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Association between polymorphisms in arsenic metabolism genes and urinary arsenic methylation profiles in girls and boys chronically exposed to arsenic

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Abstract

Disease manifestations or susceptibilities often differ among individuals exposed to the same concentrations of arsenic (As). These differences have been associated with several factors including arsenic metabolism, sex, age, genetic variants, nutritional status, smoking, and others. The present study evaluated the associations between four As metabolism-related gene polymorphisms/null genotypes with urinary As methylation profiles in girls and boys chronically exposed to As. In a total of 332 children aged 6-12 years, the frequency of *AS3MT*, *GSTO1*, *GSTT1*, and *GSTM1* polymorphisms/null genotypes and As urinary metabolites were measured. The results revealed that total As and monomethyl metabolites of arsenic (MMA) levels were higher in boys than in girls. No differences in the frequency of the evaluated polymorphisms were found between girls and boys. In *AS3MT*-Met287Thr carriers, %MMA levels were higher and second methylation levels (defined as dimethylarsinic acid divided by MMA) were lower. In children with the *GSTM1* null genotype, second methylation levels were higher. In boys, a positive association between the *AS3MT*-Met287Thr polymorphism with %MMA and between the *GSTO1*-Glu155del and As^v was found; whereas, a negative relationship was identified between *AS3MT*-Met287Thr and second methylation profiles. In girls, a positive association was found between the *GSTO1*-Ala140Asp polymorphism with second methylation levels. In conclusion, our

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Statement of author contributions

RRV and RCL conceived and designed the study. TGC, EOC, CGD, and GMR recruited study participants and collected the data. TGC processed the samples. RRV, TGC, and RCL and AJG drafted the initial manuscript, and all authors revised the manuscript for critical intellectual content and read and approved the final version for submission.

data indicate that gender, high As exposure levels, and polymorphisms in the evaluated genes negatively influenced As metabolism.

Keywords

Arsenic; Children; *AS3MT*; *GSTO1*; *GSTT1*; *GSTM1*

INTRODUCTION

Inorganic arsenic (iAs) is recognized as a potent toxicant and it causes severe health problems in populations chronically exposed through drinking water contaminated with this metalloid [De Chaudhuri et al., 2008; NRC, 2001]. The severity of the adverse effects of As on human health depends on its metabolism, which is generally assessed by the relative quantification of arsenic metabolites in urine [De Chaudhuri et al., 2008].

Humans generally eliminate As via urine, with 10-30% as iAs, 10-20% as monomethyl arsenous acid (MMA), and 60-80% as dimethyl arsenic acid (DMA) [Hopenhayn-Rich et al., 1998]. However, there is great variation in iAs metabolism between species, populations, and individuals [Valenzuela et al., 2009]. Human metabolism of As can be quite variable, producing a mixture of these As species with very different toxic potencies [Meza et al., 2007]. There are distinct populations excreting varying amounts of MMA in urine [Hughes, 2006].

Several studies carried out in *AS3MT* knockout mice indicate that an increase in the percentage of MMA (%MMA) with a decreasing percentage of DMA (%DMA) in urine is associated with increased retention of arsenic in the body [Vahter, 2002], which could increase adverse effects on health [Schläwicke-Engström et al., 2007]. Disease manifestations often differ among individuals exposed to the same concentrations of arsenic and these differences have been associated with several factors such as arsenic methylation efficiency, sex, genetic variants, nutritional status, age, smoking, and others [Heck et al., 2007; Huang et al., 2007].

Studies have reported that women have a more efficient methylation capacity than men; therefore, men are more susceptible to the development of arsenic-induced diseases [Hopenhayn-Rich et al., 1996; Pu et al., 2007; Steinmaus et al., 2005a]. In Western Bengal, India, more than 6 million individuals are exposed to iAs endemically; however, only 300,000 individuals of this population show skin lesions induced by this metalloid [De Chaudhuri et al., 2008]. It is therefore essential to determine the reasons for this marked variation in metabolism and susceptibility between individuals and population groups. One of the more important reasons for this variation in As metabolism is the existence of genetic polymorphisms in the enzymes involved in the process. This can lead to differences in arsenic metabolism and toxicity associated with exposure to the metalloid [Hughes, 2006].

To date, several genes have been identified as being involved in the biotransformation of arsenic. These include arsenic-3-methyltransferase (*AS3MT*) and glutathione S-transferase Omega 1 (*GSTO1*) [De Chaudhuri et al., 2008; Schläwicke-Engstrom et al., 2007]. In

addition, other studies have shown the involvement of two other genes belonging to glutathione S-transferases (*GST*) family: *GST*θ-1 (*GSTT1*) and *GST*μ-1 (*GSTM1*) [Schlāwicke-Engstrom et al., 2007].

