Science of the Total Environment 787 (2021) 147621

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Gestational blood levels of toxic metal and essential element mixtures and associations with global DNA methylation in pregnant women and their infants

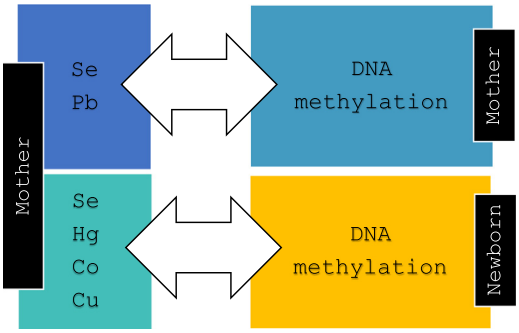
Kjell Vegard F. Weyde [a,*], Ann-Karin Olsen [a], Nur Duale [a], Jorke H. Kamstra [b], Thea S. Skogheim [a], Ida H. Caspersen [a], Stephanie M. Engel [c], Guido Biele [a,d], Yankai Xia [e,f], Helle M. Meltzer [a], Heidi Aase [a], Gro D. Villanger [a]

[a] Norwegian Institute of Public Health, Oslo, Norway
[b] Institute for Risk Assessment Sciences, Department of Population Health Sciences, Faculty of Veterinary Medicine, Utrecht University, the Netherlands
[c] Gillings School of Global Public Health, University of North Carolina at Chapel Hill, Chapel Hill, USA
[d] Department of Psychology, University of Oslo, Oslo, Norway
[e] State Key Laboratory of Reproductive Medicine, Center for Global Health, School of Public Health, Nanjing Medical University, Nanjing, China
[f] Key Laboratory of Modern Toxicology of Ministry of Education, School of Public Health, Nanjing Medical University, Nanjing, China

HIGHLIGHTS

- One of the largest studies investigating associations between metals/essential elements and global DNA methylation in humans
- 12 metals/elements included, both individually, as mixtures, and as two-way interactions.
- Mothers: possible association with DNA methylation for Se (positive) and Pb (non-linear)
- Newborns: possible associations with DNA methylation for Se (positive), Hg (negative), and Co and Cu (non-linear)
- Several possible two-way interactions for mothers and newborns, between exposures, and between exposures and covariates

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 7 January 2021
Received in revised form 24 March 2021
Accepted 3 May 2021
Available online 8 May 2021

Editor: Thomas Kevin V

ABSTRACT

Background: Pregnant women and their fetuses are exposed to multiple toxic metals that together with variations in essential element levels may alter epigenetic regulation, such as DNA methylation.
Objectives: The aim of the study was to investigate the associations between gestational levels of toxic metals and essential elements and mixtures thereof, with global DNA methylation levels in pregnant women and their newborn children.
Methods: Using 631 mother-child pairs from a prospective birth cohort (The Norwegian Mother, Father and Child Cohort Study), we measured maternal blood concentration (gestation week ~18) of five toxic metals and seven essential elements. We investigated associations as individual exposures and two-way interactions, using elastic net regression, and total mixture, using quantile g-computation, with blood levels of 5-methylcytocine (5mC)

* Corresponding author.
E-mail address: kjwe@fhi.no (K.V.F. Weyde).

https://doi.org/10.1016/j.scitotenv.2021.147621

Keywords:
Toxic metals
Essential elements
DNA methylation
Neonates
Pregnant women
The Norwegian Mother, Father and Child Cohort study (MoBa)

and 5-hydroxymethylcytosine (5hmC) in mothers during pregnancy and their newborn children (cord blood). Multiple testing was adjusted for using the Benjamini and Hochberg false discovery rate (FDR) approach.

Results: The most sensitive marker of DNA methylation appeared to be 5mC levels. In pregnant mothers, elastic net regression indicated associations between 5mC and selenium and lead (non-linear), while in newborns results indicated relationships between maternal selenium, cobalt (non-linear) and mercury and 5mC, as well as copper (non-linear) and 5hmC levels. Several possible two-way interactions were identified (e.g. arsenic and mercury, and selenium and maternal smoking in newborns). None of these findings met the FDR threshold for multiple testing. No net effect was observed in the joint (mixture) exposure-approach using quantile g-computation.

Conclusion: We identified few associations between gestational levels of several toxic metals and essential elements and global DNA methylation in pregnant mothers and their newborn children. As DNA methylation dysregulation might be a key mechanism in disease development and thus of high importance for public health, our results should be considered as important candidates to investigate in future studies.

1. Background

Environmental factors (e.g. nutrition, stress, and toxicants) during early development can influence the epigenome (Godfrey et al., 2007; Jirtle and Skinner, 2007; Skinner et al., 2011), and these gene-environment interactions are linked to many human diseases in childhood or later life-stages (Latham et al., 2012). In utero exposure through trans-placental transport of toxic metals or inadequate/excess supply of essential elements is related to several negative health effects in the child, such as birth defects, low birth weight, cancer, allergy, osteoporosis, kidney damage, neurodevelopmental disorders and cognitive impairments (Needham et al., 2011; Gorini et al., 2014; Jaishankar et al., 2014; Sun et al., 2014; Bennett et al., 2016). Also in adult populations, toxic metals are associated with a wide range of adverse health outcomes (Tchounwou et al., 2012; Jaishankar et al., 2014). The mechanistic underpinnings are largely unknown, especially when it comes to low level chronic exposures typical of the general population. Epigenetic dysregulation has been proposed as an important mechanism explaining these associations between environmental metals exposures and health outcomes, with changes in gene expression in utero that may persist into adulthood, or even across generations (Jirtle and Skinner, 2007; Bollati and Baccarelli, 2010; Perera and Herbstman, 2011a; Skinner et al., 2011; Cheng et al., 2012; Skaar et al., 2016; Tran and Miyake, 2017; Alvarado-Cruz et al., 2018; Martin and Fry, 2018).

