individual variability in methylation patterns could lead to misclassification of past methylation patterns, because we collected and analyzed metabolites from all subjects using the same protocols, the resulting bias would most likely be non-differential and towards the null, not towards the positive associations identified.

Confounding is possible, however ORs did not change with further adjustments for potential confounders such as age, sex smoking, race, and body mass index. Some selection bias may have occurred as a result of only genotyping a portion of the total Chilean population and measuring urinary metabolites in a smaller subset of these individuals. However, the age, sex and smoking status distributions within these three groups were comparable suggesting that our results are still representative of the entire study population.

In conclusion, our results highlight the involvement of AS3MT in arsenic metabolism in humans, and identify polymorphisms in this gene that account for some inter-individual variability of the metabolic process. Our study is the first to use the same population to provide evidence that *AS3MT* polymorphisms increase the risk of arsenic-induced lung and bladder cancers by reducing the metabolism of MMA to the less toxic DMA. This research may help identify subpopulations that are particularly susceptible to arsenic-induced lung and bladder cancer and who may need enhanced regulatory protection.

Statement of author contributions: Drs. Steinmaus, A Smith, M Smith, Skibola and Zhang designed the study. R de la Rosa analyzed the data and drafted the manuscript. Drs. Akers, Conde, Ferreccio, Kalman, and Mr. Zhang were involved in sample collection and data acquisition. All authors have revised and approved the final manuscript.

REFERENCES

Agusa T, Iwata H, Fujihara J, Kunito T, Takeshita H, Minh TB, Trang PTK, Viet PH, Tanabe S. 2009. Genetic polymorphisms in AS3MT and arsenic metabolism in residents of the Red River Delta, Vietnam. Toxicol Appl Pharmacol 236:131–141. doi:10.1016/j.taap.2009.01.015.

Agusa T, Fujihara J, Takeshita H, Iwata H. 2011. Individual variations in inorganic arsenic metabolism associated with AS3MT genetic polymorphisms. Int J Mol Sci 12:2351–2382. doi:10.3390/ijms12042351.

Akers NK, Curry JD, Smith MT, Bracci PM, Skibola CF. 2011. Multiplexed, ligation-dependent probe amplification for rapid and inexpensive HLA-DQB1 allelotyping. Tissue Antigens 78:275–280. doi:10.1111/j.1399-0039.2011.01737.x.

Antonelli R, Shao K, Thomas DJ, Sams R II, Cowden J. 2014. AS3MT, GSTO, and PNP polymorphisms: Impact on arsenic methylation and implications for disease susceptibility. Environ Res 132:156–167. doi:10.1016/j.envres.2014.03.012.

Barrett JC, Fry B, Maller J, Daly MJ. 2005. Haploview: Analysis and visualization of LD and haplotype maps. Bioinform Oxf Engl 21:263–265. doi:10.1093/bioinformatics/bth457.

Beebe-Dimmer JL, Iyer PT, Nriagu JO, Keele GR, Mehta S, Meliker JR, Lange EM, Schwartz AG, Zuhlke KA, Schottenfeld D, et al. 2012. Genetic variation in glutathione *S*-transferase omega-1, arsenic methyltransferase and methylene-tetrahydrofolate reductase, arsenic exposure and bladder cancer: a case-control study. Environ Health Glob Access Sci Source 11:43. doi:10.1186/1476-069X-11-43.

Buchet PJP, Lauwerys R, Roels H. 1981. Urinary excretion of inorganic arsenic and its metabolites after repeated ingestion of sodium metaarsenite by volunteers. Int Arch Occup Environ Health 48:111–118. doi:10.1007/BF00378431.

Calderon RL, Hudgens E, Le XC, Schreinemachers D, Thomas DJ. 1999. Excretion of arsenic in urine as a function of exposure to arsenic in drinking water. Environ Health Perspect 107:663–667.

Chen X, Guo X, He P, Nie J, Yan X, Zhu J, Zhang L, Mao G, Wu H, Liu Z, et al. 2017. Interactive influence of N6AMT1 and As3MT genetic variations on arsenic metabolism in the population of Inner Mongolia, China. Toxicol Sci 155:124–134. doi:10.1093/toxsci/kfw181.