Some studies in Latin America and European populations have associated the Met287Thr polymorphism in *AS3MT*(rs111191439) with a high percentage of urinary excretion of MMA and a low percentage of DMA [Hernandez-Zavala et al., 2008; Lindberg et al., 2007; Valenzuela et al., 2009]. On the other hand, in indigenous women in northern Argentina, the carriers of polymorphisms rs3740400 and rs7085104 in *AS3MT* excreted a lower percentage %MMA and greater %DMA [Schlawicke-Engstrom et al., 2007].

Another modifying factor in arsenic toxicity is age. Indeed, a study investigating the association between three arsenic metabolism candidate genes (purine nucleoside phosphorylase, glutathione-S-transferase omega, and As^{III} methyltransferase) and urinary arsenic metabolite levels on 135 arsenic-exposed subjects (90 adults and 45 children) found that three polymorphic sites in the *CYT19* gene were significantly associated with an altered DMA:MMA ratio in the total population; however, subsequent analysis of this association revealed that the association was actually caused by an extremely strong association in children only [Meza et al., 2005].

Thus, previous studies highlight the need to study variation in As metabolism and susceptibility relative to age, sex, and genetic polymorphisms. Because children are known to be susceptible to As and may have been chronically exposed, it is important to understand if their susceptibility is related to how they metabolize As and if gender modifies this. The present study examines the association between As metabolism-related gene polymorphisms/null genotypes (*AS3MT*, *GSTO1*, *GSTT1*, *GSTM1*) and methylation profiles in children by sex.

MATERIALS AND METHODS

Study population

The subjects included in this report are a subset of those reported in an earlier study [Recio-Vega et al., 2015a]. More than 500 children were evaluated; however, only 332 children met the inclusion criteria of this study. We exclusively included only healthy volunteers who were conceived in the studied rural communities, whose mothers remained throughout their entire pregnancies in these communities, and the volunteers remained as permanent residents of the same communities. The participants were girls and boys aged 6-12 years residing in four rural communities where the highest arsenic tap water levels detected in the last 20 years ranged from 104-360 ppb. These communities received groundwater through the local water supply and the high As levels in the water were due, in part, to an over-extraction of water from the ground for crops. At present, water is obtained from a depth of 200-300 meters. These communities form part of the geographic area known as Comarca Lagunera, which is located in the north-central part of Mexico and known to be an area where increased arsenic toxicity has been reported [Sampayo-Reyes et al., 2010; Recio-Vega et al., 2015a].

Written informed consent was obtained from each participant and from their parents to obtain biological samples. The study protocol was approved by the Ethics Committee of the School of Medicine at Torreon, University of Coahuila, Mexico.

Questionnaire application

Information was collected through in-person interviews and included socio-demographic variables (education, socioeconomic status, type of kitchen, the type of fuel used for cooking), lifetime residential history, lifestyle factors (secondhand smoke defined as someone smoking regularly in the same room at home, and exercise), parents' occupational history, water source types (municipal tap water, purified), current medications, medical history, and diet. Questionnaires were completed by the mothers at their own residing community. Water consumption habits were ascertained through a standardized questionnaire [Recio-Vega et al., 2015a].

As measurement in drinking water and urine

Drinking water samples (well) were collected from each rural community included in the study and analyzed for inorganic arsenic levels. No other contaminants in the drinking water were assessed. Well water samples from each rural community are representative of the water that participants drank and were provided through the unique local water supply system. Individual exposure was assessed based on urinary concentration of the total arsenic level. A first morning void urine sample was collected in sterile 120-mL screw-topped polypropylene containers. The urine samples were obtained during the late autumn and winter seasons to avoid the hottest seasons when there is much higher water consumption and children have increased outdoor activities.

Urine samples were analyzed using the methodology described by the U.S. Center for Disease Control [CDC, 2004] at the Arizona Laboratory for Emerging Contaminants, University of Arizona, Tucson, Arizona, U.S.A. Briefly, arsenic species in urine (As^{V} , As^{III} , monomethylarsonic acid (MMA^{V}), dimethylarsinic acid, (DMA^{V}) and arsenobetaine) were separated by HPLC and analyzed by ICP-MS. Arsenic concentrations in water samples and urine were analyzed by inductively coupled plasma mass spectrometry utilizing Standard Reference Water, SMR 1640 (NIST, Gaithersburg, MD, USA) and freeze-dried Urine Reference Material for trace elements (Clincheck-control; RECIPE Chemicals instruments GmbH, Munich, Germany) for urine as quality control. Urinary As concentrations were adjusted by urine creatinine levels. Additional exposure to other arsenic compounds, which usually is attributable to consumption of seafood such as bivalves and seaweeds, was considered minimal because such seafood is essentially never eaten in this area. Arsenic metabolism efficiency was calculated using the following formulas proposed by Del Razo et al. [1997]: first methylation = $\text{MMA}^{\text{V}}/(\text{As}^{\text{V}}+\text{As}^{\text{III}})$; second methylation = $\text{DMA}^{\text{V}}/\text{MMA}^{\text{V}}$.

