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Influence of genetic polymorphisms on arsenic methylation efficiency during pregnancy: Evidence from a Spanish birth cohort

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ABSTRACT

Background: Inorganic arsenic (iAs) is a widespread toxic metalloid. It is well-known that iAs metabolism and its toxicity are mediated by polymorphisms in *AS3MT* and other genes. However, studies during pregnancy are scarce. We aimed to examine the role of genetic polymorphisms in *AS3MT, GSTO2*, *N6AMT1, MTHFR*, *MTR, FTCD, CBS*, and *FOLH1* in iAs methylation efficiency during pregnancy.

Methods: The study included 541 pregnant participants from the INMA (Environment and Childhood) Spanish cohort. Using high-performance liquid chromatography coupled to inductively coupled plasma-tandem mass, we measured arsenic (iAs and the metabolites monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA)) in urine samples collected during the first trimester. iAs methylation efficiency was determined based on relative concentrations of the As metabolites in urine (%MMA, %DMA, and %iAs). Thirty-two single nucleotide polymorphisms (SNPs) in nine genes were determined in maternal DNA; *AS3MT* haplotypes were inferred. We assessed the association between genotypes/haplotypes and maternal As methylation efficiency using multivariate linear regression models.

Results: The median %MMA and %DMA were 5.3 %, and 89 %, respectively. Ancestral alleles of *AS3MT* SNPs (rs3740393, rs3740390, rs11191453, and rs11191454) were significantly associated with higher %MMA, %iAs, and lower %DMA. Pregnant participants with zero copies of the GGCTTCAC *AS3MT* haplotype presented a higher

Abbreviations: AB, arsenobetaine; As, arsenic; *AS3MT*, arsenic methyltransferase gene; BMI, body mass index; CBS, cystathionine-β-synthase gene; DMA, dimethylarsinic acid; *DNMT1a* and *DNMT3b*, DNA-methyltransferase 1a and 3b genes; FDR, false discovery rate; FFQ, food frequency questionnaire; *FOLH1*, folate hydrolase 1 gene; *FTCD*, formimidoyltransferase cyclodeaminase gene; *GSTO1* and *GSTO2*, glutathione S-transferase omega 1 and 2 genes; iAs, inorganic arsenic; ICPMS/MS, inductively coupled plasma-tandem mass spectrometry; INMA, INfancia y Medio Ambiente (Environment and Childhood Project); LOD, limit of detection; MMA, monomethylarsonic acid; MTHFR, methylenetetrahydrofolate reductase; *MTR*, 5-methyltetrahydrofolate-homocysteine methyltransferase gene; *N6AMT1*, N-6-adenine-specific DNA methyltransferase 1 gene; OCM, one-carbon metabolism; SAM, S-adenosyl methionine; SNP, single nucleotide polymorphism; STROBE, Strengthening the Reporting of Observational Studies in Epidemiology; Total As, total arsenic; VIF, variance inflation factor; ΣAs, sum of iAs, DMA, and MMA.

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%MMA. Statistically significant associations were also found for the *FOLH1* SNP rs202676 (β 0.89 95%CI: 0.24, 1.55 for carriers of the G allele vs. the A allele).
Conclusions: Our study shows that ancestral alleles in *AS3MT* polymorphisms were associated with lower As methylation efficiency in early pregnancy and suggests that *FOLH1* also plays a role in As methylation efficiency. These results support the hypothesis that As metabolism is multigenic, being a key element for identifying susceptible populations.

1. Introduction

Inorganic arsenic (iAs) is a widespread toxic metalloid. It has been estimated that around 200 million people around the world, mainly in South Asia, could be exposed to iAs through drinking water at concentrations above the maximum guideline value recommended by the World Health Organization (10 μg/L) (Podgorski and Berg, 2020; World Health Organization, 2017). In areas with low iAs levels in the drinking water, the main route of exposure is through the consumption of rice (European Food Safety Authority, 2014; Fowler et al., 2015) and, to a lesser extent, other foods, such as vegetables, fruit, and dairy products (Agencia Catalana de Seguretat Alimentaria, 2019; European Food Safety Authority, 2014).

Humans can metabolize iAs via biotransformation to less toxic forms through a series of reductions (from pentavalent to trivalent forms) and methylation processes (from iAs to monomethylarsonic acid [MMA] and dimethylarsinic acid [DMA]) (Cullen, 2014; Stýblo et al., 2021). The methylation process is catalysed via arsenic methyltransferase (AS3MT). This enzyme uses S-adenosyl methionine (SAM) as the main co-substrate, whose biosynthesis depends on one-carbon metabolism (OCM) (Abuawad et al., 2021). The As biotransformation process is considered a detoxification mechanism. However, in vitro studies have observed that trivalent intermediate compounds, such as monomethylarsonous (MMA^{III}) and dimethylarsinous acid (DMA^{III}), have a stronger potential than iAs^{III} to cause genotoxic and cytotoxic effects as well as gene expression suppressions (Stýblo et al., 2021; Thomas, 2021). The percentage of each metabolite excreted through the urine is used to estimate individual methylation efficiency, indicated by higher %DMA and lower %MMA and %iAs (Vahter, 1999). Also, other organic and complex forms of As, such as arsenosugars, arsenolipids, and arsenobetaine (AB) contribute to total As concentrations, although they are considered less toxic than iAs (Agency for Toxic Substances and Disease Registry, 2016).

Other factors influencing iAs methylation efficiency include body mass index (BMI), smoking habit, ethnicity, and levels of exposure (Gao et al., 2019a; Shen et al., 2016; Soler-Blasco et al., 2021; Tseng, 2008). Furthermore, certain nutrients could increase the efficiency of iAs methylation, especially those involved in OCM, such as vitamins B_6 and B_{12}, betaine, choline, and folic acid (Bozack et al., 2019; Gamble et al., 2006; Heck et al., 2007; Howe et al., 2017; Kurzius-Spencer et al., 2017; Laine et al., 2018; Spratlen et al., 2017), as well as other trace elements, e.g., zinc and cobalt (Clare et al., 2019; Soler-Blasco et al., 2021).

Methylation efficiency seems to increase throughout pregnancy leading to a higher %DMA and lower %MMA in this critical period for foetal development (Gao et al., 2019a; Gardner et al., 2011). This is important because iAs crosses the placental barrier and prenatal iAs exposure has been associated with several adverse effects during early life and childhood, such as increased risk of respiratory symptoms and infections (Sanchez et al., 2016), miscarriage, and foetal deaths (Quansah et al., 2015), as well as lower birth length and weight (Laine et al., 2015; Zhong et al., 2019) and impaired neuropsychological development (Freire et al., 2018; Hamadani et al., 2011; Liang et al., 2020; Soler-Blasco et al., 2022; Vahter et al., 2020).

Genetic variants appear to play an important role in an individual's iAs metabolism and, consequently, in vulnerability to arsenic-related health effects (Antonelli et al., 2014; Chi et al., 2018). In fact, increasing evidence suggests that several single nucleotide polymorphisms (SNPs) are involved in iAs methylation efficiency. In particular, polymorphisms in *AS3MT* have been associated with lower %MMA in several populations worldwide (Chen et al., 2017; de la Rosa et al., 2017; Drobná et al., 2016; Engström et al., 2011; Gao et al., 2019b; Huang et al., 2018; Stajnko et al., 2019). There is also evidence that polymorphisms in other genes, such as *N-6-adenine-specific DNA methyltransferase 1* (*N6AMT1*) and *glutathione S-transferase omega-1* and *2* (*GSTO1* and *GSTO2*), may also be involved in iAs methylation efficiency (Chen et al., 2017; de la Rosa et al., 2017; Harari et al., 2013; Huang et al., 2018; Rodrigues et al., 2012; Zakharyan et al., 2001).