DNA methylation is a key epigenetic event in which methyl groups are added at the 5'position in cytosines of CpG dinucleotides (Zeng and Chen, 2019). Methylation of cytosine (5mC) is associated with altered transcription (by regulating the transcriptional cascade), and is an important mechanism in shaping a healthy phenotype (Greenberg and Bourc'his, 2019). DNA methylation is among the most studied epigenetic markers with an essential role in cell function and maintenance of genomic stability (Godfrey et al., 2007; Latham et al., 2012; Greenberg and Bourc'his, 2019). DNA methylation is of vital importance during development with its role in regulation of gene expression, cellular differentiation, chromosome stabilization, genomic imprinting, and suppression of transposable element mobility (Gibney and Nolan, 2010; Jones, 2012; Smith and Meissner, 2013). Perinatal life is a critical time window when DNA methylation patterns are established, and a window that may confer susceptibility to disturbance from environmental factors (Jirtle and Skinner, 2007; Latham et al., 2012). During recent years it has become apparent that not only 5mC, but also the oxidized version of 5mC; the 5-hydroxymethylcytosine (5hmC), has an important role as an intermediate form in demethylation cycles (Szyf, 2016) and in the regulation of gene expression in particular in brain regions (Colquitt et al., 2013; Spiers et al., 2017). Recently it has been suggested that 5hmC is a stable epigenetic mark with an important role in DNA repair and maintaining genomic integrity (Kantidze and Razin, 2017). Both 5mC and 5hmC modifications are recognized as key players in the pathogenesis of complex disorders (Egger et al., 2004, Robertson, 2005, Dao et al., 2014). Studies have shown the importance of DNA methylation homeostasis, possibly linking various environmental exposures to human health, such as child psychopathology or breast cancer in adult women (Perera and Herbstman, 2011b; Banik et al., 2017; Alvarado-Cruz et al., 2018; Martin and Fry, 2018; Wielsøe et al., 2019). Thus, it is important to increase the knowledge about environmental factors capable of altering genomic (global) DNA methylation.

Toxic metals such as cadmium (Cd), lead (Pb), mercury (Hg), and the metalloid arsenic (As) are co-occurring, ubiquitous, and non-degradable contaminants in the environment that readily enter the food chain. Drinking water and food are the main sources of toxic metals in human populations, including fish and seafood, game meat, cereals, vegetables, and rice (Birgisdottir et al., 2013; Alexander and Oskarsson, 2019). Smoking and outdoor air pollution from traffic and combustion are also sources of some toxic metals (Tchounwou et al., 2012). The exposure levels may vary across geographical locations; due to differences in natural occurring sources and/or environmental pollution from human activities (Järup, 2003; Tchounwou et al., 2012). Toxic metals can bioaccumulate in specific tissues and increase in concentration with age (Tchounwou et al., 2012). During pregnancy, the accumulated metals may be remobilised and enter the bloodstream, and both concurrent and/or stored toxic metals expose the foetus through trans-placental transfer (Gulson et al., 2003; Reynolds et al., 2006; Chen et al., 2014). Blood levels of toxic metals in Norwegian pregnant women are generally low and comparable to those elsewhere in Europe (Caspersen et al., 2019), with the exception of total Hg and total As appearing slightly higher in Norwegian women (Haug et al., 2018).

In contrast to toxic or non-essential metals, essential elements, such as copper (Cu), cobalt (Co), molybdenum (Mo), selenium (Se), zinc (Zn), magnesium (Mg), and manganese (Mn), are important elements in human physiological and biochemical processes (Chasapis et al., 2012; Roman et al., 2014; Glasdam et al., 2016; Zoroddu et al., 2019). In addition to diet, multimineral supplements are important sources for some essential elements such as Se, Mn and Zn (Haugen et al., 2008; Caspersen et al., 2019). Essential elements generally have a narrow dose range of optimal function, and both excessive and insufficient intake may adversely affect health (Birgisdottir et al., 2013; Zoroddu et al., 2019). Pregnancy is characterized by elevated need for macro- and micronutrients in order to supply the developing foetus (King, 2000; Saunders et al., 2019), and this period presents a greater risk of insufficiencies of essential elements or other nutrients (Haugen et al., 2008; Saunders et al., 2019). Together with elevated blood concentrations of mobilized toxic metals in pregnancy, this may theoretically render pregnant women and their developing fetuses more susceptible for adverse health effects (Reynolds et al., 2006; Chen et al., 2014; Rahman et al., 2016).

Human studies report associations between exposures to toxic metals, such as As, Cd, Hg and Pb, and DNA methylation, in both children

and adults (Ruiz-Hernandez et al., 2015; Bommarito et al., 2017; Barker et al., 2018). Animal studies report associations between DNA methylation and Hg, As and other toxic metals (Rusiecki et al., 2007; Bollati and Baccarelli, 2010; Pilsner et al., 2010; Wright et al., 2010; Basu et al., 2013; Head, 2014; Cardenas et al., 2015a; Nilsen et al., 2016; Nakayama et al., 2019) as well as essential elements (Zhang et al., 2019). Underlying mechanisms may be linked to oxidative stress and altered gene expression in key pathways related to DNA repair and oxidative stress (Valko et al., 2005). To date, most studies on exposure to toxic metals and DNA methylation in human populations have included small samples and have been limited to a few metals (Bitto et al., 2014; Cardenas et al., 2015b; Cardenas et al., 2017b), and none have investigated these relationships in both pregnant women and their neonates.

The aim of the present study was to identify associations between maternal levels of toxic metals (As, Hg, Cd, Cs, and Pb) and essential elements (Se, Co, Cu, Mn, Mg, Zn, and Mo) measured mid-pregnancy, as single exposures and as mixtures, and global DNA methylation levels of both 5mC and 5hmC in maternal (measured in gestational week ~18 (mean = 18,5, SD = 1.3; Caspersen et al., 2019) and neonatal (cord) blood.

2. Methods

2.1. Study sample

2.1.1. The Norwegian Mother, Father and Child Cohort Study (MoBa)

MoBa is an on-going, prospective population-based pregnancy cohort study conducted by the Norwegian Institute of Public Health. Participants were recruited from all over Norway from 1999 to 2008. The women consented to participation in 41% of the pregnancies. The cohort now includes 114,500 children, 95,200 mothers and 75,200 fathers (Magnus et al., 2006; Magnus et al., 2016). Women were recruited prior to their first ultrasound appointment (around 18 weeks' gestation). Consenting women completed two general background and health questionnaires during pregnancy (17 and 30 weeks' gestation) and a food frequency questionnaire at 22 weeks' gestation (Magnus et al., 2016). Blood samples were taken from the mothers at around 18 week's gestation and blood was taken from children (umbilical cord) at birth (Paltiel et al., 2014; Magnus et al., 2016). MoBa is linked to the Medical Birth Registry of Norway (MBRN), providing information on pregnancy and birth records.

2.1.2. Study population

The present study utilizes data and biological samples of participants from a case-cohort study on attention-deficit/hyperactivity disorder (ADHD) nested within MoBa; the preschool ADHD sub-study. This sub-study oversampled on children at risk for ADHD based on data from the MoBa questionnaire that was administered to mothers at child age three years (Overgaard et al., 2018), and 1194 children born in 2003 and their mothers participated in this sub-study. Details of the ADHD sub-study have previously been published (Overgaard et al., 2018). Supplemental material provides a more detailed description of the study (Appendix 1).