Genotyping

AS3MT-Met287Thr (rs11191439), GSTO1-Ala140Asp (rs4925) and GSTO1-Glu155del (rs11509437)—Peripheral blood samples were collected into EDTA vacutainer tubes, and extraction of genomic DNA material was carried out with DNAzol according to the manufacturer's instructions (DNAzol[®] BD, Cincinnati, OH, USA). For

detection of SNPs, TaqMan probes (Applied Biosystems, CA, USA) were used following the supplier's instructions. Briefly, to a cap PCR tube with optical quality, 10 µL of TaqMan Genotyping Mix 0.5 µL trial were added, which included the two primers and a fluorescent probe specific for each of the polymorphisms. Two µL was further added to the solution containing the DNA probe (~10-20 ng) and brought to a final volume of 20 µL by adding molecular grade water (all reagents from Applied Biosystems, CA, USA). Thermocycling reactions were performed in a Step-One™ Real Time PCR System (Applied Biosystems, CA, USA) under the following conditions: a cycle of polymerase activation of 95 °C for 10 minutes, then 60 cycles of denaturation at 92 °C for 15 seconds and alignment/extension at 60 °C for 1 minute. Positive and negative controls were included in the same 96-well plate. Allelic discrimination was developed using the Step-One software system (Applied Biosystem, CA, USA). All SNPs passed the Hardy-Weinberg equilibrium chi-squared test with P -value > 0.05.

***GSTT1*, *GSTM1* null genotype**—All assay reagents were purchased from Applied Biosystems. The *GAPDH* probes and gene-specific primers were used as an endogenous control. The amplification of *GAPDH* and the *GST* genes in the same sample were used to normalize differences in DNA concentration between samples and ensured that no false-negative *GST**0/0 genotypes due to PCR or pipetting failure or insufficient DNA concentrations in the original sample were generated. The initial step of the PCR was set at 50 °C for 2 minutes, and the denaturation step was performed at 95 °C for 10 minutes. The amplification was performed for 40 cycles at 95 °C for 15 seconds, and at 60 °C for 1 minute. PCR was performed (Step-One™ Real Time PCR System; Applied Biosystem, CA, USA) in 96-well plates with a final sample volume of 10 µL, including 1X universal PCR master mix (TaqMan; Applied Biosystems), 25 ng DNA, and proper dilutions of primers according to the manufacturer's instruction. Data were then collected (Absolute Quantification; SDS software, ver. 1.3.1, SDS, Cary, NC). Copy number estimation was conducted by the Ct method (Copy-Caller software, ver. 1.0; AB). The detection of *GAPDH*, known to exist only in two copies in a diploid genome, was used as the calibrator to estimate the copy number of *GSTM1* and *GSTT1* [Bediaga et al., 2008; Norskov et al., 2009; Tsuchida et al., 1992]. The calculation was performed by a maximum-likelihood algorithm built into the software [Zhou et al., 2010].

Statistics

Independent and dependent variables were described according to their frequency and distribution measurements (arithmetic mean and standard deviation). The F test was used when the variable was divided into more than two categories, and the Student's t test or the Mann Whitney test was used to compare different frequencies of polymorphisms and levels of arsenic when dichotomous variable categories were analyzed. This method permitted us to establish statistical differences among groups for each dependent variable. Linear regression models were used to assess crude or independent associations between the different polymorphisms/null genotypes with arsenic urine concentrations. In all multivariable models, we included those statistically significant variables ($P < 0.05$) identified in the bivariate model and those that were biologically plausible (age, gender, body mass index, time of consumption of purified drinking water, second hand smoking and

parents', schooling). For deviation from Hardy-Weinberg equilibrium, chi-square analysis was used. All analyses were performed using the statistical software STATA 11.0 (Stata Corp., College Station, TX).

RESULTS

A total of 332 children were in the study, including 174 girls and 158 boys whose sociodemographic characteristics and lifestyle are reported in Table I. In all cases the level of As in the drinking water was much higher than the safe water Mexican standard ($< 25 \mu\text{g/L}$). Other possible sources of arsenic exposure, including diet (seafood, rice and others), agrochemicals, fuels, preservatives or other compounds containing arsenicals, were negligible. The correlation found between As tap water level with arsenic urinary level was 0.69.

When urinary As and its metabolite levels were evaluated by gender, total As and MMA^V levels were higher in boys than in girls. No differences in the other As metabolite concentrations or in the methylation profiles were found (Table II).

