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Polymorphism of *Glutathione S-Transferase* (*GST*) Variants and Its Effect on Distribution of Urinary Arsenic Species in People Exposed to Low Inorganic Arsenic in Tap Water: An Exploratory Study

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ABSTRACT. Glutathione *S*-tranferases (GST) are multigenic enzymes that have been associated with arsenic metabolism. The objective of this study was to evaluate the relationship between polymorphic variants of GST and urinary concentration of arsenic species in people exposed to low levels of arsenic. A cross-sectional study among 66 nonoccupationally exposed subjects, living in the city of Antofagasta, Chile. Polymorphic variants were analyzed by polymerase chain reaction (PCR) and arsenic species was determined by atomic absorption spectrometry. The effect of GST variants on arsenic concentration was evaluated using univariate and covariate-adjusted regressions. For both GSTT1 and GSTM1 there were no significant differences in detected arsenic relative species between carriers of the active and null polymorphic variants. There was nondefinitive evidence that polymorphic variants of GST play a role in arsenic metabolism in sample of the Chilean subjects studied.

KEYWORDS: arsenic, exposure assessment, genetic polymorphism, risk assessment

Arsenic (As) is an element broadly distributed in nature. It comprises about 0.0001% of the earth's surface and exists in the environment in both organic and inorganic forms. Of these 2 forms, inorganic arsenic has the greatest toxicity—among humans, it has been implicated in deleterious dermatologic, reproductive, cardiovascular, and neurologic health effects, and it is a well-described carcinogen.[1,2]

In humans, there is a multistep metabolic pathway to detoxify arsenic before urinary excretion. First arsenic is

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reduced from its pentavalent (As^5) to trivalent (As^3) form by a glutathione (GSH) methylation reaction.[3,4] The As^3 metabolites then undergo oxidative methylation to monomethylated As (MMA), further reduction from pentavalent MMA to trivalent, and at last methylation to dimethylated As (DMA).[5] *S*-adenosilmethionine (SAM) is the methyl donor for methylation of As, and the arsenic (+3 oxidation state) methyltrasferase (AS3MT) performs the oxidative methylation.[6–9]

For a long time, DMA^3 arsenical compounds excreted in urine have been considered less toxic to tissues than inorganic compounds.[10,11] However, recent studies have reported that trivalent arsenic species of MMA and/or DMA are more mutagenic, toxic, and carcinogenic than inorganic arsenic, especially MMA^3.[1,12–18]

To date, 3 kind of genes have been associated with arsenic metabolisms: purine nucleoside phosphorylase (PNP), glutathione *S*-transferase omega (GSTω), and arsenic (+3 oxidation state) methyltrasferase (AS3MT).[9] GST is a human family of multigenic enzymes that detoxifies mutagenic and carcinogenic compounds, via a conjugation reaction with GSH. GSTM1, GSTT1, and GSTO 1–1 are polymorphic variants of GST (μ, θ, and ω, respectively). It has been suggested that these polymorphic variants may result in different capacities to metabolize arsenic.[5,19–30]

Several research groups, including our own, have reported considerable variation in arsenic species excretion across populations with similar levels of arsenic exposure. These interindividual differences have been associated with, among other factors, age, sex, tobacco use, ethnic, and genetic polymorphic variants.[25–27,31–33] Yu et al[22] studied 2 genes involved in the methylation process: purin nucleoside phosforilase (hNP) gene and GSTω 1–1 (omega), both reductors of MMA^5 to MMA^3. These investigators found significant differences in the prevalence of genetic polymorphisms in both groups, raising the possibility that the variability of arsenic-related health effects in individuals of European and indigenous American origin may be associated with genetic-metabolic processes.[22] This present study expands on that work by examining the effect of the polymorphic variants of two other potentially important genes in the GST family in a Chilean population. Specifically, we examined the relationship between polymorphic variants of *GSTT1* and *GSTM1 on* urinary arsenic excretion following low-level environmental arsenic exposure.

