AS3MT polymorphisms, arsenic metabolites and pregnancy

A. Stajniko^{1,2}, Z. Šlejkovec¹, D. Mazej¹, M. Horvat^{1,2} & I. Falnoga¹

¹Department of Environmental Sciences, Jožef Stefan Institute, Ljubljana, Slovenia

²Jožef Stefan International Postgraduate School, Ljubljana, Slovenia

ABSTRACT: Arsenic methylating enzyme (AS3MT) genetic variations and their role in variability of As metabolism were evaluated in population of pregnant/non-pregnant women exposed to low levels of As. We have tested the associations between genotypes/haplotype of seven *AS3MT* SNPs and As metabolites in urine (methylation efficiency). Significant associations of individual SNPs and protective haplotype with arsenic metabolites have been confirmed among non-pregnant women. On the contrary, these associations have been almost absent among pregnant women – most possibly a consequence of physiological changes in pregnancy, and/or impact of external DMA due to higher seafood intake. Both factors can influence As metabolism.

1 INTRODUCTION

Arsenic (III oxidation state) methyltransferase (AS3MT) is an enzyme generating methylated (MMA) and dimethylated (DMA) arsenicals and therefore it has important role in human arsenic metabolism. Its genetic variations can have important impact on individual and population variability of As metabolism. Various studies, mainly conducted in populations exposed to high levels of As in drinking water, point on beneficial *AS3MT* genetic variants (protective haplotype) resulting in more efficient As methylation (lower AsIII% and MMA% and higher DMA%)^{1,5}. However in recent study, Xu et al. (2016) emphasised that the extent of the influence of *AS3MT* variants on As metabolism may also depends on levels of exposure to As, or better – on levels of inorganic arsenic (iAs). Therefore, in present study the influence of 7 *AS3MT* variants and pregnancy status on As metabolite patterns (urine) was evaluated in population of women exposed to low levels of As.

2 METHODS/EXPERIMENTAL

2.1 Study population

Study was conducted on Croatian-Slovenian population divided in a group of pregnant (n = 136, average age of 30 ± 5; third trimester of pregnancy) and a group with non-pregnant women (n = 209; average age of 39 ± 4). A small subset of non-pregnant women (n = 33) was consisted of women previously participating in pregnant group; they were re-sampled a few years after pregnancy. All participants were recruited between 2006 and 2017 within prospective birth cohort PHIME and follow up survey CROME-LIFE+.

2.2 Analytical methods

Total levels of metalloids in blood (As), plasma (selenium) and in urine (As) were measured using ICP-MS method, while arsenic metabolites (MMA, DMA and AsIII) in urine were determined by HPLC-HG-AFS. The percentage of metabolites were determined based on their sum (MMA+DMA+AsIII, U-SAs). Methylation efficiency was evaluated by first methylation step (PMI, MMA/AsIII), second methylation step (SMI, DMA/MMA) and total methylation rate (TMI, ((DMA+MMA)/AsIII). DNA was isolated from peripheral blood and genotyped for 7 *AS3MT* (rs7085104, rs3740400, rs3740393, rs3740390, rs11191439, rs10748835, rs1046778) and one AQP9 (rs2414539) by pre-designed TaqMan SNP Genotyping assays and qPCR method.

2.3 Statistical methods

The differences between groups and associations of genotypes/haplotypes with arsenic metabolites were tested by non-parametric Kruskal-Wallis test. Associations were further adjusted for age, weight, height, BMI, gestational age, parity, seafood intake, U-As, P-Se, smoking and AQP9 SNP in multivariable linear regression models. Statistical analysis was performed by STATA12/SE software and construction of haplotypes with Haploview 4.2.

3 RESULTS AND DISCUSSION

3.1 As exposure and urine metabolites

Arsenic exposure in studied population was low; geometric mean (GM) of total As was below 2.6 ng/g for blood and below 23 ng/ml for urine. Analysis of As urine metabolites and methylation efficiency

Table 1. As metabolites in urine and methylation efficiency.