AS3MT and GSTO1 polymorphisms; GSTT1 and GSTM1 null genotype

The genotype distributions of the polymorphisms were in accordance with Hardy-Weinberg equilibrium. More than 82% of the studied children were wild type for the *AS3MT* and *GSTO1* genes. The frequency of *GSTT1* and *GSTM1* wild type genes was $> 60\%$ and 79% , respectively. The number of subjects with the homozygous variants for *AS3MT*-Met287Thr and for *GSTO1* were one and six, respectively. Because of their low frequencies, the mutant homozygous and the heterozygous variants were combined for further analyses. More than 16% of the population had the homozygous null genotypes for *GSTT1* and $> 7\%$ for *GSTM1*. The variant with the highest allelic frequency in the overall population was Ala140Asp (0.9), and the least common was Met287Thr (0.09).

When the allelic and genotypic frequencies of polymorphisms were analyzed by sex, no differences were found, and the percentages of these frequencies were similar to those recorded in the overall studied children (Table III).

Arsenic levels and AS3MT and GSTO1 polymorphisms, and GSTT1 and GSTM1 null genotypes

Analysis of As levels and methylation profiles in wild type subjects and carriers of the *AS3MT*-Met287Thr polymorphisms CT+CC revealed that %MMA levels were higher (14.3 ± 3.7 vs 12.9 ± 3.5 ; $P=0.03$) and second methylation levels were lower (4.5 ± 2.2 vs $5.8 \pm 10.5 \mu\text{g/L}$; $P= 0.05$) in the carriers. No statistical differences were found in the other As metabolite levels, in the percent of arsenic metabolites, or in the first methylation profiles between carriers of this polymorphism (Met287Thr) compared with the wild genotype (data non-shown). No differences in urinary As metabolite levels and methylation profiles were found in comparisons of the polymorphism-carriers against individuals with wild type *GSTO1*, or the null genotype versus wild type *GSTT1* (data not shown). Subjects with *GSTM1* null genotypes had higher levels of second methylation than wild type subjects (6.05 ± 1.54 vs 5.6 ± 10.1 ; $P= 0.01$). No other differences were found in As metabolites

levels, in the percent of arsenic metabolites, or in the first methylation profiles (data not shown).

Arsenic levels and *AS3MT* and *GSTO1* polymorphisms, and *GSTT1* and *GSTM1* null genotype by sex

When the As levels were evaluated by sex and by genotype, total As and MMA^V levels were significantly higher in boys with wild types than the *AS3MT*-Met287Thr and *GSTO1*-Ala140Asp polymorphisms. MMA^V concentrations were higher in boys with the wild type of *GSTO1*-Glu155del and *GSTM1* (Table IV). No other statistical differences were found in the other As metabolites levels or in methylation profiles between carriers of polymorphisms and wild type in boys and girls (data not shown).

Correlations between arsenic levels with *AS3MT* and *GSTO1* polymorphisms, and *GSTT1* and *GSTM1* null genotypes

When associations between the *AS3MT*-Met287Thr polymorphism and arsenic urinary levels were assessed by the multivariate model, a positive association was found for this polymorphism with %MMA and a negative association with second methylation. When these data were analyzed by gender, a positive association was found with %MMA and a negative one was found with second methylation levels in boys (Table V). In carriers of the *GSTO1*-Ala140Asp polymorphism heterozygous + homozygous, a positive association was found with second methylation levels (β 4.84; $P=0.005$) in the bivariate model; however, this significant association disappeared after adjustment of the statistical model. By gender, a positive association between this polymorphism and second methylation levels was found in girls (Table V). Carriers of the *GSTO1*-Glu155del polymorphism showed a positive association between this polymorphism and As^V in boys only (Table V).

In the adjusted regression analysis, carriers of the *GSTM1* null genotype showed a positive association with %DMA. No associations were found when the data were analyzed by gender (Table V). Finally, a negative association was found between carriers of the *GSTT1* null genotype and As^V. By gender no associations were found between this polymorphism and arsenic levels (Table V).

DISCUSSION

This study assessed the frequency of As metabolism-related gene polymorphisms/null genotypes and their relationship with urinary As species concentrations and methylation profiles in girls and boys chronically exposed to high As levels through drinking water (mean 103 µg/L). An important strength of our study is that several known exposure factors that inhibit arsenic methylation, such as diet, nutritional status, smoking, and alcohol consumption were avoided because only healthy children were included.