Chung C-J, Hsueh Y-M, Bai C-H, Huang Y-K, Huang Y-L, Yang M-H, Chen C-J. 2009. Polymorphisms in arsenic metabolism genes, urinary arsenic methylation profile and cancer. Cancer Causes Control CCC 20:1653–1661. doi:10.1007/s10552-009-9413-0.

Chung JS, Kalman DA, Moore LE, Kosnett MJ, Arroyo AP, Beeris M, Mazumder DNG, Hernandez AL, Smith AH. 2002. Family correlations of arsenic methylation patterns in children and parents exposed to high concentrations of arsenic in drinking water. Environ Health Perspect 110:729–733.

Concha G, Vogler G, Nermell B, Vahter M. 2002. Intra-individual variation in the metabolism of inorganic arsenic. Int Arch Occup Environ Health 75:576–580. doi:10.1007/s00420-002-0361-1.

de Bakker PIW, Yelensky R, Pe'er I, Gabriel SB, Daly MJ, Altshuler D. 2005. Efficiency and power in genetic association studies. Nat Genet 37:1217–1223. doi:10.1038/ng1669.

Drobna Z, Naranmandura H, Kubachka KM, Edwards BC, Herbin-Davis K, Styblo M, Le XC, Creed JT, Maeda N, Hughes MF, et al. 2009a. Disruption of the arsenic (+3 oxidation state) methyltransferase gene in the mouse alters the phenotype for methylation of arsenic and affects distribution and retention of orally administered arsenate. Chem Res Toxicol 22:1713–1720. doi:10.1021/tx900179r.

Drobna Z, Styblo M, Thomas DJ. 2009b. An overview of arsenic metabolism and toxicity. Curr Protoc Toxicol Editor Board Mahin Maines Ed Chief Al 42:4.31.1–4.31.6; doi:10.1002/0471140856.tx0431s42.

Drobná Z, Martin E, Kim KS, Smeester L, Bommarito P, Rubio-Andrade M, García-Vargas GG, Stýblo M, Zou F, Fry RC. 2016. Analysis of maternal polymorphisms in arsenic (+3 oxidation state)-methyltransferase AS3MT and fetal sex in relation to arsenic metabolism and infant birth outcomes: Implications for risk analysis. Reprod Toxicol 61:28–38. doi:10.1016/j.reprotox.2016.02.017.

Engström KS, Broberg K, Concha G, Nermell B, Warholm M, Vahter M. 2007. Genetic polymorphisms influencing arsenic metabolism: Evidence from Argentina. Environ Health Perspect 115:599–605. doi:10.1289/ehp.9734.

Engström KS, Vahter M, Lindh C, Teichert F, Singh R, Concha G, Nermell B, Farmer PB, Strömberg U, Broberg K. 2010. Low 8-oxo-7,8-dihydro-2′-deoxyguanosine levels and influence of genetic background in an Andean population exposed to high levels of arsenic. Mutat Res Mol Mech Mutagen 683:98–105. doi:10.1016/j.mrfmmm.2009.10.014.

Engström K, Vahter M, Mlakar SJ, Concha G, Nermell B, Raqib R, Cardozo A, Broberg K. 2011. Polymorphisms in arsenic(+III oxidation state) methyltransferase (AS3MT) predict gene expression of AS3MT as well as arsenic metabolism. Environ Health Perspect 119:182–188. doi:10.1289/ehp.1002471.

Engström KS, Vahter M, Fletcher T, Leonardi G, Goessler W, Gurzau E, Koppova K, Rudnai P, Kumar R, Broberg K. 2015. Genetic variation in arsenic (+3 oxidation state) methyltransferase (AS3MT), arsenic metabolism and risk of basal cell carcinoma in a European population. Environ Mol Mutagen 56:60–69. doi:10.1002/em.21896.