Table 1.—Characteristic of the 66 Sampled Students, Antofagasta, Chile, 2005

Characteristics	*n*	%
Physical		
Age (years)*	66	22.71 ± 5.20
Weight (kg)*	66	65.18 ± 13.07
Height (cm)*	66	165.60 ± 9.16
Sex: male	26	39.39
BMI (kg/m^2)		
Underweight [<18.5]	2	3.03
Normal range [18.5–24.9]	47	71.21
Overweight [≥25]	17	25.75
Educational level		
Elementary	0	0.00
Middle	3	4.54
Superior	63	95.45
Smoking habit		
Never	30	45.45
Sometimes	11	16.66
Regularly	25	37.87
No. of cigarettes for day**		2.84 (1–15)
No. of cigarettes smoked the previous day**		2.33 (1–15)
Passive smoking	40	60.60
In house	37	56.06
In working area	23	34.84
Food and beverage consumption		
Water (≥1 glass)	47	71.21
Alcohol (≥1 glass)	3	5.54
Shellfish and/or fish	6	9.09
Genotypic variants		
GSTT1		
Active	54	87.09
Null	8	12.90
nd	4	—
GSTM1		
Active	32	52.45
Null	29	47.54
nd	5	—

Note. nd = no determined; BMI = body mass index.
*Mean, standard deviation.
**mean, range.

METHODS

Study design and site selection

A cross-sectional study was conducted in the northern city of Antofagasta, Chile, during the months of September and October, 2005.

Sample size

We estimated the sample size necessary to examine the effect of polymorphic variants on urinary arsenic excretion. The sample size calculation used the following assumptions, which were based on preliminary data for the *GSTT1* variant:[34,35] the *null* variant was expected to be present among 11% of the population; we desired precision of ±3.0%; and α was set at .05. Given these parameters, the target sample size was 81 people. We enrolled 81 potential subjects, but 15 were eventually excluded due to inadequate urine or blood specimens. Thus, the final study population consisted of 66 subjects. The excluded subjects had similar demographic characteristics as the total sample. Characteristics of the study population are presented in Table 1.

Study sample

The study sample included healthy students randomly selected from The University of Antofagasta. Eligible participants had to have lived in the city of Antofagasta, Chile, for at least 1 year. Although Antofogasta has a history of high levels of environmental arsenic resulting from large-scale mining operations and geological reasons, current levels of arsenic concentrations in drinking tap water during were below the Chilean national standard of 50 μg/L. Potential subjects were excluded if they reported any of the following: occupational exposure to arsenic, use of hair coloring, or renal or hepatic disease. Informed consent was obtained from all participants. Survey data and biologic samples were collected by a trained team in accordance with an existing field protocol [33]. This study was approved on December 27th of 2005 (Protocol No. 2980) by the Ethical Committee for Research in Humans of The Faculty of Medicine, University of Chile.

Exposure assessment

A survey instrument was modified to obtain demographic information, potential exposure to industrial sources of arsenic, and active and passive tobacco smoking.[33] Diet was assessed using a questionnaire of beverage and seafood consumption during the 48 hours prior to collection of urine and blood samples.

Sample collection

Collection and Pretreatment of Urine Samples

After enrollment, subjects completed the survey and were given a 250-mL plastic container along with written instructions on the hygienic collection of urine specimens. The container was previously treated with hydrochloric acid and rinsed with deionized water to avoid possible contamination with arsenic. For the following day, subjects were asked to collect their first morning void (FMV) of urine using the allotted plastic container. Participants were asked to obtain a sample of at least 250 mL. The containers were then immediately transported to the Antofagasta University laboratory, pretreated with hydrochloric acid (HCl), and put in frozen storage for later analysis.

Collection and Pretreatment of Blood Samples

Blood samples were collected from all of the participants at the time of enrollment. The samples were processed at the Center of Pharmacological and Toxicological Research in the Faculty of Medicine of the University of Chile. Samples were used to obtain genomic DNA from peripheral leucocytes using the method of Miller et al.[36] The genomic DNA was checked for purity at 260/280 nm absorption and repurified with phenol/chloroform protocol if necessary. DNA was stored at -30°C prior to genotyping.

Genotyping methods

After DNA extraction, DNA samples were analyzed for *GSTM1* and *GSTT1* genetic polymorphisms. Polymerase chain reaction (PCR) was used to examine the polymorphisms of interest. All samples were submitted to separate amplifications as described previously.[34,35] For *GSTT1* the total absence of gene *GSTT1*0* (homozygote deletion) versus the presence of this one was determined. The frequencies observed for *GSTM1*0* and *GSTT1*0* in the Chilean population previously were reported to be about 22% and 11%, respectively. For *GSTM1* or *GSTT1* the term "*null*" genotype refers to the homozygote deletion of the total gene, and the "*active*" genotype refers to the homozygote wild type and/or the heterozygote genotypes.[34,35]