Eighty five percent of the children had a urinary arsenic concentration >50 µg/L, which reflects that effectively they are highly exposed to the metalloid. It has been reported that inhibition of arsenic methylation can occur around 50 µg/L in urine. High As levels inhibit a wide range of enzymes, including methyltransferases involved in DNA methylation [Chen et al., 2004; Cui et al., 2006a, 2006b; Reichard et al., 2007; Zhou et al., 2006]. Our data (high

%MMA and low %DMA) suggest that the high As exposure levels negatively influence the metabolism of inorganic arsenic, which is in agreement with previous findings [Del Razo et al., 1997; Hopenhayn-Rich et al., 1996]. Drobná et al. [2005] suggested that the most likely explanation is that elevated exposure to arsenic inhibits the methyltransferases involved in arsenic methylation, in particular the second methylation step, as shown in vitro. Inhibition of arsenic methyltransferases has been shown to lead to longer retention times of arsenic in the body [Vahter M., 1999]; therefore, this would be expected to lead to an increased risk for arsenic-related health hazards [Huang et al., 2007; Steinmaus et al., 2006; Tseng CH., 2007]. This is consistent with our recent report in this cohort of children showing that high arsenic urinary levels negatively correlate with lung inflammatory biomarkers [Olivas-Calderon et al., 2015], and with increased frequency of abnormal spirometric patterns [Recio-Vega et al., 2015a].

Gender and As levels

There are several factors such as gender, age and exposure level that play an important role in the metabolism of arsenic and which could explain some of the variation observed in the As methylation profiles. With regard to gender, most of the studies suggest that women have a more efficient methylation capacity than men [Chung et al., 2008; Gamble et al., 2005; Hopenhayn-Rich et al., 1996; Huang et al., 2008a, 2008b; Lindberg et al., 2008a, 2008b; Pu et al., 2007; Steinmaus et al., 2005b, 2007]. Lindberg et al. [2008a] reported that in a population from Bangladesh gender and age were major factors influencing arsenic metabolism, and that women had higher arsenic methylation efficiency than men. However, sexual difference in arsenic methylation capacity was not observed in other studies [Chen et al., 2003; Chiou et al., 1997; Kurttio et al., 1998; Recio-Vega et al., 2015b]. This gender difference in arsenic methylation capacity could be explained in part by the effect of estrogen. It has been proposed that the higher methylation capacity in women may be related to the *de novo* synthesis of choline by phosphatidylethanolamine methyltransferase [Vahter et al., 2006], which is up-regulated by estrogen [Fischer et al., 2007].

In our study, second methylation and %DMA levels were slightly higher in girls than in boys; however, these differences were not statistically different ($P > 0.05$). We note that from the girls included in this study ($n = 174$), only 13 (7.4%) had started their menstrual cycle. It is known that the peak in sex hormone production in both men and women occurs in their twenties, and this could explain the lack of estrogen effects in the sexual difference in arsenic methylation capacity in our study population in support of previous results [Lindenberg et al., 2008a] demonstrating that women had higher arsenic methylation efficiency than men, but only during childbearing age. On the other hand, total As and MMA^V levels were higher in boys than in girls, which is in agreement with the results of previous studies [Hopenhayn-Rich et al., 1996; Pu et al., 2007; Steinmaus et al., 2005a]. Thus, other factors may be affecting the fate of arsenic in individuals. Indeed, Lindberg et al. [2008a] reported that arsenic exposure level, gender, and age explained at most 30% of the variation in arsenic methylation capacity in adults, and suggested that genetic polymorphisms are the most important factor influencing the metabolism of inorganic arsenic.

Polymorphisms in arsenic metabolism genes and urinary arsenic methylation profiles

Basic and epidemiological studies support the role of several enzymes in the metabolism of arsenic and probably the most widely assessed is AS3MT. The gene encoding this enzyme has some variants (polymorphisms). One of the most relevant variants is Met287Thr (T → C). The frequency reported for this polymorphism in Chile [Hernandez-Zavala et al., 2008], Eastern Europe [Lindberg et al., 2007], China [Takeshita et al., 2009], and Mexico [Valenzuela et al., 2009] is around of 5-10%. The frequency in our study population for this variant (Met287Thr) was 7%, which is similar to these frequencies and those reported in other adult Mexican populations from Sonora, Mexico (6%) [Meza et al., 2005] and Coahuila, Mexico (9%) [Sampayo-Reyes et al., 2010].

The Met287Thr polymorphism has been associated with high AS3MT enzyme activity and with increased MMA production in in vitro studies and in humans [Drobná et al., 2004, 2006; Wood et al., 2006]. Previous clinical studies carried out in Mexico [Drobná et al., 2013; Valenzuela et al., 2009], Chile [Hernández-Zavala et al., 2008] and in Europe [Lindberg et al., 2007] have shown a high %MMA and a low %DMA urinary excretion in subjects carrying the Met287Thr variant (rs111191439). Most of these studies have been carried out in adults; however, Meza et al. [2005] found a strong association with MMA urinary levels only in children but not in adults in carriers of the rs30585 polymorphism of *AS3MT*. Similarly, we found that carriers of the variant of this gene excreted a higher %MMA ($P = 0.03$) and had lower secondary methylation capacity ($P = 0.05$) compared with carriers of the wild type genotype.