Fu S, Wu J, Li Y, Liu Y, Gao Y, Yao F, Qiu C, Song L, Wu Y, Liao Y, et al. 2014. Urinary arsenic metabolism in a Western Chinese population exposed to high-dose inorganic arsenic in drinking water: Influence of ethnicity and genetic polymorphisms. Toxicol Appl Pharmacol 274:117–123. doi:10.1016/j.taap.2013.11.004.

Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, Higgins J, DeFelice M, Lochner A, Faggart M, et al. 2002. The structure of haplotype blocks in the human genome. Science 296: 2225–2229. doi:10.1126/science.1069424.

Gamble MV, Liu X, Ahsan H, Pilsner JR, Ilievski V, Slavkovich V, Parvez F, Chen Y, Levy D, Factor-Litvak P, et al. 2006. Folate and arsenic metabolism: A double-blind, placebo-controlled folic acid-supplementation trial in Bangladesh. Am J Clin Nutr 84:1093–1101.

Gebel TW. 2002. Arsenic methylation is a process of detoxification through accelerated excretion. Int J Hyg Environ Health 205: 505–508. doi:10.1078/1438-4639-00177.

Harari F, Engström K, Concha G, Colque G, Vahter M, Broberg K. 2013. *N*-6-adenine-specific DNA methyltransferase 1 (N6AMT1) polymorphisms and arsenic methylation in Andean women. Environ Health Perspect 121:797–803. doi:10.1289/ehp.1206003.

Hernández A, Xamena N, Sekaran C, Tokunaga H, Sampayo-Reyes A, Quinteros D, Creus A, Marcos R. 2008. High arsenic metabolic efficiency in AS3MT287Thr allele carriers. Pharmacogenet Genom 18:349–355. doi:10.1097/FPC.0b013e3282f7f46b.

Hopenhaynrich C, Smith AH, Goeden HM. 1993. Human studies do not support the methylation threshold hypothesis for the toxicity of inorganic Arsenic. Environ Res 60:161–177. doi:10.1006/enrs.1993.1024.

IARC (International Agency for Research on Cancer). Working Group on the Evaluation of Carcinogenic Risks to Humans. 2012. Arsenic, Metals, Fibres and Dusts 100:11–465.

Kalman DA, Dills RL, Steinmaus C, Yunus M, Khan AF, Prodhan MM, Yuan Y, Smith AH. 2014. Occurrence of trivalent monomethyl arsenic and other urinary arsenic species in a highly exposed juvenile population in Bangladesh. J Expo Sci Environ Epidemiol 24:113–120. doi:10.1038/jes 2013. 14.

Kile ML, Hoffman E, Hsueh Y-M, Afroz S, Quamruzzaman Q, Rahman M, Mahiuddin G, Ryan L, Christiani DC. 2009. Variability in biomarkers of arsenic exposure and metabolism in adults over time. Environ Health Perspect 117:455–460. doi:10.1289/ehp.11251.

Lesseur C, Gilbert-Diamond D, Andrew AS, Ekstrom RM, Li Z, Kelsey KT, Marsit CJ, Karagas MR. 2012. A case-control study of polymorphisms in xenobiotic and arsenic metabolism genes and arsenic-related bladder cancer in New Hampshire. Toxicol Lett 210:100–106. doi:10.1016/j.toxlet.2012.01.015.

Lindberg A-L, Kumar R, Goessler W, Thirumaran R, Gurzau E, Koppova K, Rudnai P, Leonardi G, Fletcher T, Vahter M. 2007. Metabolism of low-dose inorganic arsenic in a central European population: Influence of sex and genetic polymorphisms. Environ Health Perspect 115:1081–1086. doi:10.1289/ehp.10026.

Marshall G, Ferreccio C, Yuan Y, Bates MN, Steinmaus C, Selvin S, Liaw J, Smith AH. 2007. Fifty-year study of lung and bladder cancer mortality in Chile related to arsenic in drinking water. J Natl Cancer Inst 99:920–928. doi:10.1093/jnci/djm004.