Phenotypic analysis

The excretion of arsenic was determined by assessing nondietary arsenic species in urine. Using the methodology of Burattie et al, we examined inorganic arsenic ($As^5 + As^3$), monomethylarsenic acid (MMA) and dimethylarsenic acid (DMA).[37] Total urinary arsenic (TuAs) and arsenic species (inorganic arsenic [InAs: $As^3 + As^5$] + monomethyarsonate [MMA] + dimethylarsinate [DMA]) were analyzed using an atomic absorption spectrophotometer, Perkin-Elmer Model 4000, coupled with hydride-generator system MHS-20, an electric oven, and a discharge lamp EDL System 2. For arsenic species separation, a column with Biorad AG 50W-X8 cationic interchange resin was used. The quality control of these determinations were made using standards solution of 100 ng/mL of I-As, MMA, and 200 ng/mL of DMA certificated by National Institute of Standard and Technology (NIST). Once the arsenic species were determined, we calculated the relative proportions of InAs/TuAs (%InAs), MMA/TuAs (%MMA), and DMA/TuAs (%DMA), allowing us to infer participants' methylation capacity. The%MMA and%DMA reflect the first and second passages of methylation, respectively, in each subject.[25,38–40] Arsenic species are presented adjusted by creatinine urine concentration. Creatinine was determined in accord with the method of Newman and Price.[41]

Statistical analysis

We began by estimating standard descriptive statistics (mean, median, proportion, standard deviation, minimum and maximum values) for all our variables. Nonparametric tests (Kruskal-Wallis and Mann-Whitney) were then used to compare arsenic metabolites across levels of each variable. Genotype frequencies for GST were calculated as the proportion of individuals with a given genotype divided by the total number of participants. To examine associations between GST variants and phenotypic expression, univariate and multivariate analyses were conducted. We fit an exploratory multiple linear regression model to evaluate the relationships between the percentage of arsenic species

Table 2.—Median Total and Relative Urinary Arsenic Species*, Antofagasta, Chile, 2005

Arsenic fraction	*N* (%)	T-In As (μg/g) Median (range)	%InAs Median (range)	%MMA Median (range)	%DMA Median (range)
Total sample	66(100.0)	15.82(4.37–69.56)	9.09(3.22–20)	11.76(3.12–33.33)	77.77(50.00–90.32)
Sex					
Women	34 (51.5)	16.85 (21.43–58.33)	9.09 (3.22–10.27)	14.83 (4.16 – 15.47)	76.16 (74.25–90.32)
Men	32 (48.5)	15.00 (17.81 – 69.56)	9.09 (4.34–9.41)	9.30 (3.12–10.78)	80.00 (62.50–79.79)
p value		.169	.429	.003	.009
BMI (kg/m^2)					
Underweight [<18.5]	3 (4.5)	17.37 (11.00–27.27)	9.09 (7.31–16.66)	9.75 (9.09–33.33)	81.81 (50.00–82.92)
Normal range [18.5–24.9]	43 (65.2)	15.21 (4.37–69.56)	9.09 (3.22–20.00)	12.50 (3.12–28.57)	77.77 (57.14–89.79)
Overweight [≥25]	20 (30.3)	21.06 (7.14–58.33)	9.52 (4.34–17.24)	9.52 (4.08–28.57)	77.52 (57.14–89.79)
p value		.453	.916	.894	.999
Smoking (no. cigarettes)					
Never	31 (46.9)	15.68 (5.55–69.56)	9.09 (3.22–20.00)	13.33 (3.12–33.33)	77.77 (50.00–90.32)
≤2	18 (27.3)	15.58 (4.37–36.66)	8.71 (4.34–13.63)	13.33 (5.40–27.77)	78.63 (61.11–89.18)
3–5	9 (13.6)	14.40 (11.00–36.36)	11.76 (6.12–16.66)	10.00 (4.08–16.66)	76.47 (66.66–89.79)
>5	8 (12.2)	20.94 (6.87–30.86)	9.90 (6.97 – 14.28)	10.10 (4.65–28.57)	77.77 (57.14–88.37)
p value		.888	.172	.596	.948
Beverage and seafood					
Water					
No consumption	11 (16.7)	20.93 (7.52–69.56)	10.00 (6.97–20.00)	11.11 (3.12–17.85)	77.77 (66.66–89.06)
<1 glass	9 (13.6)	15.21 (8.84–27.27)	11.11 (3.22–17.24)	9.09 (6.41–33.3)	77.77 (50.00–90.32)
1–2 glass	8 (12.1)	18.34 (4.37–48.71)	6.78 (4.34–12.50)	9.22 (4.16–25.00)	84.88 (62.5–89.58)
>2 glass	38 (57.6)	15.15 (5.55–58.33)	9.09 (4.76–16.00)	13.33 (4.08–28.57)	77.09 (57.14–89.79)
p value		.682	.061	.333	.208
Alcohol					
Yes	5 (7.6)	22.34 (13.15–69.56)	9.52 (7.81–16.00)	8.00 (3.12–9.52)	80.95 (76.00–90.32)
No	61 (92.4)	15.38 (4.37–58.33)	9.09 (3.22–20.00)	12.50 (4.08–33.33)	77.77 (50.00–90.32)
p value		.092	.369	.018	.254
Shellfish and/or fish					
Yes	6 (9.0)	16.00 (11.78–30.86)	8.45 (6.25–17.24)	7.04 (4.16–25.00)	76.92 (62.5–89.58)
No	60 (91.0)	15.38 (4.37–69.50)	9.09 (3.22–20.00	11.76 (3.12–33.33)	77.77 (50.00–90.32)
p value		.474	.932	.356	.874