For the present study, the preschool ADHD sub-study participants constituted a convenience sample. We selected mother-child pairs based on the following inclusion criteria; singleton birth of child, no congenital malformation or affected by Down's syndrome or Cerebral palsy, no high scores on ASD symptoms, available maternal whole blood and DNA (from blood) around pregnancy week 18, and available DNA from child at birth (from cord blood). Of these, 652 mother-child pairs met the inclusion criteria (Fig. 1). Due to lacking child DNA samples at retrieval or insufficient material to perform DNA methylation analyses, the final newborn sample comprised 631 children (Fig. 1).

The establishment and data collection in MoBa was previously based on a licence from the Norwegian Data Protection Agency and approval from The Regional Committee for Medical Research Ethics, and it is now based on regulations related to the Norwegian Health Registry Act. Parents enrolled in MoBa and the ADHD sub-study gave written consent for the use of this data. This study was approved by The Regional Committee for Medical Research Ethics (ref. nu. 2012/985-1).

2.2. Determination of toxic metals and essential elements in maternal blood

In this study, we used maternal blood samples from approximately week 18 (mean = 18.5, SD = 1.3; $N = 2982$; Caspersen et al., 2019) of gestation to measure metal and essential element concentrations. Details about the sampling procedure, handling and storage in the MoBa biobank is described in detail elsewhere (Paltiel et al., 2014). Twelve metals and essential elements were determined in maternal whole blood using inductively coupled plasma-sector field mass spectrometry (ICP-SFMS); Total As, Cd, Cs, Pb, Total Hg, and essential elements; Co, Cu, Mg, Mn, Mo, Se and Zn. We measured total Hg and As in maternal blood, which included both inorganic and organic forms. For most metals/elements the concentrations above limit of quantification (LOQ) are reported, except for As, Cd, Pb and Hg where concentrations above limit of detection (LOD) are reported. Metals/elements concentrations are given in μg/L, except for Mg, which is given in mg/L.

The main part of the analysis was conducted at ALS laboratory group of Norway, while a small proportion of the blood samples was analysed at the University of Lund as a part of another MoBa project. The Norwegian Institute of Public Health has a framework agreement with ALS and they have until now analysed ~2000 samples of maternal whole blood from MoBa. Internal quality control samples and procedure blanks were analysed along with each batch of samples to ensure high quality of the determinations throughout the project and across laboratories. The samples were randomized to batch. See more detailed information on analytical procedures, LODs, LOQs and quality control in Appendix 2.1 and Table S1.

2.3. Determination of global DNA methylation in maternal and child blood

DNA was isolated from EDTA-blood from cord blood and maternal whole blood collected at approximately week 18 weeks' gestation (Paltiel et al., 2014). After sample retrieval from the MoBa-repository, the integrity and concentration of each sample was assessed in 2–4 technical parallels using Nanodrop 1000 spectrometry (ND-1000, Thermo Scientific, Germany) prior to analyses of 5mC and 5hmC.

Determination of the whole genome (global) levels of 5mC and 5hmC in extracted DNA were performed by liquid chromatography-mass spectrometry/mass-spectrometry (LC-MS/MS) as previously described (Kamstra et al., 2017). Briefly, DNA was digested to nucleosides. Internal standards were added to samples, standard calibrators and controls (pooled blood sample DNA) to yield a final volume of 200 μL. A standard curve was made within the expected range of human blood (0–5% 5mC and 0–0.08% 5hmC, relative to G). A volume of 5 μL was injected on an Agilent 1200 μHPLC coupled with a triple quadruple (QQQ) MS (6490, Agilent). Conditions for LC/MS-MS analysis, calculation of samples concentrations based on the calibration curve, and quality control are described previously (Kamstra et al., 2017). See also supplemental material for more detailed information on the procedure (Appendix 2.2). Every run included a control sample consisting of pooled DNA from human peripheral blood, ensuring that inter-run accuracy was within acceptable limits. The resulting 5mC and 5hmC levels are given in percentage (%).

2.4. Covariates

The covariates were obtained from the prenatal MoBa questionnaires (Magnus et al., 2016) and the MBRN. We considered the following covariates: Maternal intake of folate, iodine, vitamin B12 and seafood during pregnancy, smoking during pregnancy, maternal