Several studies report that SNPs in several OCM-related genes, such as methylenetetrahydrofolate reductase (*MTHFR*), 5-*methyltetrahydrofolate-homocysteine methyltransferase* (*MTR*), *folate hydrolase 1* (*FOLH1*), *formimidoyltransferase-cyclodeaminase* (*FTCD*), and *cystathionine-β-synthase* (*CBS*), may also influence iAs methylation efficiency; however, the evidence is not consistent (Gamboa-Loira et al., 2018; Hsueh et al., 2020; Niedzwiecki et al., 2018; Pierce et al., 2019; Porter et al., 2010; Spratlen et al., 2018). For instance, Niedzwiecki et al. (2018) found that rs1801133 in *MTHFR* was associated with As metabolism, whereas this association was not observed by Gamboa-Loira et al. (2018), Hsueh et al. (2020), or Porter et al. (2010). In the same way, the G allele of rs1805087 in *MTR* was associated with lower %iAs in Gamboa-Loira et al. (2018), but not in Porter et al. (2010). Differences in levels of As exposure, characteristics of the studied population, and SNPs could explain the heterogeneity of the results.

Evidence concerning the role of genetic factors in As methylation efficiency during pregnancy is still scarce (Engström et al., 2007, 2011; Gao et al., 2019b; Gardner et al., 2011; Stajnko et al., 2019). These studies, however, only examined SNPs in *AS3MT, DNA-methyltransferases 1a* and *3b* (*DNMT1a* and *DNMT3b*), *MTHFR*, *MTR*, and *GSTO2*, and the results are still inconclusive. Therefore, the objective of the present study was to explore the influence of several maternal polymorphisms in the *AS3MT*, *N6AMT1, GSTO, MTHFR, MTR, FOLH1, FTCD*, and *CBS* genes on maternal iAs methylation efficiency during pregnancy in a Spanish cohort.

2. Material and methods

2.1. Study population

Our study was based on data from participants of the multicentre birth cohort INMA. The INMA (Childhood and Environment) Project involves pregnant women from different geographical areas of Spain (http://www.proyectoinma.org). In the present study, data from Gipuzkoa (northeast region of Spain) and Valencia (eastern region of Spain) were used. The study protocol was described in Guxens et al. (2012). Briefly, 1493 pregnant participants were recruited during their first antenatal visit in 2003–2008 ($n = 638$ in Gipuzkoa and $n = 855$ in Valencia). The final study samples included 541 subjects with data on both urinary As species concentrations in the first trimester of pregnancy and genetics. The main reasons for non-participation were withdrawal, loss to follow-up ($n = 28$), or unavailability of As measurements and/or genetics ($n = 924$).

This study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline for cohort studies. The study protocol was approved by the corresponding Ethics Committees (University Hospital La Fe in Valencia, the Public Health

Table 1
Sociodemographic and environmental characteristics of study participants, and calibrated[a] As percentages according to the characteristics during the first trimester of gestation.

	All population (n = 541)	%MMA$_{cal}$	%DMA$_{cal}$	%iAs$_{cal}$
	N (%) or mean (SD)	Median (percentile 5, percentile95)		
Study area				
Gipuzkoa	113 (21)	6.4 (0.3, 13.1)[*]	84.8 (66.6, 5.2)	7.7 (0.9, 30.01)
Valencia	428 (79)	7.6 (3.1, 15.1)[*]	83.7 (66.0, 94.0)	7.9 (2.7, 19.1)
Gestational age at sampling (weeks)	12.7 (1.1)	−0.13 (−0.21, 0.05)[*]	0.09 (−0.03, 0.14)	−0.08 (−0.08, 0.09)
Age (years)	30.6 (4.2)	−0.04 (−0.10, 0.07)	0.01 (−0.11, 0.06)	−0.02 (−0.05,0.12)
Country of birth				
Spain	532 (98)	7.4 (2.7, 14.6)	84.12 (66.01, 94.1)	7.9 (2.6, 20.4)
Others	9 (2)	5.3 (3.1, 13.5)	79.1 (69.7, 93.1)	11.3 (3.2, 24.1)
BMI before pregnancy (Kg/m2)				
<25	402 (74)	7.5 (2.8, 14.7)[*]	83.4 (65.9, 93.9)[*]	8.09 (2.6, 21.3)[*]
25- <30 (Overweight)	94 (17)	7.2 (2.8, 14.7)[*]	84.7 (67.6, 94.7)[*]	7.86 (2.5, 18.1)[*]
≥ 30 (Obesity)	45 (8)	6.3 (2.8, 11.4)[*]	86.5 (69.6, 95.5)[*]	6.07 (2.3, 23.4)[*]
Parity				
0	308 (57)	7.6 (3.00, 15.1)	83.4 (66.5, 94.1)	8.1 (2.5, 19.9)
≥1	233 (43)	7.2 (2.7, 14.0)	84.55 (65.1, 94.4)	7.56 (2.6, 23.9)
Parental social class				
I + II (high)	150 (28)	6.77 (1.6, 13.8)	85.04 (58.9, 94.9)	7.23 (1.7, 35.7)
III	152 (28)	7.5 (2.9, 14.5)	83.1 (67.4, 93.5)	8.6 (3.00., 19.9)
IV + V (low)	239 (44)	7.6 (3.3, 14.7)	84.2 (66.2, 93.2)	7.8 (3.1, 19.9)
Educational level				
Up to primary	150 (28)	7.8 (3.9, 14.7)	83.9 (66.4, 91.5)	7.8 (3.8, 17.5)
Secondary	238 (44)	7.2 (2.3, 13.9)	84.4 (66.34, 94.8)	8.00 (2.4, 22.9)
University	153 (28)	7.1 (2.2, 15.9)	84.1 (64.4, 94.4)	7.9 (2.2, 21.4)
Working status				
Non- worker	125 (23)	7.6 (2.9, 14.6)	84.25 (66.4, 94.0)	7.6 (3.2, 17.8)
Worker	415 (77)	7.4 (2.6, 14.6)	84.1 (66.1, 94.2)	8.00 (2.5, 21.9)
Tobacco consumption				
No	410 (77)	7.2 (2.5, 14.5)[*]	84.4 (66.3, 94.5)[*]	7.6 (2.4, 22.0)
Yes	129 (24)	7.8 (3.4, 14.7)[*]	82.1 (67.1, 93.0)[*]	8.7 (3.6, 19.8)
Alcohol consumption				
No	463 (86)	7.4 (2.8, 14.3)	84.0 (66.5, 94.1)	7.93 (2.6, 20.6)
Yes	77 (14)	7.4 (2.6, 15.8)	84.3 (65.2, 94.8)	7.8 (2.4, 18.9)
Rice consumption (g/week)[b]	361.2 (176.6)	0.07 (0.01, 0.18)	−0.06 (−0.14, 0.03)	0.04 (−0.07, 0.10)
Fish consumption (g/week)[b]	554.2 (245.4)	−0.04 (−0.12, 0.05)	0.03 (−0.06, 0.11)	−0.02 (−0.09, 0.07)
Estimated folate intake[*] (μg/day)[b]	2329.7 (3080.2)	−0.10 (−0.15, 0.02)	0.05 (−0.05, 0.12)	−0.05 (−0.10, 0.07)
Urinary As levels	Median (percentiles 5, 95)			
∑As (μg/ g creatinine)	7.1 (2.8, 24.8)			
DMA (μg/ g creatinine)	6.2 (2.3, 24.0)			
MMA (μg/ g creatinine)	0.4 (0.1,1.1)			
iAs (μg/ g creatinine)	0.3 (0.1, 1.3)			
%DMA$_{cal}$	84.1 (66.2, 94.2)			
%MMA$_{cal}$	7.4 (2.7, 14.6)			
%iAs$_{cal}$	7.9 (2.5, 21.3)			

Note: BMI, Body mass index; wg, weeks of gestation; g/week: grams per week. The percentages of each metabolite were calculated: levels of calibrated metabolite/(calibrated DMA + calibrated MMA + calibrated iAs).

[*] Variables with p-value <0.05. P-values comparing As percentages between the different categories of the variables using the Kruskal Wallis Test, and for evaluating the correlation between As percentages and continuous variables (Spearman correlation).

[a] Calibrated percentages were calculated based on As metabolite concentrations corrected by arsenobetaine and creatinine concentrations (denoted by %MMA$_{cal}$, %DMA$_{cal}$, %iAs$_{cal}$).