Note. *N* = number; BMI = body mass index; T-In = total inorganic arsenic; InAs = inorganic arsenic; MMA = monomethylarsenic; DMA = dimethylarsenic. *Creatinine-adjusted.

(dependent variables) and GST polymorphic variants (exposure variable), adjusted by potential confounders identified in the literature. These potential confounders included age, sex, body mass index (BMI), passive smoke exposure, number of cigarettes per day, and consumption of water, alcohol, and seafood for the day prior to specimen collection.[25,26,42–44] Analyses were performed with Stata V10 software.[45]

RESULTS

Table 1 reports demographic characteristics of the study populations. The mean age, weight and height were 22.7 years (± 5.2), 65.1 kg (± 13.0), 165.6 cm (± 9.1), respectively. Males comprised 39.39% of the study population and 71.21% of subjects had normal body mass index (BMI); 27.75% were overweight. Nearly all subjects (95.45%) had a university-level education. About 55% of participants were smokers with a mean of 2.84 cigarettes per day. Three quarters of subjects reported exposure to passive smoke. The prevalence of *GSTM1* and *GSTT1* genotypic polymorphic *null* variants were 12.9% and 47.5%, respectively.

Table 2 reports the urinary profile of absolute inorganic arsenic and median percentage species adjusted by creatinine for different variables in univariate analyses. The median of total inorganic arsenic (T-InAs) concentration in urine of all individual was 15.82 μg/g, with a range of 4.37 to 69.56 μg/g. Men had significantly lower %MMA than women ($p = .003$). For the %DMA, the relation was inverse, men had a higher level than women ($p = .009$). BMI, number of cigarettes smoked the previous day, and selfish and/or fish consumption was not statistically associated with any measure of arsenic. Water consumption level was not associated with any measure of arsenic species, but the association for %InAs did approach statistical significance ($p = .061$). Participants reporting alcohol consumption had significantly lower %MMA than participants reporting no alcohol consumption ($p = .018$).

Table 3.—Median Total and Relative Urinary Arsenic Species* by GST Polymorphic Variant, Antofagasta, Chile, 2005

All subjects	*N* (%)	T-In As (μg/g) Median (range)	%InAs Median (range)	%MMA Median (range)	%DMA Median (range)
Genotypic variant					
GSTT1					
Active	49 (89.00)	15.68 (4.37–69.56)	9.09 (3.22–17.24)	11.76 (3.12–33.33)	77.77 (50.00–90.32)
Null	6 (11.00)	29.68 (13.23–36.66)	10.10 (6.89–20.00)	10.10 (6.89–20.00)	79.79 (66.66–86.20)
p value		.062	.304	.324	.807
GSTM1					
Active	29 (53.70)	20.93 (6.87–50.00)	9.09 (3.22–20.00)	9.75 (4.08–33.33)	80.00 (50.00–90.32)
Null	25 (46.30)	15.09 (4.37–69.56)	9.09 (4.76–17.24)	13.33 (3.12–28.57)	76.92 (57.14–89.06)
p value		.199	.476	.390	.262
Women					
GSTT1					
Active	28 (87.50)	0.42 (0.36–8.33)	4.76 (3.22–17.24)	5.26 (4.16–33.33)	57.14 (50.00–90.32)
Null	4 (12.50)	1.47 (1.47–2.37)	9.09 (9.09–20.00)	9.09 (9.09–13.33)	66.66 (66.66–81.80)
p value		.209	.110	.171	.864
GSTM1					
Active	15 (46.88)	0.36 (0.36–8.33)	4.76 (4.76–17.24)	6.89 (6.89–28.57)	57.14 (57.14–85.71)
Null	17 (53.12)	0.60 (0.60–7.14)	3.22 (3.22–20.00)	4.16 (4.16–33.33)	50.00 (50.00–90.32)
p value		.096	.496	.205	.226
Men					
GSTT1					
Active	21 (91.30)	0.79 (0.70–5.43)	5.55 (4.34–13.63)	4.08 (3.12–25.00)	66.66 (62.50–89.79)
Null	2 (8.70)	1.40 (1.40–1.71)	6.89 (6.89–9.09)	6.89 (6.89–9.09)	81.81 (81.81–86.20)
p value		.190	.662	.4126	.444
GSTM1					
Active	10 (45.45)	0.87 (0.87–5.43)	5.88 (5.88–13.04)	3.12 (3.12–25.00)	62.50 (62.50–89.06)
Null	12 (54.54)	0.70 (0.70–2.32)	4.34 (4.34–13.63)	4.08 (4.08–22.22)	66.66 (66.66–89.79)
p value		.947	.792	.552	.644