Both of these observations (high MMA levels and *AS3MT*-Met287Thr polymorphism) have important human health implications because this variant has been associated with an increased risk of malignant skin lesions [Engström et al., 2011; Valenzuela et al., 2009], and genetic damage [Hernandez-Zavala et al., 2008; Sampayo-Reyes et al., 2010] presumably because MMA is more cyto- and genotoxic than DMA. Therefore, carriers of this variant should be assessed by health institutions because of their expected increased risk in developing several arsenic-related diseases including cancer.

When arsenic species levels were evaluated by gender and by carriers of the Met287Thr polymorphism, higher total As and MMA urinary concentrations ($P < 0.04$) were found in boys with the wild type genotype. These data are in contrast to our previously mentioned results, because when the results from the all children were assessed, high %MMA levels were found in the carriers of the polymorphism, and when the population was divided by gender, higher %MMA was found in boys with the wild type gene only. This difference may be explained by the reduced number of girls ($n = 21$) and boys ($n = 26$) carrying the polymorphism compared with wild type children. Our results suggest that gender (independent of sexual hormonal influence) plays an important role in the variation observed in the metabolism of arsenic at this specific age, and that the evaluated genetic polymorphism partly explains this variation. However, further studies are needed comparing differences between gender and the influence of age and sex steroids on the metabolism of arsenic.

AS3MT is unlikely to be the only methyl As methyltransferase involved in metabolism, since there are over 100 different methylation genes in the human body [Linderberg et al., 2007]. Another important enzyme in As metabolism is GSTO1, which is an arsenic-reductase enzyme that is able to reduce both As^{III} to As^V as well as MMA^{III} to MMA^V [Schlätwicke-Engström et al., 2007] and has a thiol-transferase GST activity. Tanaka-Kagawa et al. [2003] reported that the Ala140Asp polymorphism reduces the thiol transferase activity of GSTO1. Several polymorphisms of the gene encoding this enzyme have been reported; however, we assessed two of the more common polymorphisms of the gene: Ala140Asp (rs4925) and Glu155del (rs11509437).

The frequency reported for Ala140Asp in Europe is 0.34, in Sub-Saharan African is 0.49 and in African American is 0.109 [NLM.NIH.GOV, 2015]. In our study the frequency was slightly higher (0.9) to that reported in Europe and Sub-Saharan Africans, and lower to that in African American, and these small differences may be due mainly to ethnicity. Rodrigues et al. [2012] showed an association between the *GSTO1* rs4925 homozygous wild type and higher MMA and DMA urinary concentrations; however, other studies assessing *GSTO* polymorphisms have not shown associations with methylation profiles or iAs-associated health outcomes [Antonelli et al., 2014]. In our study, we did not find any association between the *GSTO1* polymorphism and As species or methylation profiles, suggesting that this polymorphism did not play an important role in the As metabolism in our study population and supporting the results of previous studies.

The Glu155del polymorphism (–/AGG) (rs11509437) has been reported to be associated with decreased inorganic arsenic metabolism. The frequency of this polymorphism in our population was 0.09. Marnell et al. [2003] reported that heterozygous subjects for Glu155del and Glu208Lys had a significantly higher percentage of %iAs in urine (67-79%). Agusa et al. [2010] reported a higher urinary concentration of As^V in *GSTO1*-Glu155del heterozygotes than wild types. We found that the carriers of the Glu155del variants (heterozygotes plus mutant homozygotes) had slightly higher %iAs, MMA and DMA levels, but these levels were not different when compared to the children with the wild-type allele.

Few studies have identified other variants of the *GSTs*, such as, *GSTμ1* (*GSTM1*) and *GSTθ1* (*GSTT1*), which also have adverse effects on As metabolism [Schlätwicke-Engström et al., 2007]. According to a report from pooled data of 12,525 Caucasians, 2,136 Asians, and 996 African Americans, the frequency of the *GSTM1* null genotype is 0.53 (range 0.42–0.60) in Caucasians, 0.27 (range 0.16–0.36) in African-American subjects, and 0.53 (range: 0.42–0.54) in Asians. The frequency of the *GSTT1* gene deletion is 0.20 (range 0.13–0.26) and 0.47 (range 0.35–0.53) for Caucasians and Asians, respectively [Garte et al., 2001]. In our subjects the frequencies of both variants were relatively lower (0.2 and 0.1, respectively) compared to what has been reported previously, which is probably due to ethnicity.

Schlätwicke-Engström et al. [2007] reported that carriers of the *GSTM1* and *GSTT1* null variants showed different urinary patterns of As metabolites. These authors found that carriers of the *GSTM1* variant have modified %MMA levels and DMA/MMA ratio, whereas carriers of the *GSTT1* variant have altered levels of %MMA, %DMA and DMA/MMA ratio. Marcos et al. [2006] found that carriers of the *GSTM1* null variant excreted more MMA.