[b] Adjusted for calories.

Research Centre of Valencia, and the Donostia Hospital in Gipuzkoa). Informed consent was obtained from all participants.

2.2. Study variables and information sources

2.2.1. Outcome variables: urinary arsenic speciation analysis

Spot urine samples were collected in the first trimester of pregnancy (mean = 12.7 weeks of gestation). Total As concentrations in urine were measured using inductively coupled plasma-tandem mass spectrometry (ICPMS/MS, 8800, Agilent Technologies, Waldbronn, Germany). As speciation was performed using high-performance liquid chromatography (1200, Agilent Technologies) coupled to ICPMS/MS (8800, Agilent Technologies). Further details regarding laboratory techniques can be found in Soler-Blasco et al. (2021). Limits of detection (LOD) were: 0.02 μg/L for AB and 0.03 μg/L for DMA, MMA, and iAs. Two per cent of samples had MMA levels below the LOD, while 1.3 % had iAs levels below the LOD. For these samples, ½ LOD was calculated. All samples had DMA levels above the LOD.

2.2.2. Genetic analysis

DNA was obtained from whole blood at the Spanish National Genotyping Center (CEGEN-Barcelona) using the Chemagen kit (PerkinElmer) for the Valencia cohort, and at the Basque Biobank using the Flexigene DNA kit (Qiagen) for the Gipuzkoa cohort. Further details regarding the genetic analysis can be found in Supplemental Material Appendix S1 (A.1.1 Methodology: description of genetic analysis). SNPs were selected based on their functional impact on As metabolism and OCM according to previously published literature. The selected SNPs were: rs9527, rs7085104, rs3740400, rs3740390, rs3740392, rs3740393, rs3740394, rs11191439, rs12768205, rs11191453,

rs11191454, rs10748835 and, rs1046778 (*AS3MT*); rs2297235 and rs156697 (*GSTO2*); rs1695 and rs1138272 (Glutathione S-Transferase Pi 1, [*GSTP1*]), rs1006903, rs1003671, rs7282257, rs1048546, rs2065266, rs16983411, and rs2205449 (*N6AMT1*); rs1801131, and rs1801133 (*MTHFR*); rs1805087 (*MTR*); rs202676 (*FOLH1*); rs61735836 (*FTCD*); rs4920037, and rs234709 (*CBS*).

We performed linkage disequilibrium analysis and inferred haplotypes from *AS3MT* rs7085104, rs3740400, rs3740393, rs3740390, rs11191439, rs11191453, rs10748835, and rs1046778 via PHASE software (Stephens and Donnelly, 2003) (Fig. S1).

2.2.3. Covariates and potential confounders

Sociodemographic, environmental, and lifestyle information was collected through questionnaires administered by trained interviewers during the first and third trimesters of gestation (mean = 12.8 and 32.4 gestational weeks, respectively). A semi-quantitative food frequency questionnaire (FFQ) was administrated during the first trimester (Vioque et al., 2013). Dietary folate was estimated using food composition tables (Palma et al., 2008; U.S. Department of Agriculture: Agricultural Research Service USDA, 2007) as well as data on the intake of supplements from the FFQ (Vioque et al., 2013). The variables used in the present analysis were study area (Gipuzkoa, Valencia), age at conception (years), place of birth (Spain, other), tobacco consumption during the first trimester of pregnancy (yes, no), BMI (kg/m^2) before pregnancy, weeks of gestation at sample collection, parental social class (I + II [high], III, IV + V [low]), parity (0, ≥1), education level (up to primary, secondary, university), working status at first trimester of pregnancy (non-worker, worker), season of sample collection (spring, summer, winter, autumn), rice and fish consumption (grams per day), and estimated dietary folate intake (μg/day) at the first trimester of pregnancy. Creatinine concentrations were measured in the same urine samples by the DRI® Creatinine-Detected® Test using AV680 from Beckman Coulter.

2.3. Statistical analysis

Descriptive and bivariate analyses were performed to detect differences between included and excluded populations.

As methylation efficiency was calculated as relative concentrations of individual iAs, MMA, and DMA over the sum of these species (∑As). Because organic arsenic (oAs) from seafood contributes to DMA concentrations and could lead to an inappropriate estimation of iAs methylation, we estimated the calibrated percentages of iAs, MMA, and DMA, corrected for AB and creatinine concentrations (%iAs_{cal}, %MMA_{cal}, and %DMA_{cal}) using the mathematical method proposed by Jones et al. (2016). More information about this method can be found in Soler-Blasco et al. (2021) and Supplemental Material Appendix S1 (A.1.2 Methodology: description of arsenic species calibration methodology).

Deviation from Hardy–Weinberg equilibrium by comparing expected vs. observed genotype was tested using chi-square analysis. Two SNPs in *AS3MT* (rs12768205 and rs1046778) and one in *N6AMT1* (rs7282257) were not in Hardy-Weinberg equilibrium ($p < 0.05$) and, therefore, excluded from further analysis.

We initially performed descriptive and bivariate analyses of maternal %MMA_{cal}, %DMA_{cal}, and %iAs_{cal} (median and 5–95 percentiles) according to sociodemographic, nutritional, and lifestyle covariates. Thereafter, we assessed the association between maternal genotypes and haplotypes, and maternal As methylation efficiency using multivariate regression models. Genotypes and haplotypes were modelled as categorical variables (0, 1, or 2 alleles for genotypes and 0, 1, or 2 copies for haplotypes) with the genotypes/haplotypes associated with lower %MMA_{cal} denoted first and used as the reference group. When the frequency of a homozygous genotype or copy number included <5 % of individuals, this group was pooled with the heterozygotes or, alternatively, with the 1 copy haplotype group.

For multivariate regression models, we first built a core model using all covariates associated with %MMA_{cal}, %DMA_{cal}, and %iAs_{cal} with a p-value <0.20 in the bivariate analysis. Using a backward elimination procedure, the covariates associated with percentages of As metabolites at p-value <0.10 in the likelihood ratio test were retained in the model. The final core model included the following variables: study area, maternal tobacco consumption during the first trimester of pregnancy, BMI before pregnancy, folate and folic acid intake during the first trimester of pregnancy, and ∑As concentrations. Gestational age at sampling was included in the core model regardless of its statistical significance. Subsequently, we modelled associations between each genotype for *AS3MT, N6AMT1, GSTO, MTHFR, MTR, FOLH1, FTCD*, and *CBS* SNPs and each *AS3MT* haplotype with each calibrated As percentage and adjusted the model for study area (Valencia and Gipuzkoa). Finally, models were adjusted for all variables included in the core model. We used the q-value step-up false discovery rate (FDR) method to adjust for inflation of p-values (Storey and Tibshirani, 2003).

Linear regression assumptions were checked via visual inspection of residuals for normality and homoscedasticity. Collinearity among variables and influential data in the final models were examined using variance inflation factors (VIFs) and Cook's distance, respectively. No influential data were identified and all VIFs were < 2.5. Confidence intervals were calculated based on robust standard error in the final results to manage the possibility of minor deviations from normality and homoscedasticity. R statistical package version 4.1.3 was used for statistical analyses (R Core Team, 2021).

3. Results

3.1. Study population

Characteristics of the study participants are shown in Table 1. Subjects were 30 years old on average, 28 % completed university studies, 24 % smoked during pregnancy, 74 % had a BMI below 25, and 57 % were primipara. Participants from Valencia, smokers, and those with BMI <25 had higher %MMA_{cal}.

Study participants included in this study differed somewhat from those not included. In particular, included participants ($n = 541$) were mostly from the Valencia cohort, mostly born in Spain, and had a slightly lower educational level and socioeconomic class than those not included in the study. Also, the participants' intake of rice and folate was slightly higher and lower, respectively, than in non-participants. There was no difference in fish consumption (Table S1).