Note. N = number; BMI = body mass index; T-In = total inorganic arsenic; InAs = inorganic arsenic; MMA = monomethylarsenic; DMA = dimethylarsenic. *Creatinine-adjusted.

Table 3 reports the median arsenic species by GST variant for all subjects and separately for each gender. For both *GSTT1* and *GSTTM1,* there were no significant differences in percentage of arsenic species between *active* and *null* polymorphic variant in the total study population. However, the absolute level of T-InAs was higher among *null GSTT1* carriers than among those carrying the *active* gene ($p = .062$). When we compared arsenic species concentration stratified by sex, we did not observe significant differences between *active* and *null* variants of either GST polymorphism. However, women and men with the *active* variant of *GSTT1* had higher median values of %InAs, %MMA, and %DMA compared with carriers of *null* variant. In women, carriers of *active GSTM1* had higher median values of %InAs, %MMA, and %DMA compared with carriers of *null* variant. Conversely, men had lower median values of %MMA and %DMA compared with carriers of *null* variant.

We constructed separate multivariate linear regression models in which each urinary arsenic metabolite was designated as the dependent variable (Table 4). None of the covariate-adjusted associations with arsenic metabolites were statistically significant. Participants carrying the *active* variant of GSTT1 tended to have lower levels of %InAs and %DMA and higher levels of %MMA than *null* carriers. And carriers of *active GSTM1* tended to have lower levels of %InAs and %MMA and higher levels of %DMA than *null* carriers. These relationships approached statistical significance.

COMMENT

Traditionally, the methylation of arsenic was thought to be an important step in its detoxification. There is, however, some evidence that mono- and dimethylated forms of arsenic, MMA and DMA, are more toxic than their precursor, arsenite (As^{+3}). Accordingly, interpersonal differences in the capacity to methylate arsenic may explain some variation in arsenic-related disease susceptibility. Therefore, it is of great importance to identify factors that affect methylation capacity. In this study, we examined the effect of polymorphic variants within the GST family on the methylation of excreted arsenic.

The genotypic frequencies of null variants for GSTT1 and GSTM1 (12.9% and 47.54%, respectively) in this study

Table 4.—Covariate-Adjusted* Effect of GST Polymorphic Variant on Relative Arsenic Species, Antofagasta, Chile, 2005

Genotypic variant	B	Standard error	p value	R^{2*}
GSTT1—Active versus Null				
%InAs	−1.69	1.62	.303	.26
%MMA	5.01	2.86	.088	.41
%DMA	−3.32	3.95	.406	.34
GSTM1—Active versus Null				
%InAs	−0.73	1.04	.483	.24
%MMA	−2.47	1.83	.185	.40
%DMA	3.21	2.48	.203	.35

*Covariates included total inorganic arsenic, age, sex, BMI, number of cigarettes the previous day, consumption of tap water, beverage and seafood. R^{2*} adjusted. I-As = inorganic arsenic; MMA = monomethylarsonic acid; DMA: dimethylarsinic acid.

were similar to those described in other Chilean populations.[46–48] For example, Marcus et al[49] recently found similar frequencies of *null* variants of GSTT1 (15%) and GSTM1 (42.5%) in a population of smelting workers in northern Chile.