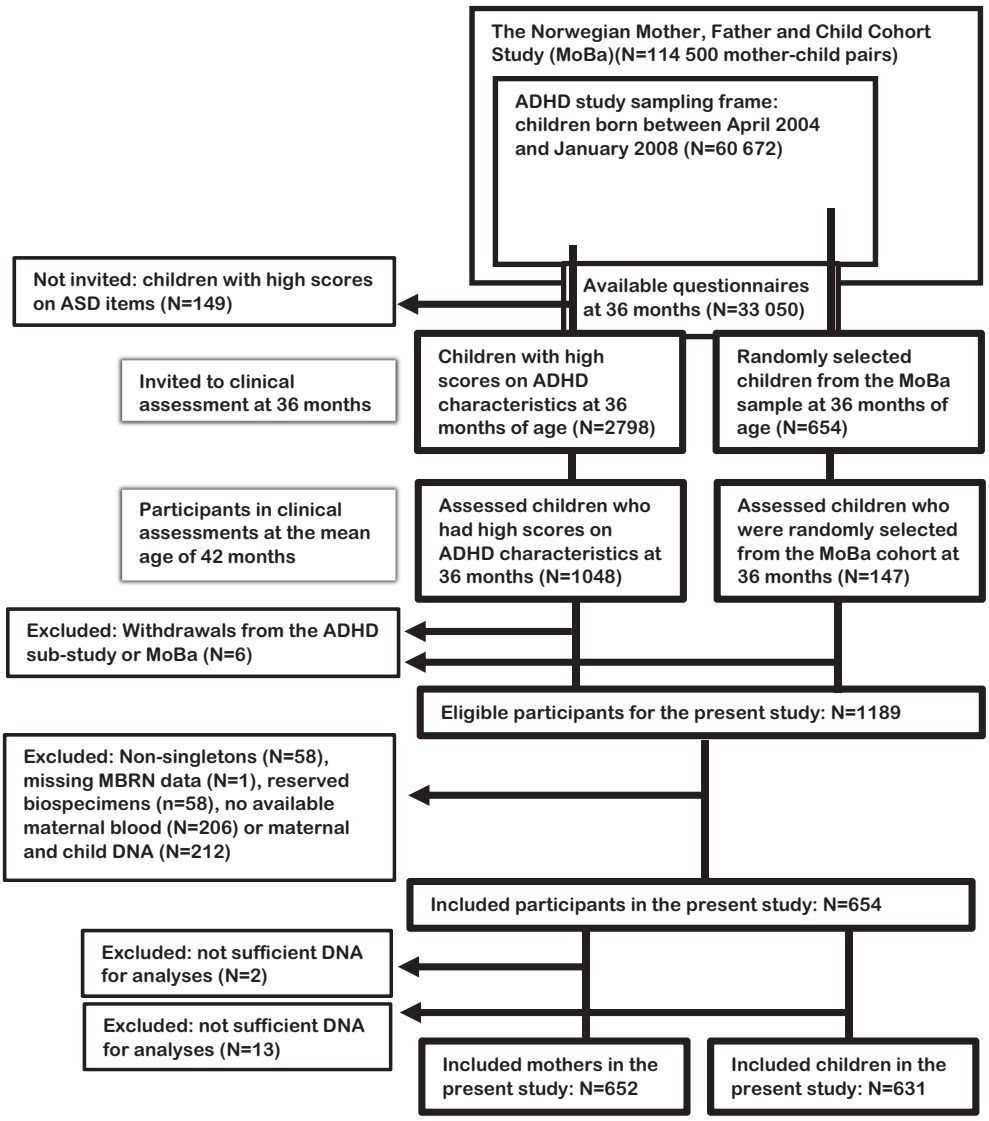


Fig. 1. Flow chart of the recruitment of MoBa-participants to the ADHD study and inclusion of mother-child pairs in the present study. Abbreviations: ADHD: Attention-deficit/hyperactivity disorder, ASD: Autism Spectrum Disorder, MoBa: The Norwegian Mother, Father and Child Cohort Study.

education, maternal age, parity and sex of the child. Maternal intake of vitamins and seafood was calculated based on a maternal semi-quantitative food frequency questionnaire (FFQ) given around gestation week 22. The FFQ include questions regarding habitual dietary intake since becoming pregnant (Brantsaeter et al., 2008; Meltzer et al., 2008) and has demonstrated good validity for estimates of intake of food and nutrients (Brantsaeter et al., 2008).

Based on literature-informed a priori directed acyclic graphs (DAGs; Greenland et al., 1999; Shrier and Platt, 2008; Textor et al., 2011) we identified a minimal adjustment set of covariates, including maternal age (continuous, years), maternal education (less than university vs university), parity (0 vs 1+), maternal smoking (no vs sometimes/daily), maternal seafood intake (continuous), and child sex (Fig. S1). As folate is an important methyl donor that could influence methylation status (Boeke et al., 2012; Tapp et al., 2013), we chose to include maternal folate intake (continuous, from diet and supplement combined) in the final adjustment set (Fig. S1).

2.5. Statistical analyses

The current analysis is based on version 9 of the MoBa quality assured data files. The data analyses were done in Stata 15.0 (StataCorp, 2017; descriptive tables and Welch two-sample test) and R version 4.0 (R Core Team, 2017). Only metals or essential elements with concentrations above LOQ (above LOD for Hg, As, Cd and Pb) in >80% of the samples were included in the present analyses (Table S1). All metals and essential elements were natural log transformed before analyses to approximate normal distribution.

We investigated correlations among the measured metals and essential elements in maternal blood, as well as the correlation between 5mC and 5hmC in the maternal and child samples using Spearman correlation. We also tested for mean difference of 5mC and 5hmC between girls and boys in the child sample using independent group *t*-tests (Welch two-sample test; significance set at $p < 0.05$).

Prior to further analyses, outliers were removed; defined as observations with both a Cook's d larger than four divided by the sample size (Bollen and Jackman, 1990) *and* a standardized residual absolute value above three. Missing exposure data and covariates were multiple imputed using the Amelia II package in R (Honaker et al., 2011) (m = 20; Johnson and Young, 2011). For missing As, Co, and Cd (below LOD/LOQ), lower (≈0) and upper limits (LOD/LOQ) were specified, so that imputed values would fall into these intervals. Missing Mg and Cs results from the University of Lund were imputed based on their log-normal distributions, that is, without bounds. Imputations were based

Table 1
Characteristics of the mother and newborn child populations.

Characteristics	Children				Mothers			
	%	%C_5mC [median (IQR)]	%C_5hmC [median (IQR)]	%C_5hmC/ C_5mC [median(IQR)]	%	%M_5mC [median (IQR)]	%M_5hmC [median (IQR)]	%M_5hmC/ M_5mC [median(IQR)]
		N = 631	N = 631	N = 631		N = 652	N = 652	N = 652
Overall	100	3.6686(0.1152)	0.0211(0.0040)	0.0057(0.0010)	100	3.7233(0.1874)	0.0166(0.0022)	0.0044(0.0005)
Sex								
Boy	52.3	3.6872(0.1060)	0.0212(0.0044)	0.0057(0.0011)	–	–	–	–
Girl	47.7	3.6455(0.1101)	0.0208(0.0036)	0.0057(0.0009)	–	–	–	–
Maternal age								
<28	24.2	3.6673(0.1245)	0.0211(0.0037)	0.0057(0.0009)	24.2	3.711(0.195)	0.0166(0.0021)	0.0045(0.0005)
28–33	43.3	3.6742(0.1021)	0.0209(0.0040)	0.0057(0.0010)	42.6	3.727(0.184)	0.0167(0.0025)	0.0045(0.0005)
≥ 33	32.5	3.6621(0.1195)	0.0212(0.0038)	0.0058(0.0009)	33.1	3.725(0.187)	0.0163(0.0020)	0.0044(0.0005)
Maternal education								
High school	23.5	3.6826(0.1371)	0.0211(0.0038)	0.0057(0.0010)	23.3	3.723(0.185)	0.0166(0.0022)	0.0045(0.0005)
High school+	74.6	3.6672(0.1058)	0.0211(0.0039)	0.0057(0.0010)	74.5	3.724(0.196)	0.0165(0.0022)	0.0044(0.0005)
Missing	1.9				2.1			
Parity								
0	89.2	3.6669(0.1139)	0.0209(0.0039)	0.0057(0.0010)	88.8	3.723(0.192)	0.0166(0.0022)	0.0045(0.0005)
1+	10.8	3.6866(0.1235)	0.0218(0.0043)	0.0059(0.0010)	11.2	3.723(0.193)	0.0163(0.0022)	0.0044(0.0005)
Smoking								
No/sometimes	92.2	3.6685(0.1077)	0.0210(0.0040)	0.0057(0.0010)	92.3	3.719(0.189)	0.0166(0.0021)	0.0045(0.0005)
Daily	7.8	3.6702(0.1515)	0.0215(0.0032)	0.0059(0.0008)	7.7	3.754(0.231)	0.0170(0.0023)	0.0045(0.0005)
Maternal folate intake								
<25th pctile	22.3	3.6731(0.0985)	0.0212(0.0036)	0.0058(0.0009)	22.5	3.734(0.176)	0.0166(0.0022)	0.0045(0.0005)
25-75th pctile	45.2	3.6687(0.1201)	0.0209(0.0039)	0.0057(0.0010)	45.1	3.725(0.189)	0.0167(0.0023)	0.0045(0.0005)
≥ 75th pctile	22.7	3.6623(0.1160)	0.0211(0.0040)	0.0057(0.0010)	22.5	3.717(0.211)	0.0164(0.0021)	0.0044(0.0005)
MISSING	9.8				9.8			