3.2. Description of As species concentrations and percentages and genotypes/haplotypes

The median (5–95 percentile) of urinary ∑As concentrations and % MMA, %DMA, and %iAs were 7.1 (2.8, 24.8) μg/g of creatinine, 5.3 % (1.4, 11.2), 89.3 % (77.2, 97.1), and 4.9 % (1.2, 14.6), respectively. The calibrated percentages of As metabolites were lower, with medians (p5–95) of 7.4 % (2.7, 14.6) for %MMA_{cal}, 84.1 % (66.2, 94.1) for %DMA_{cal}, and 7.9 % (2.5, 21.3) for %iAs_{cal} (Table 1).

The allelic frequencies of the different SNPs were similar to those observed in European populations in the ALPHA Project (Table S2), except for rs234709 in *CBS*, where a higher T-allele frequency was observed in our study population.

Twelve *AS3MT* haplotypes were inferred: 1) ATGCTTGT, 2) ATGCTTAC, 3) ATGCCTAT, 4) ATCCTTAC, 5) AGGCTTAC, 6) AGCTTCAC, 7) GGGCTTGT, 8) GGGCTTAC, 9) GGGCCTAT, 10) GGGCCCAC, 11) GGCCTTAC, and 12) GGCTTCAC; of which only haplotypes 1, 4, 8, 9, and 12 were common enough to be used in subsequent analyses (present in 56.8 %, 5.4 %, 14.4 %, 10.0 %, and 10.0 % of samples, respectively) (Table S3).

Table 2
Beta coefficient (95%CI) of the multivariate linear regression between methylation As efficiency and maternal polymorphisms.

	$\%MMA_{cal}$ [a]				$\%DMA_{cal}$ [a]				$\%iAs_{cal}$ [a]			
	Beta	(95%CI)	p [b]	p-FDR [c]	Beta	(95%CI)	p [b]	p-FDR [c]	Beta	(95%CI)	p [b]	p-FDR [c]
AS3MT (arsenic (+3 oxidation state) methyltransferase)												
rs9527 (ref. CC)												
CT	−0.28	(−0.95, 0.39)	0.33	0.46	0.71	(−0.81, 2.22)	0.38	0.63	−0.43	(−1.60, 0.76)	0.66	0.75
TT	0.87	(−0.74, 2.48)			−1.68	(−5.57, 2.21)			0.82	(−2.63, 4.26)		
rs7085104 (ref. GG)												
AG	−0.61	(−1.70, 0.47)	**0.01**	**0.04**	0.29	(−2.16, 2.74)	**0.03**	0.11	0.32	(−1.66, 2.31)	0.18	0.69
AA	0.42	(−0.72, 1.56)			−1.86	(−4.47, 0.75)			1.44	(−0.68, 3.56)		
rs3740400 (ref. GG)												
TG	−0.63	(−1.65, 0.38)	**0.01**	**0.02**	0.09	(−2.22, 2.40)	**0.03**	0.11	0.54	(−1.34, 2.42)	0.19	0.69
TT	0.52	(−0.56, 1.60)			−2.05	(−4.52, 0.42)			1.54	(−0.45, 3.52)		
rs3740394 (ref. AA)												
AG + GG	−0.40	(−1.21, 0.41)	0.33	0.46	1.13	(−0.75, 3.00)	0.24	0.51	−0.72	(−2.29, 0.84)	0.36	0.69
rs3740393 (ref. CC + CG)												
GG	1.35	(0.67, 2.04)	**<0.001**	**<0.001**	−2.75	(−4.32, -1.17)	**0.001**	**0.01**	1.39	(0.13, 2.66)	**0.03**	0.16
rs3740392 (ref.TT)												
TC	−0.54	(−1.22, 0.13)	0.11	0.22	1.06	(−0.46, 2.59)	0.24	0.51	−0.52	(−1.70, 0.67)	0.62	0.75
CC	0.69	(−0.75, 2.13)			−1.17	(−4.65, 2.31)			0.48	(−2.56, 3.53)		
rs3740390 (ref. TT + TC)												
CC	1.40	(0.55, 2.25)	**0.001**	**0.004**	−3.53	(−5.39, -1.68)	**<0.001**	**0.002**	2.13	(0.72, 3.54)	**0.003**	**0.03**
rs11191439 (ref. CC + CT)												
TT	0.33	(−0.48, 1.13)	0.43	0.46	−1.07	(−2.92, 0.79)	0.26	0.51	0.74	(−0.80, 2.28)	0.35	0.69
rs11191453 (ref. CC + CT)												
TT	1.40	(0.56, 2.25)	**0.001**	**0.004**	−3.57	(−5.40, -1.73)	**<0.001**	**0.002**	2.16	(0.76, 3.56)	**0.003**	**0.03**
rs11191454 (ref. GG + GA)												
AA	1.54	(0.73, 2.35)	**0.001**	**0.002**	−3.80	(−5.60, -2.00)	**<0.001**	**0.001**	2.26	(0.86, 3.66)	**0.002**	**0.03**
rs10748835 (ref. AA)												
GA	−0.37	(−1.26, 0.53)	**<0.001**	**0.004**	0.13	(−1.90, 2.17)	**0.02**	0.11	0.23	(−1.47, 1.94)	0.38	0.69
GG	1.16	(0.14, 2.18)			−2.21	(−4.45, 0.04)			1.05	(−0.74, 2.83)		
GSTP1 (glutathione S-transferase Pi 1)												
rs1695 (ref. AA)												
AG	0.74	(−0.58, 2.06)	0.52	0.46	0.27	(−2.47, 3.02)	0.51	0.67	−1.01	(−3.21, 1.18)	0.27	0.69
GG	0.53	(−0.78, 1.84)			1.13	(−1.57, 3.82)			−1.66	(−3.83, 0.52)		
rs1138272 (ref. CC)												
CT + TT	0.52	(−0.79, 1.83)	0.44	0.46	−0.05	(−2.33, 2.23)	0.96	0.87	−0.47	(−1.76 0.82)	0.48	0.69
GSTO2 (glutathione S-transferase omega-2)												
rs2297235 (ref. GG)												
AG	0.52	(−0.64, 1.69)	0.56	0.46	0.27	(−2.28, 2.81)	0.78	0.77	−0.79	(−2.93, 1.35)	0.70	0.75
AA	0.22	(−0.93, 1.36)			0.73	(−1.83, 3.30)			−0.95	(−3.14, 1.24)		
rs156697 (ref.GG)												
AG	−0.20	(−1.41, 1.01)	0.54	0.46	1.11	(−1.55, 3.77)	0.41	0.63	−0.91	(−2.85, 1.03)	0.49	0.69
AA	−0.54	(−1.78, 0.71)			1.79	(−1.00, 4.58)			−1.26	(−3.34, 0.83)		
N6AMT1 (N-6-adenine-specific DNA methyltransferase 1)												
rs1006903 (ref.GG)												
GC + CC	−0.02	(−0.77, 0.74)	0.96	0.74	0.52	(−1.14, 2.17)	0.54	0.67	−0.50	(−1.73, 0.73)	0.43	0.69
rs1003671 (ref. TT)												
GA	0.59	(−0.38, 1.56)	0.48	0.46	−0.69	(−2.99, 1.61)	0.68	0.72	0.10	(−1.62, 1.82)	0.74	0.75
GG	0.51	(−0.51, 1.52)			−1.10	(−3.57, 1.37)			0.60	(−1.27, 2.47)		
rs1048546 (ref. TT)												
GT	1.34	(0.26, 2.43)	**0.04**	0.09	−1.80	(−4.52, 0.91)	0.42	0.63	0.46	(−1.53, 2.46)	0.88	0.83

(*continued on next page*)

Table 2 (*continued*)