Melak D, Ferreccio C, Kalman D, Parra R, Acevedo J, Pérez L, Cortés S, Smith AH, Yuan Y, Liaw J, et al. 2014. Arsenic methylation and lung and bladder cancer in a case-control study in Northern Chile. Toxicol Appl Pharmacol 274:225–231. doi:10.1016/j.taap.2013.11.014.

Naujokas MF, Anderson B, Ahsan H, Aposhian HV, Graziano JH, Thompson C, Suk WA. 2013. The broad scope of health effects from chronic arsenic exposure: Update on a worldwide public health problem. Environ Health Perspect 121:295–302. doi: 10.1289/ehp.1205875.

Petrick JS, Ayala-Fierro F, Cullen WR, Carter DE, Vasken Aposhian H. 2000. Monomethylarsonous acid (MMA(III)) is more toxic than arsenite in Chang human hepatocytes. Toxicol Appl Pharmacol 163:203–207. doi:10.1006/taap.1999.8872.

Pierce BL, Kibriya MG, Tong L, Jasmine F, Argos M, Roy S, Paul-Brutus R, Rahaman R, Rakibuz-Zaman M, Parvez F, et al. 2012. Genome-wide association study identifies chromosome 10q24.32 variants associated with arsenic metabolism and toxicity phenotypes in Bangladesh. M.I. McCarthyed. PLoS Genet 8:e1002522. doi:10.1371/journal.pgen.1002522.

Porter KE, Basu A, Hubbard AE, Bates MN, Kalman D, Rey O, Smith A, Smith MT, Steinmaus C, Skibola CF. 2010. Association of genetic variation in cystathionine-β-synthase and arsenic metabolism. Environ Res 110:580–587. doi:10.1016/j.envres.2010.05.001.

R Core Team. 2015. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing.

Ren X, Aleshin M, Jo WJ, Dills R, Kalman DA, Vulpe CD, Smith MT, Zhang L. 2011. Involvement of N-6 adenine-specific DNA methyltransferase 1 (N6AMT1) in Arsenic biomethylation and its role in Arsenic-induced toxicity. Environ Health Perspect 119:771–777. doi:10.1289/ehp.1002733.

Schläwicke Engström K, Nermell B, Concha G, Strömberg U, Vahter M, Broberg K. 2009. Arsenic metabolism is influenced by polymorphisms in genes involved in one-carbon metabolism and reduction reactions. Mutat Res 667:4–14. doi:10.1016/j.mrfmmm.2008.07.003.

Schlebusch CM, Gattepaille LM, Engström K, Vahter M, Jakobsson M, Broberg K. 2015. Human adaptation to Arsenic-rich environments. Mol Biol Evol 32:1544–1555. doi:10.1093/molbev/msv046.

Schlebusch CM, Lewis CM, Vahter M, Engström K, Tito RY, Obregón-Tito AJ, Huerta D, Polo SI, Medina ÁC, Brutsaert TD, et al. 2013. Possible positive selection for an arsenic-protective haplotype in humans. Environ Health Perspect 121:53–58. doi: 10.1289/ehp.1205504.

Schouten JP, McElgunn CJ, Waaijer R, Zwijnenburg D, Diepvens F, Pals G. 2002. Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. Nucleic Acids Res 30:e57. doi:10.1093/nar/gnf056.

Smith AH, Hopenhayn-Rich C, Bates MN, Goeden HM, Hertz-Picciotto I, Duggan HM, Wood R, Kosnett MJ, Smith MT. 1992. Cancer risks from arsenic in drinking water. Environ Health Perspect 97: 259–267.

Smith AH, Marshall G, Yuan Y, Ferreccio C, Liaw J, von Ehrenstein O, Steinmaus C, Bates MN, Selvin S. 2006. Increased mortality from lung cancer and bronchiectasis in young adults after exposure to arsenic in utero and in early childhood. Environ Health Perspect 114:1293–1296.

Smith AH, Steinmaus CM. 2009. Health effects of arsenic and chromium in drinking water: Recent human findings. Annu Rev Public Health 30:107–122. doi:10.1146/annurev.publhealth.031308.100143.