The median percentage of arsenic species in our sample population for InAs, MMA, and DMA were 9.09%, 11.67%, and 77.77%, respectively. These concentrations are consistent with published levels of arsenic species, which range from 10% to 30% for InAs, 10% to 20% for MMA, and 60% to 80% for DMA.[11,32,50,51]

In the univariate analysis, sex, water consumption, and alcohol consumption were significant predictors for level of arsenic species excretion. Women had a higher median urine excretion of %MMA and lower %DMA compared with men, suggesting that women have a less robust methylation capacity than men. However, we note that Lindberg et al[44] reported the opposite findings in a study of a Bangladeshi population; women had a lower %MMA and higher %DMA than men, but only in childbearing age, suggesting an influence of sex hormones. They reports that exposure level of arsenic, gender, and age explain about 30% of the variation observed.[44] In our population, BMI, exposure to cigarette smoke, and consumption of seafood the day prior to specimen collection were not statistically significant predictors of arsenic species excretion. In the Lindberg et al study, there was not a relationship between BMI and arsenic methylation, but other covariates were not examined.[44]

When we evaluated the effect of GST polymorphic variant on the distribution of percent arsenic species, we found that there were not statistically significant differences between the *active* and *null* genotypes. However, when we stratified this analysis by sex, we found that women with the *active* genes had higher levels of arsenic species than those with the *null* genes. This finding was strongest for GSTT1. Steinmaus et al[52] reported a similar finding: for both GSTM1 and GSTT1, they reported no significant differences in excreted arsenic species when comparing active and null carriers; however, when they stratified their analysis by sex, they found that women carrying the active GSTM1 variant had higher %DMA and lower %MMA than null carriers.[52] Some authors suggest that these differences observed could be explained for water consumption or nutrition, the role of the hormones, and ethnic background over arsenic metabolisms.[4,53–55]

In order to further assess the effect of the GST polymorphic variants, we fit a multivariable model that included the variables that were significant in our univariate analyses and variables from prior studies that were reported to be associated with arsenic excretion. We did not observe a significant effect for any of the variables in our model. Nevertheless, when we fit separate regression models for each arsenic species, we observed a negative association between with the *active GSTT1* and both %InAs and %DMA. Although these findings were not statistically significant, this suggests that subjects with this variant may be more efficient metabolizers of arsenic species than *null* carriers. Conversely, the *active* variant of *GSTM1* was negatively associated with both %InAs and % MMA, suggesting a low rate of metabolisms. On the other hand, similar results were reported by Choiu et al[21] in one of the first published studies to evaluate the association between GST variants and arsenic metabolism. In that study, variants of *GSTM1* and *GSTT1* were significantly associated with arsenic methylation, suggesting that subjects carrying the *active* genotype of *GSTM1* had an decreased %InAs, while those with *active* genotype of *GSTT1* had diminished percentage of %DMA.[21]

In contrast, Schlawicke Engstrom et al[8] reported that *GSTT1* was negatively associated with %MMA and *GSTM* was positively associated with %MMA. For %DMA they found a negative association for *GSTT1* adjusting for age and total urinary arsenic.[8] Marcos et al,[49] studying a Chilean population of workers occupationally exposed to arsenic, found a relationship between *GSTM1* polymorphism and urinary profiles (MMA excretion) and between *GSTP1* (val/val) and DMA excretion. We did not detect a similar association, probably due to the small size of our sample. McCarty et al[60] reported an association between the *active* variant of *GSTT1* and the second passage of methylation (%DMA).

Several investigations have been developed to evaluate the effect of GST polymorphisms on susceptibility to different pathologies and arsenic exposure, especially those related to cancer of the urinary system and skin. Carriers of *GSTT1 null* and *GSTM1 null* genotype have been associated a higher risk bladder cancer and skin lesions.[56–58] However, some researchers did not find relationships between arsenic exposures a genetic polymorphism of *GSTM1* and *GSTT1* with skin lesions following arsenic exposure.[59,60]

In the present study, a limitation is that the sample size were not sufficient to evaluate gene-environment interactions, we explored the relationships between arsenic species excreted by urine and GST polymorphic variants over each gene. It is

necessary to study a larger population in order to assess the effect of the interactions.

In conclusion, our exploratory results shown that GST polymorphic variants *(T1 and M1)* were not clearly associated with arsenic metabolite excretion levels; however, it will be necessary include in this research genes that are participating in arsenic biotransformation, as for example AS3MT and SAM, in order to have a more complete view of the As excretion profile in Chilean exposed population for geologic and anthropogenic reasons.

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