	%MMA$_{cal}$[a]				%DMA$_{cal}$[a]				%iAs$_{cal}$[a]			
	Beta	(95%CI)	p[b]	p-FDR[c]	Beta	(95%CI)	p[b]	p-FDR[c]	Beta	(95%CI)	p[b]	p-FDR[c]
GG	0.80	(−0.25, 1.84)			−1.29	(−3.97, 1.38)			0.49	(−1.48, 2.47)		
rs2065266 (ref.CC)												
TC	0.81	(−0.17, 1.79)	0.20	0.36	−1.03	(−3.29, 1.23)	0.64	0.71	0.22	(−1.45, 1.88)	0.71	0.75
TT	0.36	(−0.66, 1.38)			−1.08	(−3.53, 1.38)			0.72	(−1.14, 2.57)		
rs16983411 (ref. GG)												
AG	0.90	(−0.68, 2.48)	0.53	0.46	−1.66	(−5.10, 1.78)	0.60	0.70	0.76	(−1.56, 3.08)	0.72	0.75
GG	0.79	(−0.72, 2.31)			−1.67	(−4.96, 1.61)			0.88	(−1.26, 3.02)		
rs2205449 (ref.TT)												
AT	0.78	(−0.22, 1.77)	0.24	0.40	−0.92	(−3.22, 1.38)	0.71	0.72	0.15	(−1.55, 1.84)	0.76	0.75
AA	0.36	(−0.68 0.1.40)			−0.97	(−3.45, 1.51)			0.61	(−1.27, 2.48)		
MTHFR (methylenetetrahydrofolate Reductase)												
rs1801131 (ref. TT)												
TG	−0.01	(−0.71, 0.70)	1.00	0.74	−0.51	(−2.15, 1.14)	0.20	0.50	0.51	(−0.79, 1.82)	**0.03**	0.16
GG	−0.04	(−1.17, 1.08)			1.57	(−0.57, 3.70)			−1.53	(−2.94, -0.12)		
rs1801133 (ref. GG)												
GA	0.03	(−0.71, 0.77)	1.00	0.74	−1.02	(−2.71, 0.67)	0.49	0.67	0.99	(−0.34, 2.32)	0.34	0.69
AA	0.02	(−0.96, 1.01)			−0.50	(−2.63, 1.63)			0.48	(−1.06, 2.02)		
MTR (5-methyltetrahydrofolate-homocysteine methyltransferase)												
rs10495387 (ref. CC)												
CA + AA	−0.48	(−1.81, 0.84)	0.48	0.46	1.04	(−2.40, 4.48)	0.55	0.67	−0.56	(−3.51, 2.39)	0.71	0.75
rs1805087 (ref. AA)												
AG + GG	−0.48	(−1.81, 0.84)	0.48	0.46	0.11	(−1.46, 1.68)	0.89	0.84	−0.50	(−1.84, 0.84)	0.46	0.69
FOLH1 (folate hydrolase 1)												
rs202676 (ref. AA)												
AG + GG	0.89	(0.24, 1.55)	**0.01**	**0.02**	−1.49	(−3.02, 0.04)	0.06	0.18	0.59	(−0.67, 1.86)	0.36	0.69
CBS (cystathionine-β-synthase)												
rs4920037 (ref. GG)												
GA + AA	0.24	(−0.48, 0.95)	0.52	0.46	−0.68	(−2.29, 0.93)	0.41	0.63	0.44	(−0.80, 1.68)	0.48	0.69
rs234709 (ref. CC)												
CT	0.40	(−0.35, 1.16)	0.55	0.46	−0.09	(−1.85, 1.68)	0.99	0.87	−0.32	(−1.75, 1.12)	0.91	0.83
TT	0.35	(−0.58, 1.27)			−0.17	(−2.34, 2.01)			−0.18	(−1.97, 1.61)		
FTCD (formimidoyltransferase-cyclodeaminase)												
rs61735836 (ref. CC)												
CT + TT	1.42	(0.04, 2.81)	**0.04**	0.09	−2.21	(−4.83, 0.41)	0.10	0.28	0.79	(−0.77, 2.34)	0.32	0.69

Note: 95%CI, 95 % confidence intervals; %DMA$_{cal}$: calibrated percentage of dimethylarsinic acid; %MMA$_{cal}$: calibrated percentage of methylarsonic acid; %iAs$_{cal}$: calibrated percentage of inorganic As. Ref, reference category,

The percentages of each metabolite were calculated: levels of calibrated metabolite/(calibrated DMA + calibrated MMA + calibrated iAs).

All models were adjusted for creatinine, study area, pre-pregnancy body mass index, tobacco consumption at first trimester of pregnancy, gestational age at sampling, estimated folate intake and ∑As (sum of urinary MMA, DMA and iAs). Values in bold indicate p-values <0.05.

DNA sequence has been read in the 5′ to 3′ direction.

[a] Calibrated percentages were calculated with As metabolite concentrations corrected by arsenobetaine and creatinine concentrations (denoted by %MMA$_{cal}$, %DMA$_{cal}$, %iAs$_{cal}$).

[b] p value from ANOVA F-test; [c]Corrected p-value for FDR of 5 %.