IQR = interquartile range. Maternal age and maternal folate intake are continuous in the analyses, but are categorized here for illustrative purposes. The population was selected from The Norwegian Mother, Father and Child Cohort Study (MoBa).

on the following variables: natural log-transformed concentrations of metals and essential elements (As, Cd, Hg, Mn, Pb, Cs, Cu, Co, Mo, Zn, Mg, and Se), maternal iodine intake, maternal dietary intake of folate and seafood, maternal pre-pregnancy body mass index, maternal age, maternal education, parity, maternal smoking, child sex, child birth year, and maternal and child 5mC and 5hmC levels (see Tables 1 and 2). Kernel density plots were used to confirm that the imputed values followed the expected distributions (data not presented). All regression modelling is performed in multiple imputed datasets (MI), unless otherwise mentioned, and estimates were combined using Rubin's rules (Rubin, 1987).

2.5.1. Mixture analyses

We applied different methods to investigate the associations between 12 toxic metals and essential elements measured in maternal blood during pregnancy on DNA methylation outcome variables in pregnant women at the same time point (cross-sectional) and their children at birth (prospective). One approach aimed to identify the most important metals/elements explaining global DNA methylation levels in these groups and assess their independent relationship with the outcomes (including their multiplicative two-way interactions), while a complimentary approach investigated the total effect of the metals/elements in the mixture.

2.5.1.1. Selecting the most predictive metals/elements for DNA methylation outcomes. The multiple exposure data in this study, in which some exposures may be intercorrelated (Caspersen et al., 2019), can produce unstable estimates and inflated standard errors when running traditional exposure regression models that mutually co-adjust for exposure variables (Gibson et al., 2019). Also, multiple comparisons using single-pollutant models increase the chance of type I error (false positive), while traditional multiple testing adjustment methods (e.g. Bonferroni) are often too conservative and the chance of type II error (false negative) (Streiner, 2015). We used a method for regularization and variable selection, elastic net regression (Zou and Hastie, 2005) (see Appendix 3 in Supplementary material), to identify metal/element exposures

Table 2
Distribution of metal concentrations (ug/ml*) analysed in whole blood of pregnant women sampled in approximately week 17–18 of pregnancy (N = 652).

	Mean (SD)	10th%	25th%	50th%	75th%	90th%	Min	Max	n < LOQ(%)/n < LOD(%)	n missing (%)
Arsenic	2.23 (3.07)	0.53	0.77	1.36	2.53	4.75	0.30	48.4	25(3.8)/16(2.5)	–
Mercury	1.47 (1.04)	0.53	0.81	1.22	1.83	2.64	0.07	11.9	0	0
Cadmium	0.28 (0.26)	0.10	0.13	0.18	0.32	0.63	0.04	2.35	0	0
Manganese	10.5 (7.81)	6.19	7.49	9.04	11.6	14.1	3.50	112	0	0
Lead	9.07 (9.04)	4.76	6.06	7.87	10.5	13.0	2.19	160	0	0
Selenium	94.7 (18.0)	73.1	82.2	92.7	106	118.	48.3	181	0	0
Copper	1462 (245)	1180	1300	1440	1600	1740	807	3700	0	0
Cesium	2.18 (0.71)	1.42	1.69	2.09	2.51	3.08	0.82	6.84	0	95(14.6)
Cobalt	0.21 (0.22)	0.08	0.10	0.17	0.24	0.33	0.05	2.44	39(6.0)/32(4.9)	–
Molybdenum	1.25 (4.95)	0.44	0.56	0.69	0.91	1.55	0.24	98.4	0	0
Zinc	4677 (826)	3710	4120	4628	5180	5710	1720	7950	0	0
Magnesium[a]	27.5 (3.12)	23.8	25.3	27.2	29.4	31.5	17.0	39.0	0	95(14.6)

N = 652.
[a] For magnesium, concentrations are in mg/ml.

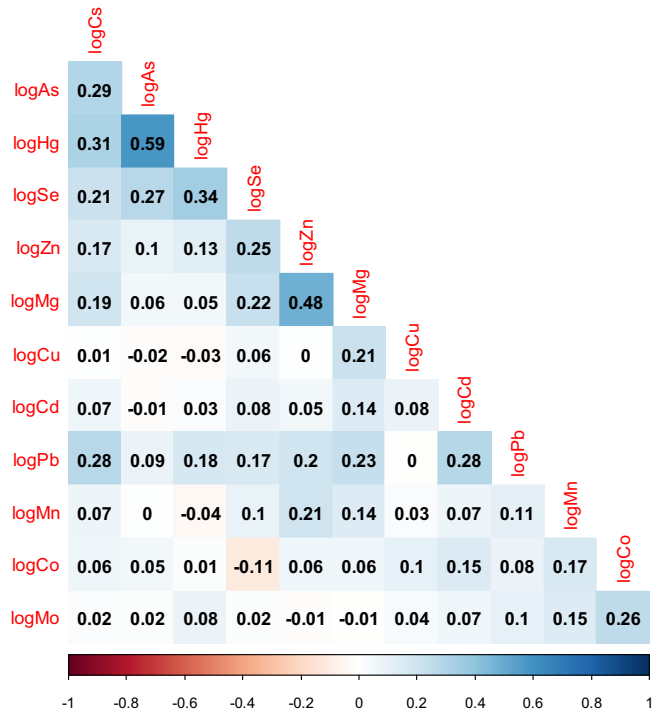


Fig. 2. Spearman correlations between the 12 metals/elements included in the study, based on the maternal sample ($N = 652$).

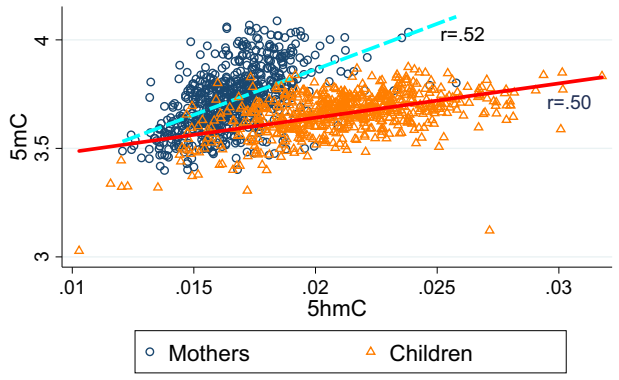


Fig. 3. Scatterplot of 5hmC versus %-5mC in children and mothers. Correlation coefficients are Pearson's r. Based on N = 652 for mothers and $N = 631$ for children.

important for DNA methylation outcomes. Alpha was set to 0.9. Lambda was selected using cross validation, and we selected the values that minimized the mean squared error. The covariates were not penalized. In order to ensure robustness of results, elastic net regression was performed using a stability selection approach. In stability selection, variables that are only weakly related to the outcome are more likely to be filtered out, due to more noise being introduced into the datasets (Meinshausen and Bühlmann, 2010). Indirectly, this will also reduce the probability of spurious findings from multiple testing. Briefly, random sampling from the original data with replacement was done 100 times, yielding 100 new datasets. In each of the randomly sampled datasets, 20 MI datasets were made (see Johnson and Young, 2011 for a discussion on the number of imputed datasets). Elastic net was run in every MI dataset, and it was calculated how often, on average, the exposures were selected and results were combined using Rubin's rules (Rubin, 1987). Thus, each randomly drawn dataset produced one selection probability estimate for each exposure. The mean selection probabilities for each exposure variable across the 100 randomly drawn datasets were then calculated (according to Meinshausen and Bühlmann, 2010).