Chiou et al. [1997] reported a positive association between high concentrations of %iAs with the *GSTM1* null variant, but did not find any association between %DMA with the *GSTT1* null variant. In contrast, Agusa et al. [2010] found a higher %DMA in the urine of wild type *GSTM1* subjects compared with the null type. In our study, we did not find any association between the *GSTM1* null variant and As metabolites, probably due to the lower frequency of these genetic variants in our population, and probably because epidemiological studies have suggested that Native Americans methylate As with greater efficiency [Gomez-Rubio et al., 2010]. In addition, it is noteworthy that in our study an analysis of ancestry was not carried out, which could change our results and perhaps reveal differences in methylation profiles.

Overall, our results emphasize the need for further studies on As metabolism with a larger number of participants, evaluated for the influence of age, gender, interaction of genetic polymorphisms and known exposure factors that influence the arsenic methylation are required.

In conclusion, our data indicate that gender and high As exposure levels negatively influence the metabolism of As, and that polymorphisms in the evaluated genes play a role in the metabolism and excretion of this metalloid. Carriers of these genetic variants should be followed-up by health institutions because these variants could increase risk for arsenic-related diseases.

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Table I

Socio-demographic, anthropometric characteristics and lifestyle factors of the participants (results shown as arithmetic mean, standard deviation and percent).

	Total (N = 332)	Low exposure (N = 166)	High exposure (N = 166)	P-value
Age (years)	8.96 ± 1.78	9.01 ± 1.8	8.90 ± 1.7	0.5
Gender (%)				0.79
Girls	174 (52.4)	95 (57.2)	79 (47.5)	
Boys	158 (47.6)	71 (42.7)	87 (52.4)	
BMI *	17.87 ± 3.8	18.42 ± 4.1	17.326 ± 3.4	0.05
Time of consumption of purified drinking water (months)	22.81 ± 36.5	29.25 ± 41.9	16.21 ± 28.6 **	0.001
Type of water used at home (%)	145 (45.4)			
Purified	167 (52.3)	145 (45.4)	85 (53.1)	0.01
Tap water	7 (2.1)	167 (52.3)	71 (44.3)	
Both		7 (2.1)	4 (2.5)	
Type of water used at school (%)				0.007
Purified	33 (10.4)	24 (15.0)	9 (5.7)	
Tap water	275 (87.0)	129 (81.1)	146 (92.9)	
Both	8 (2.5)	6 (3.7)	2 (1.2)	
Second hand smoking (%)				0.6
Father	81 (27.0)	39 (26.0)	42 (28.1)	
Mother	14 (4.5)	5 (3.2)	9 (5.8)	
As in drinking water (µg/L)	103.2	31 ± 12.4	111 ± 22.7	0.001
Urinary As levels (µg/L)	144.04 ± 116.2	65.64 ± 27.3	222.45 ± 118.2 **	0.0001

* BMI: calculated as: weight/height².

** $P < 0.05$; Mann Whitney test.

Table II

Urinary arsenic levels by sex (results shown as arithmetic mean and standard error).

	Girls n=174	Boys n=158	P-value
Total As ($\mu\text{g/L}$)	132.4 ± 7.6	156.8 ± 10.3	0.05 *
As ^{III} ($\mu\text{g/L}$)	16.1 ± 1.3	19.2 ± 1.6	0.22
As ^V ($\mu\text{g/L}$)	7.2 ± 0.9	10.4 ± 1.7	0.16
MMA ^V ($\mu\text{g/L}$)	17.3 ± 1.1	21.8 ± 1.6	0.02 *
DMA ^V ($\mu\text{g/L}$)	85.8 ± 5.4	99.5 ± 6.8	0.08
First methylation	0.8 ± 0.0	0.9 ± 0.0	0.71
Second methylation	6.1 ± 1.0	5.0 ± 0.2	0.44
%iAs	20.5 ± 1.4	22.1 ± 1.7	0.73
%MMA ^V	13.0 ± 0.3	13.4 ± 0.2	0.39
%DMA ^V	62.2 ± 1.5	62.0 ± 1.8	0.20

* $P = 0.05$; Mann Whitney test.

Allelic and genotyping frequencies of *AS3MT* and *GSTO1* and polymorphisms, and *GSTT1* and *GSTM1* null genotype by gender (results shown as percentage).