	Pregnant women	Non-pregnant women	
N	136	33*	176
AsIII%	3.83 (0.13–71.4)	4.58 (0.63–24.0)	5.78 (0.31–29.6)
MMA%	5.13 (0.26–98.0)	9.94 (0.62–28.6)	10.9 (0.74–40.0)
DMA%	82.7 (1.34–99.6)	80.1 (57.1–98.1)	77.1 (40.0–98.9)
PMI	1.34 (0.01–146)	2.17 (0.33–6)	2.08 (0.16–19.6)
SMI	16.1 (0.01–384)	8.05 (2.0–158)	8.58 (1.0–98.7)
TMI	24.1 (0.04–770)	20.4 (3.17–156)	15.9 (2.38–325)

B-blood, U-urine, U-SAs (MMA+DMA+AsIII); data presented as GM (range), * women from pregnant group re-sampled during follow up study.

(Table 1) showed significantly higher DMA% (and SMI) accompanied with lower MMA% (and PMI) for pregnant group in comparison with non-pregnant women. Results are consistent with previous studies and indicate more efficient and changed As methylation in pregnancy, what could be partially explained by hormonal changes (estrogen up-regulates other methylation pathways) and dietary supplementation (folic acid increases urine DMA%)³, TMI was higher in pregnant women, but the difference was lower when compared to re-sampled non-pregnant women. Explanation could be possible higher (DMA containing)-seafood intake in re-sampled subgroup⁵.

3.2 *AS3MT* genotypes/haplotypes and As metabolites

After adjustment, the significant associations of individual *AS3MT* SNPs with As metabolites were found for 4 SNPs (rs7085104, rs3740400, rs11191439 and rs10748835) among pregnant women and for 6 out of 7 SNPs among non-pregnant women (exception was rs11191439; re-sampled women were excluded due to missing data). Interestingly, when associations were estimated among participants with blood-As level above median (≥ 2.7 ng/g and ≥ 0.53 ng/ml for pregnant and non-pregnant women, respectively) the associations became stronger for non-pregnant group. On other hand, three associations were lost for pregnant group; only rs11191439 stayed associated with As metabolites. Obtained results are generally consistent with literature data where protective alleles (6 minor alleles, and one common – rs11191439) were found associated with more efficient methylation (lower AsIII% and MMA% and higher DMA%, SMI and TMI).

Metabolites were also marginally affected by AQP9 SNP.

Due to high LD (linkage disequilibrium) for SNPs, we constructed *AS3MT* haplotypes. Calculated frequency of protective haplotype was 9.5 % what is similar to the frequencies reported for European countries with comparable low iAs exposure (10–14%; Slovakia, Hungary and Romania)²; percentage was much lower from frequencies reported for populations adapted to historic exposure to high iAs levels in drinking water (50–70%; Argentina, Peru, Chile)^{1,4}.

Figure 1. MMA%, DMA% and SMI in non-pregnant women stratified by the presence of protective haplotype.

In present study a protective haplotype was significantly associated with lower MMA% and with higher DMA% and SMI in group of non-pregnant women (Figure 1), while among pregnant group no significant associations were observed.

4 CONCLUSIONS

Our results indicate that pregnancy, due to physiological changes, and DMA containing seafood may had significant influence on As metabolites and therefore, blur the associations with genetic variants at such low level of exposure. On the other hand, among non-pregnant women – with more stable physiology, significant associations with As metabolites were found for separate SNPs as well as for protective haplotype.

ACKNOWLEDGEMENTS

Study was supported by Slovenian Research Agency (P1 043), and PHIME, CROME-LIFE+, HEALS projects.

REFERENCES

- Apata M. et al., 2017, Human adaptation to arsenic in Andean populations of the Atacama Desert, *Am J Phys Antropol*, 163:192–199.
- Engström K. et al., 2015, Genetic Variation in Arsenic (+3 Oxidation State) Methyltransferase (*AS3MT*), ArsenicMetabolism and Risk of Basal Cell Carcinoma in a European Population, *Environ Mol Mutagenesis* 56:60–69.
- Li et al., 2008, Nutritional status has marginal influence on the metabolism of inorganic arsenic in pregnant Bangladeshi Women. *Environ Health Perspect*, 116(3):315–321.
- Schleibush M.C. et al., 2013, Possible positive selection for an arsenic-protective haplotype in humans, *Environ Health Perspect*, 121(1):53–58.
- Taylor et al., 2017, Human exposure to organic arsenic species from seafood, *Science of the total Environ*, 580: 266–282.
- Xu et al., 2016, Associations between variants in Arsenic (+3 Oxidation State) Methyltransferase (*AS3MT*) and urinary metabolites of Inorganic Arsenic: Role of Exposure level. *Tox Sci*: 153(1):112–123.