Since multiple exposures were tested, we performed analyses to control the false discovery rate (FDR) at $\alpha = 0.05$, according to a method proposed by Ahmed et al. (2011) where the FDR approach is modified for stability selection using permutations (see Appendix 3). Briefly, a permutation procedure was used to calculate *p*-values from the elastic net regression with stability selections. The ranked (small-to-large) p-values was then compared to the Benjamini and Hochberg FDR threshold (p_{FDR})(Benjamini and Hochberg, 1995). Exposures were considered important for the outcome (i.e., selected) if they had a selection probability (P_s) of 0.6 or higher (Meinshausen and Bühlmann, 2010) and a calculated *p* value of 0.05 or lower. This finding was further strengthened if p-value was less than the p_{FDR}. The selected exposures ($P_s > 0.6$, $p \leq 0.05$) were entered in linear regression model (ordinary least square; co-adjusted for other selected exposures), along with the covariates of the final adjustment set (no CIs or p-values were considered).

In order to assess multiplicative interactions, two-way interaction terms were created between all exposure variables and between exposures and covariates (from the final adjustment set). Continuous variables were standardized. We performed elastic net regression with stability selection followed by permutations to calculated p-values and FDR thresholds as described above. Interaction terms with a p-value of 0.1 or lower were considered important. We also considered if p-values were lower than the respective FDR thresholds (p_{FDR}). Selected two-way interactions from elastic net regression were illustrated in line plots.

2.5.1.2. Variable importance. To assess the importance of metals and essential elements exposures during pregnancy relative to other known predictors of maternal or child DNA methylation levels (e.g. folate intake and smoking; (Boeke et al., 2012, McKay et al., 2012, Joubert et al., 2016), we calculated variable importance using random forest analysis (Breiman, 2001) with all metals/elements and covariates of final adjustment set (in addition to maternal 5mC and 5hmC levels in newborns) as exposures, and maternal and child 5mC or 5hmC as outcomes (500 trees in one MI dataset; R package randomForestSRC).

2.5.1.3. Total mixture effects. The effect of individual metals or essential elements may be small and challenging to identify. This makes it difficult to predict the total effect of the mixture's metals/elements based

Table 3
Distribution and correlation of DNA methylation outcomes in mother-child pairs.

	Children					Mothers					Pearson's *r*
	N	Mean (SD)	Median	Min	Max	*N*	Mean (SD)	Median	Min	Max	($N = 609$)
5mC, %	631	3.6581 (0.0991)	3.6684	3.0276	3.8747	652	3.7234 (0.1416)	3.7233	3.3984	4.0877	0.092
5hmC, %	631	0.0211 (0.0031)	0.0211	0.0103	0.0318	652	0.0166 (0.0018)	0.0166	0.0121	0.0258	0.038
5hmC: 5mC ratio	631	0.0058 (0.0008)	0.0057	0.0034	0.0087	652	0.0045 (0.0004)	0.0044	0.0035	0.0068	0.039

on modelling of single compounds. Thus, as a secondary mixture approach, we assessed the joint effect of the metal/element mixture on DNA methylation in mothers and newborn children using a quantile-based g-computation approach (R package qgcomp) (Keil et al., 2019). This novel method, combining weighted quantile sum (WQS) regression and g-computation, estimates the simultaneous effect on the outcome of an increase of all exposures in the mixture by one quantile (Keil et al., 2019; Niehoff et al., 2020). In our study, we investigated three different mixtures a priori based on the literature (Tchounwou et al., 2012; Zoroddu et al., 2019): A mixture containing all 12 metals and essential elements (MixAll), a mixture containing only essential elements (MixEss; Se, Mn, Co, Cu, Mo, Zn, and Mg) and a mixture containing only toxic (or non-essential) metals (MixTox; As, Hg, Cd, Pb, and Cs). The quantile was set to one quartile increase in natural log-metal/element concentrations and the estimates with 95% CIs are reported. The mixture analyses were run in multiple imputed datasets, and results were combined using Rubin's rules.

2.5.2. Analyses of individual toxic metals and elements

For comparison with the mixture results, we performed multivariable adjusted linear regression models with individual metal/elements and DNA methylation outcomes. Estimates are given in as the change in 5mC or 5hmC methylation level per interquartile (IQR) range increase in natural log-metal/element concentration with 95% CIs. We also assessed if the functional form of the dose-relationships of the individual elastic net selected metals/element differed from linearity by comparing with linear regression model of single exposures with natural splines with knots at 10th, 50th and 90th percentiles (Harrell Jr, 2015). Significant splines with significant non-linear forms (likelihood-ratio test; significance at $p \leq 0.05$) was fed back into the elastic net regression with the appropriate term based on the shape of the dose-response function.

2.5.3. Sensitivity analyses

We performed several sensitivity analyses of the main results of the selected metals/elements and interaction terms from the elastic net regression in both the maternal and child samples. All models were restricted to maternal non-smokers ($N_{children} = 564$, $N_{mothers} = 588$) and maternal folate supplement users ($N_{children} = 431$, $N_{mothers} = 452$) during pregnancy, and to children without ADHD symptoms ($N_{children} = 421$). We also examined if the findings were robust to inclusion of previously omitted outliers ($N_{children} = 631$), to analyses of complete cases (i.e. without imputed data; $N_{children} = 474/445$, $N_{mothers} = 490/460$) and to exclusion of metals/elements analysed at the