Table III

Gene	wt/wt		wt/vt		vt/vt		wt/vt + vt/vt		Allelic frequency	
	Girls	Boys	Girls	Boys	Girls	Boys	Girls	Boys	Girls	Boys
<i>AS3MT</i>										
Met287Thr	87.6	83.3	12.3	16.0	0	0.6	12.3	16.6	0.06	0.08
<i>GSTO1</i>										
Ala140Asp	86.3	87.3	11.1	11.2	2.4	1.4	13.6	12.6	0.13	0.14
Glu155del	84.3	81.3	15.0	17.2	0.6	1.3	15.6	18.6	0.08	0.10
<i>GSTT1</i>	59.7	60.7	22.4	23.5	17.8	15.6	--	--	0.29	0.27
<i>GSTM1</i>	79.0	80.8	10.4	14.6	10.4	4.4	--	--	0.15	0.11

wt = wild type; vt = variant type.

Table IV

Relationship between urinary As levels with *AS3MT* and *GSTO1* polymorphisms, and with the *GSTT1* and *GSTMI* genotypes, by sex (results shown as arithmetic mean and standard deviation).

	Girls (n=174)	Boys (n=158)	P-value
<i>AS3MT-Met287Thr</i>			
Total As (µg/L)			
TT	129.9 ± 100.6	156.7 ± 118.1	0.03 *
C (CT + CC)	141.2 ± 107.4	163.9 ± 181.7	0.73
MMA^V (µg/L)			
TT	16.8 ± 14.5	21.0 ± 17.0	0.02 *
C (CT + CC)	19.4 ± 18.3	26.2 ± 33.7	0.81
<i>GSTO1-Ala140Asp</i>			
Total As (µg/L)			
AA	133.5 ± 100.7	159.9 ± 132.5	0.05 *
C (CA + CC)	125.3 ± 117.8	115.4 ± 85.6	0.52
MMA^V (µg/L)			
AA	17.6 ± 14.2	22.0 ± 21.2	0.04 *
C (CA + CC)	16.2 ± 20.7	16.5 ± 13.5	0.10
<i>GSTO1-Glu155del</i>			
MMA^V (µg/L)			
AGGAGG	17.6 ± 15.7	23.3 ± 22.5	0.03 *
AGG/- + -/-	15.8 ± 12.3	18.2 ± 12.7	0.67
<i>GSTMI</i>			
MMA^V (µg/L)			
Wild type	17.5 ± 15.3	21.8 ± 21.0	0.05 *
Null	15.2 ± 12.7	19.0 ± 9.7	0.12

* $P < 0.05$; Mann Whitney test (girls vs boys).

Table V

Multivariate analysis between urinary As levels and *AS3MT*, *GSTO1* polymorphisms, and *GSTT1* and *GSTMI* null genotypes.

	<i>AS3MT</i> Met287Thr			<i>GSTO1</i> Ala140Asp			<i>GSTO1</i> Glu155del			<i>GSTMI</i> null			<i>GSTT1</i> null		
	β	P-	value	β	P-	value	β	P-	value	β	P-	value	β	P-	value
Total As															
All subjects	11.3	0.37		-22.1	0.27		-28.7	0.12		-0.73	0.97		6.44	0.72	
Girls	11.53	0.647		-5.29	0.836		-27.9	0.244		-3.29	0.903		11.64	0.607	
Boys	20.86	0.483		-51.1	0.140		-28.4	0.372		28.12	0.615		9.79	0.758	
As^V															
All subjects	-1.1	0.68		-2.67	0.41		4.76	0.10		-4.19	0.27		-4.65	0.009*	
Girls	1.37	0.560		-1.53	0.596		-1.21	0.666		-1.34	0.662		0.04	0.986	
Boys	-1.39	0.785		-4.86	0.447		12.39	0.024*		-5.58	0.563		-6.41	0.241	
Second methylation															
All subjects	-0.90	0.01*		0.56	0.17		0.24	0.52		0.88	0.08		0.28	0.45	
Girls	-0.70	0.176		1.27	0.017*		0.23	0.631		0.69	0.223		0.07	0.878	
Boys	-1.14	0.050*		-0.17	0.794		0.009	0.987		1.01	0.363		0.04	0.939	
%MMA^V															
All subjects	1.80	0.001*		-0.17	0.77		0.10	0.85		-0.41	0.58		0.33	0.54	
Girls	1.26	0.141		-0.99	0.247		0.19	0.803		-0.27	0.762		-0.06	0.935	
Boys	2.30	0.002*		0.54	0.557		0.48	0.592		-0.38	0.808		1.10	0.215	
%DMA^V															
All subjects	-3.4	0.30		4.35	0.26		1.06	-0.73		10.4	0.02*		5.72	0.09	
Girls	-4.81	0.317		6.17	0.217		2.22	0.622		9.59	0.070		1.89	0.672	
Boys	-3.38	0.509		2.70	0.661		-1.17	0.822		11.69	0.233		5.00	0.367	

* $P < 0.05$; linear regression.