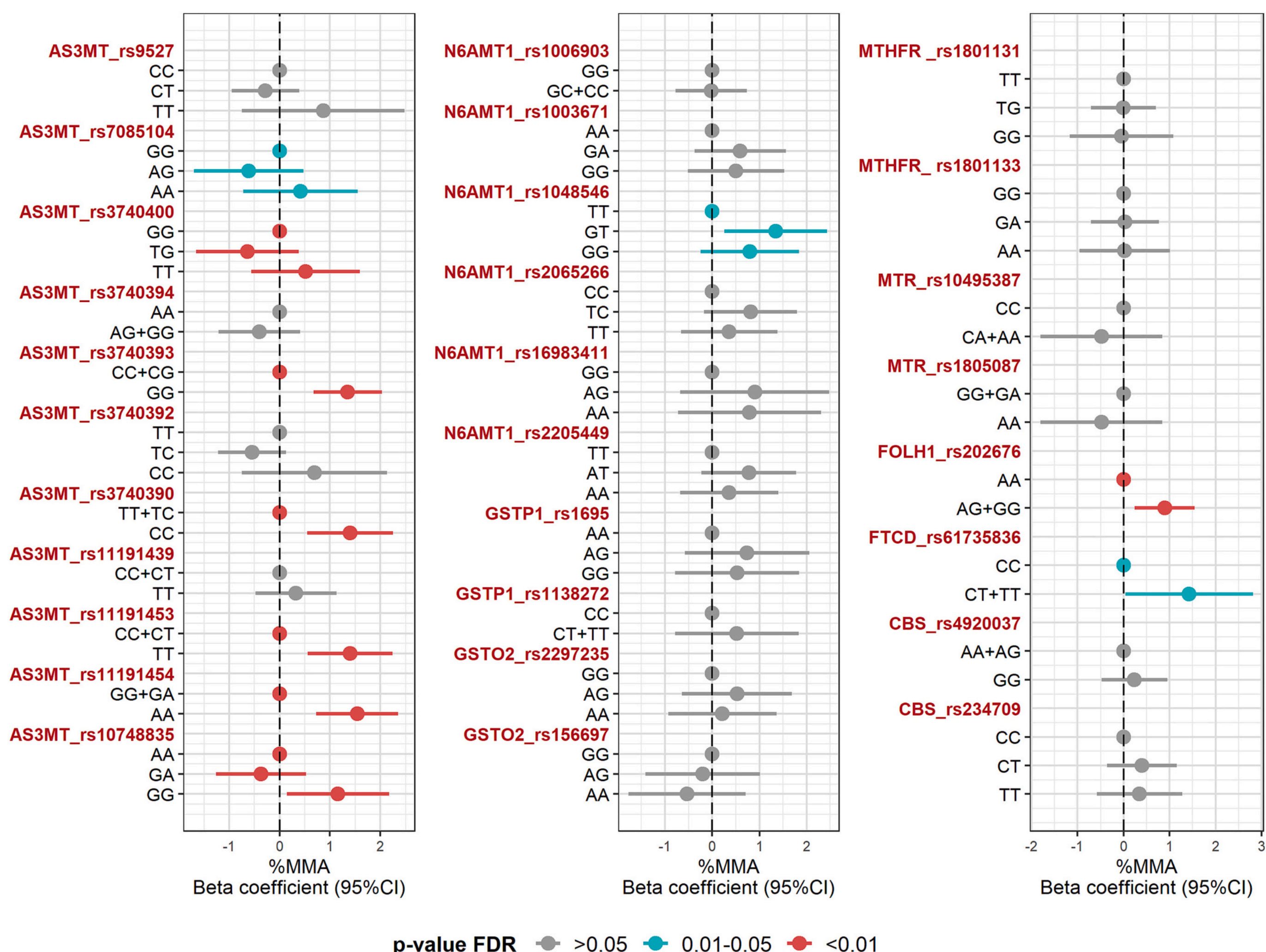

Fig. 1. Beta coefficients (CI95%) of the multivariate linear regression between methylation efficiency and maternal polymorphisms.
Note: *AS3MT*: arsenic methyltransferase gene; *GSTP1*: glutathione S-transferase pi 1 gene; *GSTO2*: Glutathione S-transferase omega-2 gene; *N6AMT1*: N-6-Adenine-Specific DNA Methyltransferase 1 gene; *MTRF*; Methylenetetrahydrofolate Reductase gene; *MTR*: 5-methyltetrahydrofolate-homocysteine methyltransferase gene; *FOLH1*: Folate Hydrolase 1 gene; *FTCD*: Formimidoyltransferase-Cyclodeaminase gene; *CBS*: cystathionine beta-synthase gene; %MMAcal: calibrated percentage of monomethylarsonic acid.
[a]Calibrated %MMA was calculated with urinary MMA concentrations corrected for arsenobetaine and creatinine concentrations (denoted by $\%MMA_{cal}$); [b]*p*-value from ANOVA F-test; [c]Corrected *p*-value for FDR of 5 %. All models were adjusted for creatinine, study area, pre-pregnancy body mass index, tobacco consumption at first trimester of pregnancy, gestational age at sampling, estimated folate intake, and $\sum$As (sum of urinary MMA, DMA, and iAs).

3.3. Association between genotypes/haplotypes and arsenic methylation efficiency

Several SNPs in *AS3MT* (rs3740393, rs3740390, rs11191453, rs11191454, and rs10748835) were associated with As metabolism in models adjusted for the study area (Table S4). All associations remained significant even after adjusting for creatinine, study area, pre-pregnancy BMI, tobacco consumption during the first trimester of pregnancy, gestational age at urine sampling, estimated folate intake, and $\sum$As in multivariate linear regression models (see Table 2 for associations with %MMA, %DMA, and %iAs, and Fig. 1 for %MMA). The genotypes GG in rs3740393, CC in rs3740390, TT in rs11191453, and AA in rs11191454 were associated with higher $\%MMA_{cal}$ and $\%iAs_{cal}$ and lower $\%DMA_{cal}$. All associations remained statistically significant even after adjustment for FDR. The GG genotype in rs10748835 was also associated with higher $\%MMA_{cal}$, even after adjusting for FDR, however, not with $\%DMA_{cal}$ or $\%iAs_{cal}$. For rs3740393, associations remained significant for $\%MMA_{cal}$ and $\%DMA_{cal}$. Concerning the other genes examined, statistically significant associations were found between carriers of genotypes AG + GG in *FOLH1* rs202676 and higher $\%MMA_{cal}$ (β [95%CI]: 0.89 [0.24, 1.55]), which remained statistically significant even after adjustment for FDR. Carriers of CT + TT in *FTCD* rs61735836 also had higher $\%MMA_{cal}$ (β = 1.42, 95%CI = 0.04, 2.81) but associations became non-significant after adjustment for FDR. We found no associations between the different calibrated percentages of As metabolites and the remaining genotypes examined (see Table 2).

Regarding *AS3MT* haplotypes, $\%MMA_{cal}$ was significantly higher in participants with 0 copies (vs. 2 copies) of haplotype GGCTTCAC (β = 1.54; 95 % CI = 0.72, 2.36) as well as in those with 0 copies (vs. 2 copies) of haplotype ATCCTTAC (β = 0.92; 95 % CI = 0.04, 1.79) (Table 3). Haplotype GGCTTCAC was also associated with a lower %DMA (β = −3.77; 95 % CI = −5.59, −1.95) and higher %iAs (β = 2.23; 95 % CI: 0.82, 3.64).

4. Discussion

This study adds evidence regarding the influence of genetic *AS3MT* variants on As methylation efficiency in pregnant women, specifically in

Table 3
Beta coefficient (95%CI) of the multivariate linear regression between As methylation and AS3MT haplotypes.

	Sequence	Copies[b]	Freq (%)	Calibrated[a] %MMA				Calibrated[a] %DMA				Calibrated[a] %iAs			
				Beta	(95%CI)	p[c]	p-FDR[d]	Beta	(95%CI)	p[c]	p-FDR[d]	Beta	(95%CI)	p[c]	p-FDR[d]
Haplotype 1	ATGCTTGT	0 copies	11.1												
		1 copy	58.0	−0.97	(−2.03, 0.09)	**<0.01**	**<0.01**	2.03	(−0.51, 4.57)	**0.01**	**0.02**	−1.06	(−3.32, 1.20)	0.24	0.61
		2 copies	30.9	0.58	(−0.61, 1.77)			−0.56	(−3.35, 2.22)			−0.02	(−2.38, 2.35)		
Haplotype 4	ATCCTTAC	1 copy	9.8												
		0 copies	90.2	0.92	(0.04, 1.79)	**0.04**	0.07	−0.63	(−3.04, 1.78)	0.61	0.65	−0.29	(−2.53, 1.96)	0.80	0.82
Haplotype 8	GGGCTTAC	0 copies	75.0												
		1 + 2 copies	25.0	0.47	(−0.30, 1.24)	0.23	0.29	−1.05	(−2.76, 0.66)	0.23	0.38	0.58	(−0.76, 1.91)	0.40	0.66
Haplotype 9	GGGCCTAT	1 + 2 copies	16.6												
		0 copies	83.4	0.27	(−0.59, 1.14)	0.54	0.54	−0.47	(−2.50, 1.56)	0.65	0.65	0.20	(−1.54, 1.94)	0.82	0.82
Haplotype 12	GGCTTCAC	1 + 2 copies	17.9												
		0 copies	82.1	1.54	(0.72, 2.36)	**<0.01**	**<0.01**	−3.77	(−5.59, −1.95)	**<0.01**	**<0.01**	2.23	(0.82, 3.64)	**<0.01**	**<0.01**

Note: 95%CI, 95 % confidence intervals; %DMA, percentage of dimethylarsinic acid; %MMA, percentage of methylarsonic acid; %iAs, percentage of inorganic As. Ref, reference category; Freq: frequency.
The percentages of each metabolite were calculated: levels of calibrated metabolite/(calibrated DMA + calibrated MMA + calibrated iAs.
All models were adjusted for creatinine, study area, pre-pregnancy body mass index, tobacco consumption at first trimester of gestation, gestational age at sampling, estimated folate intake and ∑As (sum of urinary MMA, DMA and iAs). Values in bold indicate p-values <0.05.
AS3MT haplotypes listed in the following order: rs7085104, rs3740400, rs3740393, rs3740390, rs11191439, rs11191453, rs10748835, rs1046778.
[a] Calibrated percentages were calculated with As metabolite concentrations corrected by arsenobetaine and creatinine concentrations.
[b] Number of copies of each haplotype associated with lower %MMA are denoted first.
[c] p value from ANOVA F-test.
[d] Corrected p-value for FDR of 5 %.

a Spanish area with low iAs levels in the drinking water and high rice and seafood consumption. Homozygotes for ancestral alleles in rs3740393, rs3740390, rs11191453, rs11191454, and rs10748835 in *AS3MT* were associated with a lower methylation efficiency in early pregnancy (denoted by higher %MMA and lower %DMA). Interestingly, the G allele in rs202676, an SNP in the OCM-related gene *FOLH1,* also associated with higher %MMA. Unexpectedly, no associations were found for any other SNP studied in genes previously reported to be related to As metabolism (*N6AMT1*, *GSTP1*, *GSTO2*) or OCM (*MTR*, *MTHFR*, *CBS*, *FTCD*).