Steinmaus C, Carrigan K, Kalman D, Atallah R, Yuan Y, Smith AH. 2005. Dietary intake and arsenic methylation in a U.S. population. Environ Health Perspect 113:1153–1159.

Steinmaus CM, Ferreccio C, Romo JA, Yuan Y, Cortes S, Marshall G, Moore LE, Balmes JR, Liaw J, Golden T, et al. 2013. Drinking

water arsenic in Northern Chile: High cancer risks 40 years after exposure cessation. Cancer Epidemiol Biomark Prev Publ Am Assoc Cancer Res Cosponsored Am Soc Prev Oncol 22:623–630. doi:10.1158/1055-9965.EPI-12-1190.

Steinmaus C, Moore L, Hopenhayn-Rich C, Biggs ML, Smith AH. 2000. Arsenic in drinking water and bladder cancer: Environmental carcinogenesis. Cancer Invest 18:174–182.

Steinmaus C, Yuan Y, Kalman D, Rey OA, Skibola CF, Dauphine D, Basu A, Porter KE, Hubbard A, Bates MN, et al. 2010. Individual differences in arsenic metabolism and lung cancer in a case-control study in Cordoba, Argentina. Toxicol Appl Pharmacol 247:138–145. doi:10.1016/j.taap.2010.06.006.

Stephens M, Donnelly P. 2003. A comparison of Bayesian methods for haplotype reconstruction from population genotype data. Am J Hum Genet 73:1162–1169.

Stevenson M, Nunes T, Heuer C, Marshall J, Sanchez J, Thornton R, Reiczigel J, Robison-Cox J, Sebastiani P, Solymos P, et al. 2015. epiR: Tools for the Analysis of Epidemiological Data.

Stýblo M, Drobná Z, Jaspers I, Lin S, Thomas DJ. 2002. The role of biomethylation in toxicity and carcinogenicity of arsenic: A research update. Environ Health Perspect 110:767–771.

The 1000 Genomes Project Consortium. 2012. An integrated map of genetic variation from 1,092 human genomes. Nature 491:56–65. doi:10.1038/nature11632.

Thomas DJ, Li J, Waters SB, Xing W, Adair BM, Drobna Z, Devesa V, Styblo M. 2007. Arsenic (+3 oxidation state) methyltransferase and the methylation of Arsenicals. Exp Biol Med Maywood NJ 232:3–13.

Tingley D, Yamamoto T, Hirose K, Keele L, Imai K. 2014. Mediation: R package for causal mediation analysis. J Stat Softw 1.

Tseng C-H. 2007. Arsenic methylation, urinary arsenic metabolites and human diseases: Current perspective. J Environ Sci Health C 25: 1–22. doi:10.1080/10590500701201695.

Vahter M. 1999. Variation in human metabolism of arsenic. In: Chappell WR, Abernathy CO, Calderon RL, editors. Arsenic Exposure and Health Effects III. London: Elsevier Sci. Ltd 267–279.

Vahter M. 2000. Genetic polymorphism in the biotransformation of inorganic arsenic and its role in toxicity. Toxicol Lett 112/113:209–217. doi:10.1016/S0378-4274(99)00271-4.

Vahter M. 2002. Mechanisms of arsenic biotransformation. Toxicology 181/182:211–217. doi:10.1016/S0300-483X(02)00285-8.

Valenzuela OL, Drobná Z, Hernández-Castellanos E, Sánchez-Peña LC, García-Vargas GG, Borja-Aburto VH, Stýblo M, Del Razo LM. 2009. Association of AS3MT polymorphisms and the risk of premalignant arsenic skin lesions. Toxicol Appl Pharmacol 239:200–207. doi:10.1016/j.taap.2009.06.007.

Zhang H, Ge Y, He P, Chen X, Carina A, Qiu Y, Aga DS, Ren X. 2015. Interactive effects of N6AMT1 and As3MT in Arsenic biomethylation. Toxicol Sci kfv 101. doi:10.1093/toxsci/kfv101.

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