

Blood and urinary arsenic levels are associated with methylation of promoters of genes involved in molecular processes related to arsenic toxicity.

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Background:

World Health Organization lists arsenic (As) as human toxicant and group one carcinogen. Chronic exposure to As increases the risk of numerous health conditions, including skin lesions, impaired intellectual function, cardiovascular disease, diabetes, inflammation, and cancers including bladder, lung, kidney, liver, skin, and possibly prostate (1, 2). Previous studies have shown that As exposure is associated with changes of both global DNA methylation levels and gene specific methylation. Nevertheless, the molecular mechanism of As exposure related dysregulation of the epigenome are still to be elucidated.

Methods:

Our study included 56 cancer free women from West Pomerania region of Poland with total arsenic concentrations in blood in the range of 0.22-5.77 $\mu\text{g/L}$ and 34 women diagnosed with breast cancer and blood As levels 0.65-4.31 $\mu\text{g/L}$. The As was measured with ICP-MS (Elan DRC-e, PerkinElmer, USA) and DNA was extracted from diagnostic blood samples using the salting-out method. Genome-wide DNA methylation profiling was performed using the Infinium MethylationEPIC array (Illumina). Raw data were processed with the ChAMP R package. Methylation values were logit-transformed to M-values for robust linear association analysis (limma R package), adjusted for age and cell fraction proportions (calculated based on methylation data). Statistical significance was determined with FDR-corrected p-values, with a cutoff set at $10\text{e-}8$ and 0.05. The physiological significance of the identified methylation changes was approximated using annotations of regions marked with histones modifications in 11 cell types from 15 state core model. We also conducted GSEA using the FUMA platform and "GO biological processes" and "Hallmark gene sets" databases. We validated our results performing identical analyses on methylation profiling data from 38 cord blood samples for which maternal urinary arsenic was measured and was in the range 6.18-319.74 $\mu\text{g/L}$.

Results:

Our initial analysis identified 2,453 CpG sites associated (1,794 DMPs positively and 659 negatively) with blood arsenic levels (0.22-5.77 µg/L). The identified CpGs very specifically mapped to the genomic regions marked by histones occupying transcription start sites and gene bodies of actively transcribed genes. The gene ontology terms analyses linked these methylation changes to the physiological processes that have previously been shown to be affected by As exposure such as: cell cycle, mitosis, G2M checkpoint, chromatin organization, or mitotic spindle. Identical analyses performed for the cohort of women diagnosed with cancer and cord blood samples linked CpG sites associated with As levels in these cohorts to the same genomic regions and similar molecular processes.

Conclusions:

Our study performed in three cohorts of individuals exposed to arsenic, uniformly showed that arsenic exposure induces methylation changes specifically at promoters and bodies of actively transcribed genes involved in key molecular processes previously associated with As toxicity. The presence of those changes in blood cells of women exposed to As who developed cancer as well as cord blood, not only validates our results but also suggests that those changes may contribute to the increased risk of cancer observed in exposed individuals.

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References:

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