Several studies analysed the influence of *AS3MT* polymorphisms during pregnancy on As methylation efficiency (Table 4). In agreement with our results, in a study carried out in Mexico (median urinary ∑As: 23.3 μg/L at delivery), women carrying ancestral alleles in rs10748835 (G carriers) and rs3740390 (C carriers) presented a lower methylation efficiency (denoted by higher urinary %MMA and lower %DMA) (Drobná et al., 2016). In the same way, Gao et al. (2019b) found an association between *AS3MT* rs3740393 and lower %DMA at mid-late pregnancy in Bangladeshi G-allele-carrier women (median urinary total As = 79.9 and 92.2 mg/g creatinine at early and mid-late pregnancy, respectively). However, contrary to our results, no relationship was found between rs10748835 genotypes and maternal As methylation efficiency. In another Bangladeshi study (median urinary ∑As = 100 μg/L), homozygotes for the ancestral *AS3MT* alleles rs3740393, rs3740390, rs11191439, and rs11191453 were associated with higher %MMA and lower %DMA (denoting lower methylation efficiency) during early pregnancy, and rs10748835 G carriers presented higher %MMA, in consonance with our results (Engström et al., 2011). Finally, in a study carried out in a low-As-exposed population (pregnant and non-pregnant women) from Croatia and Slovenia (geometric mean urinary ∑As: 3.23 μg/mL), no associations were observed between *AS3MT* rs7085104, rs3740400, rs3740393, rs3740390, rs11191439, rs10748835, rs1046778, and rs7897654 and As methylation efficiency among women in the third trimester of pregnancy (Stajnko et al., 2019). Nevertheless, in the same study, non-pregnant women carrying ancestral alleles in rs3740393 and rs10748835 showed higher %MMA, in agreement with our results.

The influence of *AS3MT* on As metabolism appears to be well-established, however, there are discrepancies between studies. Multiple factors might account for these heterogeneous results from different studies. Differences in sample size, As internal dose, and allele frequencies of each SNP across populations are some of the factors that may explain these differences. Also, the strong linkage disequilibrium between alleles in the polymorphisms analysed challenges the interpretation of the individual effect of each SNP. Additionally, As methylation has been reported to increase during pregnancy (Gardner et al., 2011; Hopenhayn et al., 2003), and differences in weeks of gestation across the studied populations could also explain the inconsistency in the results between studies.

Pregnancy itself has been suggested to have a stronger impact on As metabolism than the *AS3MT* genotype. The study of Gardner et al. (2012) conducted on pregnant Bangladeshi women, observed that the absolute change in As metabolites (measured as percentages) attributable to gestational age (between 8 and 30 weeks of gestation) was greater than the influence of the genetic background. The reason for this was suggested to be OCM upregulation during pregnancy, due to the high demand of methyl groups to support foetal and placental development (Kalhan, 2016). Thus, it is possible that genetic influences could be more clearly observed in early stages of pregnancy and remain constant throughout gestation as genetic variants themselves remain unchanged during pregnancy. However, As-related epigenetic changes have been observed during pregnancy. Experimental and epidemiological studies have reported both DNA hyper- and hypomethylation, explained by competition for the methyl-donor SAM (Kile et al., 2012; Liu et al., 2020; Martinez and Lam, 2021). Nevertheless, the results are still inconclusive.

Table 4
Studies of genetic influences on As metabolism during pregnancy.

Study	Localization	n	Gestational age	Urinary As concentrations	SNPs related to As metabolism during pregnancy
Present study	Spain	541	1st trim	∑As:7.05 (2.76, 24.75) μg/g creatinine[a]	*AS3MT* rs7085104 (AA), rs3740400 (TT), and rs10748835 ↑%MMA *AS3MT* rs3740394 (G carriers) ↓%MMA *AS3MT* rs3740393 (GG), rs11191454 (AA), rs10748835 (GG): ↑%MMA%, ↓%DMA. *AS3MT* rs3740390 (CC) and rs11191453 (TT), rs11191453 (TT), and rs11191454 (AA): ↑%MMA%, ↓%DMA, ↑%iAs%. *FOLH1* rs202676 (G carriers) ↑%MMA%.
Stajnko et al., 2019	Croatia-Slovenia	136	3rd trim	∑As: 3.23 (2.84–3.68) μg/L[b]	*AS3MT*: rs7085104, rs3740400, rs3740393, rs3740390, rs11191439, rs10748835, rs1046778, and rs7897654, ns association *MTHFR*: rs1801131, G carriers: ↓ %As^{III}, and ↓%DMA (significant in bivariate analysis, non in adjusted models).
Gao et al., 2019a, 2019b	Bangladesh	1613 (1st trim) 1228 (3rd trim)	1st trim and 3rd trim	TAs: 1st Trim: 79.9 (43.2–185.1) mg/ g creat[c] 3rd trim: 92.2 (52.2–202.2) mg/ g creat[c]	*AS3MT*: rs9527 T alelle ↓%DMA (adjusted by As water exposure, in early gestation). *AS3MT*, rs1046778 (C allele) ↑ %DMA (adjusted by As water exposure, in early gestation). *AS3MT*, rs3740393 (C allele) and rs1046778 (C allele) ↑ %DMA (adjusted by As water exposure, in mid-late gestation). *AS3MT*: rs11191439, rs10748835, rs3740400: ns relationship between any SNP and DMA% in early nor mild-late gestation (*p*-values under multiple testing adjustment, FDR). *GSTO2*: rs156697: Ns relationship between any SNP and DMA% in early nor mild-late gestation (p-values under multiple testing adjustment, FDR) Ns interaction SNP*As exposure (low/high) early nor mild-late gestation (p-values under multiple testing adjustment, FDR).
Drobná et al., 2016	Mexico	200	Delivery	∑As: 23.3 (4.3–319.7) μg/L[d] iAs: 1.3 (<LOD-23.0) μg/L[d] MMA: 1.4 (0.12–18.2) μg/L[d] DMA: 20.6 (1.4–292.5) μg/L[d]	*AS3MT*: rs7085104 (G carriers); rs3740400 (G carriers); rs3740393 (C carriers); rs3740390 (T carriers); rs1046778 (C carriers): ↓MMA, and ↑DMA, ↓%MMA, and ↑%DMA, only in women pregnant with a male. *AS3MT*: rs10748835 (A carriers): ↓MMA, ↓%MMA, and ↑%DMA, only in women pregnant with a male. *AS3MT*: rs11191439 (C carriers): ↑ MMA, ↓ DMA and, ↑%MMA, only in women pregnant with a male. *AS3MT*: rs10748835 (A carriers): ↓MMA, and ↓%MMA, only in women pregnant with a male.
Engström et al., 2011	Bangladesh	361	Early pregnancy	∑As: 100 (21–390) μg/L[e]	*AS3MT*: rs3740400 (C carriers): ↓%iAs, ↓%MMA. *AS3MT*: rs3740393 (C carriers); *AS3MT*: rs3740390 (A carriers): ↓%iAs, ↓%MMA, and ↑%DMA. *AS3MT* rs10748835 (G carriers): ↑ %MMA. *AS3MT*: rs11191439 (MetThr carriers): ↑%iAs, ↑%MMA, and ↓%DMA.
Engström et al., 2007	Argentinean Andes	33		∑As: 233 (111–401)[d]	*MTHFR*: rs1801133 (Ala222Val): variant allele carriers (one or two copies)↑ %iAs, ↓ %DMA ↓%MMA. *MTR*: rs1805087 (Asp919Gly) heterozygotes ↓%MMA.

Note: trim: trimester of pregnancy; ns: no significant, FDR: False discovery rate; creat: creatinine; *AS3MT*: *arsenic (+3 oxidation state) methyltransferase*; *GSTO2*: *glutathione S-transferase omega-2*; *N6AMT1*: *N-6-adenine-specific DNA methyltransferase 1*; *MTRF*: *methylenetetrahydrofolate reductase*; *FOLH1*: *folate hydrolase 1*; *FTCD*: *formimidoyltransferase-Cyclodeaminase*; DMA, dimethylarsinic acid; MMA, monomethylarsonic acid; iAs, inorganic As.

[a] Median (5–95 percentile).
[b] Geometric mean (CI%95).
[c] Median (Interquartil Range).
[d] Median (range).
[e] Median (10–90 percentile).