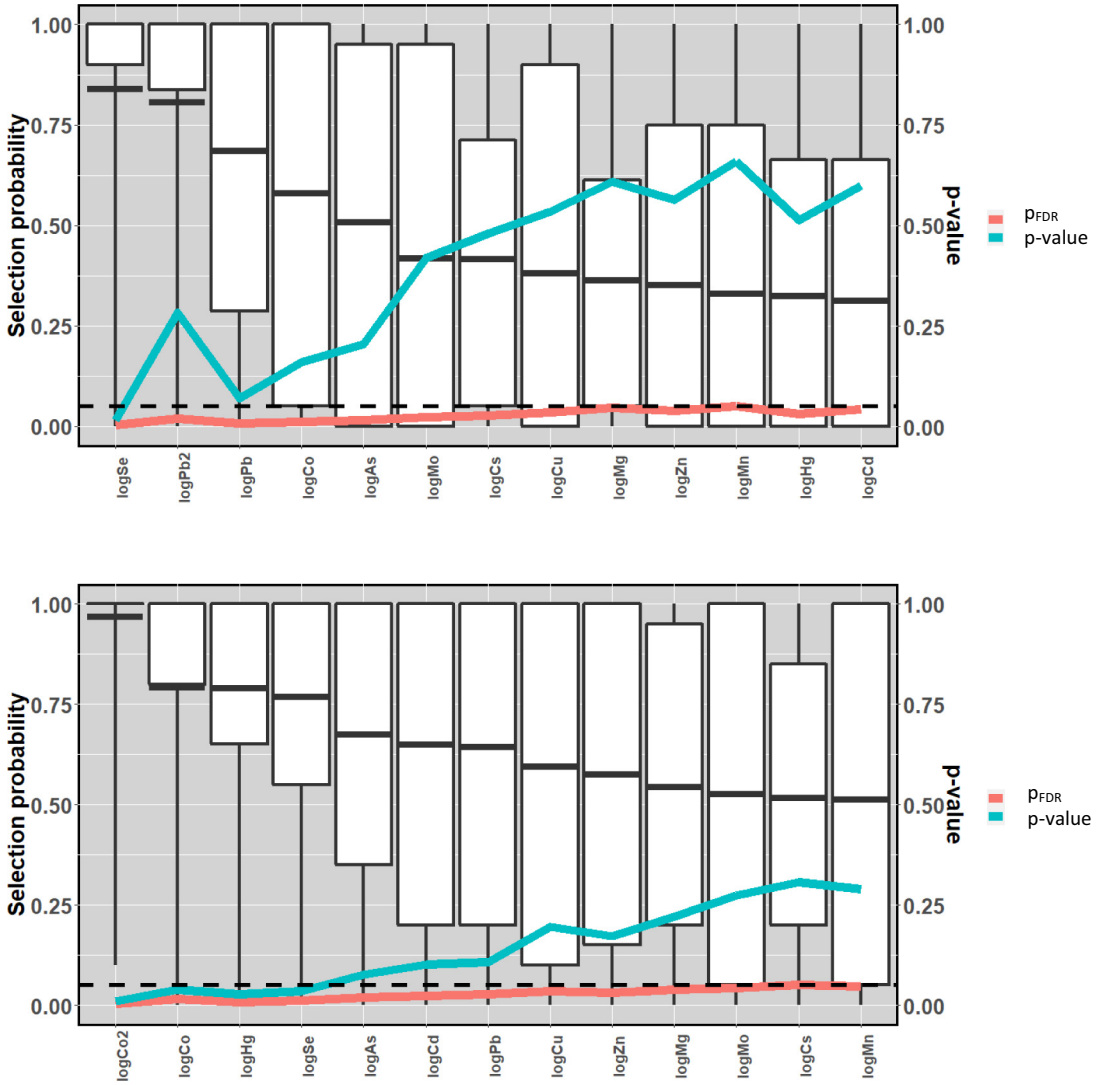


Fig. 4. Mean selection probability (boxplot), based on elastic net regression in 2000 datasets (20 multiple imputed datasets in each of 100 randomly drawn datasets with replacement), and calculated *p*-values and Benjamini and Hochberg false discovery rate thresholds, based on 1.000.000 elastic net runs. All analyses adjusted for minimal adjustment set of covariates. Upper figure: M_5mC (N = 652); lower figure: C_5mC (*N* = 625).

University of Lund ($N = 95$). Since child DNA methylation may be genetically linked to maternal DNA methylation (McKay et al., 2012), we investigated if adjusting for maternal DNA methylation levels altered the results.

3. Results

The population characteristics are presented in Table 1. Among them, 52.3% of the children were males, and 89.2% of the children were firstborns. The distribution of metal concentrations is presented in Table 2. The highest correlations (Spearman) between the natural log-transformed metals were between As and Hg ($r = 0.59$), Mg and Zn ($r = 0.48$) and Se and Hg ($r = 0.34$) (Fig. 2).

The distribution of DNA methylation variables is presented in Table 3, with children and mothers exhibiting means of 3.66 and 3.72% 5mC, and 0.021 and 0.017% 5hmC, respectively. The correlation between methylation markers in mother-child pairs were 0.11 for 5mC ($p = 0.006$), and 0.04 for %-5hmC ($p = 0.26$). The correlation between 5mC and 5hmC in children was 0.50 ($p < 0.0001$), and in mothers 0.52 ($p < 0.0001$; Fig. 3). Among the newborn children, males had higher 5mC levels than females (Welch two sample *t*-test, $p < 0.0001$), but there were no differences for 5hmC ($p = 0.10$).

3.1. Associations between single metals and essential elements in the mixture

3.1.1. Pregnant mothers

Using the elastic net regression in conjunction with stability selection identified Se ($P_s = 0.84$, $p = 0.016$) and Pb ($P_s = 0.84$, $p = 0.07$) as important maternal 5mC in pregnant mothers as the outcome (Fig. 4, Table S2a; Fig. S2). The *p*-values were above the FDR thresholds (Se: $p_{FDR} = 0.004$; Pb: $p_{FDR} = 0.008$). Although close, Pb was not below the set significance levels (0.05; nor the FDR threshold 0.008). Nonetheless, its selection probability was high and analyses of single metals/elements revealed significant non-linearity (LR-test, linearity: $p = 0.04$; LR-test of model with Pb splines vs model without Pb: $p = 0.04$) for the Pb-5mC relationship. Thus, we included Pb along with Se as the selected variables. When these selected exposures were included in a multivariable adjusted linear regression model, the estimates showed that Se was positively associated with 5mC, with an IQR increase in Se associated with 0.02 times increase in 5mC-levels. In the single exposure model, Se was linearly associated with 5mC ($\beta = 0.026$, 95% CI = [0.010,0.041]) (Fig. 5a; Table S2b), while Pb showed a non-linear U-shaped relationship with 5mC levels in pregnant mothers ($F = 2.74$, $p = 0.04$; Fig. 6a; Table S2b).

In sensitivity analyses, excluding smokers, excluding mothers who did not take folate supplement during pregnancy, or using complete cases only, did not change the results (Fig. S3a and b; Table S3).