In our study, we built a haplotype using the most relevant *AS3MT* SNPs (rs7085104, rs3740400, rs3740393, rs3740390, rs11191439, rs11191453, rs10748835, and rs1046778), finding that pregnant participants carrying zero copies (vs. two copies) of haplotype GGCTTCAC (18 % with at least one copy) had lower As methylation efficiency, denoted by higher $\%MMA_{cal}$ and $\%iAs_{cal}$, and lower $\%DMA_{cal}$. Other epidemiological studies have analysed the influence of these same *AS3MT* haplotypes on As methylation efficiency in different populations. In Andean women from Northern Argentina, a population exposed to very high As levels over several generations, the haplotype GCCATCAC (90 % with at least one copy) associated with higher %DMA and lower %MMA and %iAs (Engström et al., 2011). Nevertheless, in the same study, the same haplotype (GCCATCAC) in pregnant Bangladeshi women, (29 % carriers of at least one copy) was associated with a higher %DMA and lower %iAs but not with %MMA. The authors also evaluated the influence of the haplotype sequence AAGGTTGT on As methylation efficiency. They observed an association with higher %MMA and %iAs and lower %DMA in Argentinian women, and with higher %MMA in Bangladeshi women. In our study population, this haplotype was not present. In a case-control study carried out in Chile, the haplotype GCCATC inferred from six *AS3MT* SNPs (rs7085104, rs3740400, rs3740393, rs3740390, rs11191439, rs1046778) was associated with lower %MMA (de la Rosa et al., 2017). A similar association was found for haplotype GGCTAC (33 % with at least one copy) inferred from the *AS3MT* SNPs rs7085104, rs3740400, rs3740393, rs3740390, rs10748835, and rs1046778, in a population of non-pregnant women exposed to low As concentrations. In this study, pregnant participants with zero copies of the haplotype presented higher %MMA and lower % DMA (Stajnko et al., 2019).

Results from all these studies, and others conducted across other populations (Chen et al., 2017; Drobná et al., 2016; Harari et al., 2013), highlight the strong influence of *AS3MT* on individual As methylation efficiency, even in populations that are not exposed to high As concentrations. The protein encoded by this gene, the methyltransferase AS3MT, is the main enzyme catalysing the transfer of a methyl group from SAM to iAs^{III} and MA^{III} (BRENDA The Comprehensive Enzyme Information System, 2022). It has been suggested that genetic variants in the 10q24.32 region, which contains the *AS3MT* gene, decrease the expression and activity of the AS3MT enzyme, causing lower As methylation efficiency and higher As retention in tissues (Chernoff et al., 2020). Conversely, other study found that C allele in rs3740400 associated with higher methylation efficiency, was inversely associated with *AS3MT* expression (Engström et al., 2011). More research is needed to elucidate this relation and the specific AS3MT mechanism.

Interestingly, we also observed a significant association between *FOLH1* rs202676 and As metabolism. Specifically, carriers of the variant G allele had higher %MMA. *FOLH1* encodes the glutamate carboxypeptidase 2 protein, an enzyme required for folate uptake in the intestine. Several studies observed an influence of folate status on As metabolism, reporting an inverse association between plasma folate and %iAs (Abuawad et al., 2021). A polymorphism in this gene (1561C > T) has been associated with low blood folate levels and hyperhomocysteinemia (Devos et al., 2009). As far as we know, only one study has evaluated the relationship between this polymorphism and As methylation efficiency. In that study, carried out in 1027 healthy Mexican women (median urinary TAs: 20.2 μg/g creatinine), the homozygous *FOLH1* rs202676 variant allele (in this study CC) had higher %DMA, and lower %MMA and %iAs than T-allele carriers, however, only the latter was statistically significant (Gamboa-Loira et al., 2018).

The present study has some limitations: 1) the participants included in the present study were slightly different compared with the non-included women, i.e. among the included participants there was a slightly lower percentage of participants from the high socioeconomic classes (I + II and III), participants had a lower educational level and a higher rice consumption compared with non-participants. Although statistically significant, for most of these factors, differences were, however, small (Table S1). For example, among the included women, weekly rice consumption was only 30 g higher compared with non-included women. It is important to point out that other factors that may affect arsenic metabolism (e.g. BMI, parity, age and fish consumption) did not differed among the included and non-included participants. Although we still believe our study population could not be representative for the whole study cohort, our results provide valuable insight on the influence of genetic polymorphisms on the arsenic methylation efficiency among low-exposed Spanish women. To some extent, our results on the associations with some of the *AS3MT* SNPs are in agreement with other studies, which also give validity to our results. Moreover, the size of our study is considerable in comparison with other birth cohort studies investigating this research question; 2) we measured As methylation efficiency only once during pregnancy and we, therefore, cannot evaluate the influence of genetics on As metabolism across pregnancy. It has been suggested that As methylation efficiency increases in the final stages of pregnancy (Gao et al., 2019a; Gardner et al., 2011), thus, it would have been valuable to also measure As efficiency at a later stage of pregnancy; 3) The present study contains information about folate intake based on questionnaires; however, the use of plasma folate concentrations would have been more useful to determine whether the *FOLH1* SNP found to be associated in our study (rs202676) also associated with lower folate levels. On the other hand, a noteworthy strength of our study is the considerable sample size in comparison with other studies evaluating the influence of maternal polymorphisms on As methylation efficiency. Furthermore, we analysed several As metabolites (both organic and inorganic) and used calibrated As percentages to minimize the influence of organic As species, like AB, and more precisely evaluate As methylation efficiency in this low–level iAs-exposed population with high seafood intake.

5. Conclusions

In our study, SNPs in *AS3MT* and *FOLH1* were associated with lower As methylation efficiency in pregnant Spanish participants, whereas we found no impact on As metabolism for SNPs in other genes forming part of one-carbon metabolism. Altogether, our results support the hypothesis that As metabolism is multigenic, even in populations with low environmental exposure to As through drinking water and food. This study not only provides basic knowledge about gene-environment interactions, a key element for determining susceptibility, but also explains why some individuals have lower iAs methylation efficiency and, thereby, probably a higher susceptibility to As-related health effects.

The present study is one of the few to analyse genetic influences on As methylation during pregnancy, considering variants in genes both related to As and OCM metabolism. In addition, multiple significant factors have been adjusted to obtain more accurate results. Nevertheless, As metabolism is a complex process that is not yet fully understood; further epidemiological studies are necessary to understand the possible impact of genetics on both As metabolism and vulnerability to arsenic-related health effects. Taken together, all this information can be useful to propose new public-health strategies.

Ethics approval and consent to participate

The study protocol was approved by the Ethics Committee of the University Hospital La Fe (Valencia), the Ethics Committee of the Public Health Research Centre in Valencia (CSISP), and the Ethics Committee of Donostia Hospital (Gipuzkoa). All participants were properly informed about the study protocol and objectives and provided informed consent. The study has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. The study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline for cohort studies.

Consent for publication

We confirm that the manuscript has been read and approved by all named authors, who agreed that the research was ready for submission to a journal.

CRediT authorship contribution statement

RSB: study conceptualization and methodology, formal analysis, methodology, writing - original draft preparation, writing - review and editing; **FH**: study conceptualization and methodology, supervision, formal analysis, writing - original draft preparation, writing - review and editing; **GRM**: formal analysis; writing - review & editing; **MM**: formal analysis, data curation; writing - review and editing; **ML**: data curation; writing - review and editing; **AI**: writing - review and editing; **LSM**: funding acquisition, study conceptualization and methodology, Writing - review and editing; **MBZ**: writing - review and editing; **NFJ**: writing - review and editing; **SB**: writing - review and editing; **FB**: funding acquisition, study conceptualization and methodology, project administration; writing - original draft preparation, writing - review and editing; **SL**: funding acquisition, study conceptualization and methodology, Supervision, project administration; formal analysis, writing - original draft preparation, writing - review and editing.

Declaration of competing interest

The authors declare that they have no competing interests.

Data availability

The datasets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.165740.

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