The most important two-way interaction terms uncovered for 5mC included the following (in decreasing selection probability): Se*Zn ($P_s = 0.99$, $p = 0.007$), Cd*Mo ($P_s = 0.94$, $p = 0.018$), Se*Maternal education ($P_s = 0.90$, $p = 0.006$), Se*Cs ($P_s = 0.87$, $p = 0.003$), and Pb*Cu ($P_s = 0.86$, $p = 0.068$) (Fig. 7 and 8a and Tables S4). However, *p*-values for these interaction terms were above the FDR thresholds ($p_{FDR} = 0.001–0.003$). In line-plots of bivariate, linear regression models with the selected interaction terms, maternal Se concentrations appeared positively associated with 5mC levels among highly educated mothers, but negatively related to the same outcome among less educated mothers (Fig. 8a). Se was more strongly, positively associated with 5mC in the higher Zn and Cs strata. Cd showed a positive association with 5mC in the low, none in the mid, and negative in the high Mo strata. For Pb there was a stronger negative association with 5mC in the high Cu strata (Fig. 8a). The elastic net regression did not identify any important associations with metals/elements or their two-way interactions with 5hmC levels in pregnant mothers (Figs. S4-S6; Table S5).

3.1.2. Newborn children

In elastic net regression with stability selection, maternal blood levels of the following metals/elements had high mean selection probabilities (P_s) and low p-values for newborn 5mC levels; Co/Co^2 ($P_s = 0.79/0.97$, $p = 0.04/0.01$), Hg ($P_s = 0.79$, $p = 0.03$), and Se ($P_s = 0.77$, $p = 0.04$;

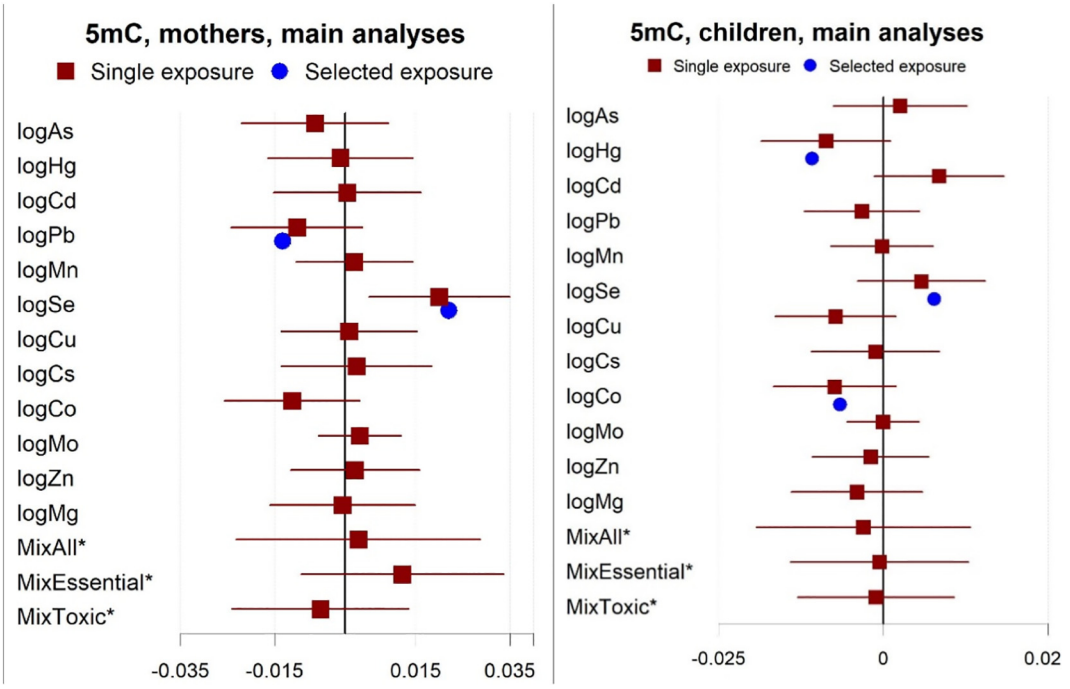


Fig. 5. Linear regression estimates for metal/element-DNA methylation associations for a) mothers, and b) children. Based on multiple imputed data. Increase in outcome per interquartile range increase in exposure. The Mix terms represent change in 5mC for a quartile increase in the metal mixture using qgcomp, where MixAll includes all 12 metals/elements, *) MixEssential includes Se, Mn, Cu, Co, Mo, Zn, and Mg, and MixToxic includes As, Hg, Cd, Pb, and Cs. Outliers, as defined by a Cook's d larger than 4/sample size AND a standardized residual with an absolute value above 3, were removed prior to analyses (6 observations for children, 0 for mothers). Analyses were adjusted for maternal age, maternal education, parity, maternal smoking, maternal seafood and folate intake, and child sex (only for children).

Fig. 4; Table S6a). All p-values were above the FDR thresholds (p_{FDR} = 0.004–0.012; Fig. 4, Table S6a). When Co, Se and Hg were included in a multivariable adjusted linear regression model, the estimates showed that Se was positively associated with 5mC, with an IQR increase associated with 0.01 times increase in 5mC levels (Fig. 5b; Table S6b). Maternal Hg and Co levels showed negative associations with newborn 5mC; one IQR increase in gestational levels of Hg and Co were associated with 0.01 and 0.05 times reduction in 5mC, respectively (Fig. 5b; Table S6b). For Se and Hg this pattern of associations with 5mC was similar in linear regression models with single exposures; Se (β = 0.01, 95% CI = [0.00,0.02]) and Hg (β = −0.01, 95% CI = [−0.02,0.00]) (Fig. 5b; Table S6b), while Co was non-linearly associated with 5mC levels (Fig. 6b; Table S6b: F = 2.73, p = 0.04) with a U-shaped exposure-response relationship.

With 5hmC as outcome, the only important exposure was the quadratic term of Cu (Cu^2) (P_s = 0.95 p = 0.029; Figs. S5; S6; Table S7a), though the p-value was above the FDR threshold (p_{FDR} = 0.004). When included in single exposure model, Cu displayed a non-linear relationship with 5hmC (Fig. 6b, Table S7b; F = 3.95, p = 0.008), with a U-shape in the area including most of the datapoints.

None of the sensitivity analyses changed in the results (Fig. S7; Table S8).

Elastic net regression with stability selection identified several important metals/element and covariate two-way interactions associated with 5mC levels in newborns (in decreasing selection probability); Co*Zn (P_s = 0.93, p = 0.03), Hg*As (P_s = 0.91, p = 0.04), Hg*Parity P_s = 0.73, p = 0.03), Se*Smoking (P_s = 0.70, p = 0.03), Hg*Sex (P_s = 0.66, p = 0.05), and Hg*Maternal education (P_s = 0.63, P = 0.05)

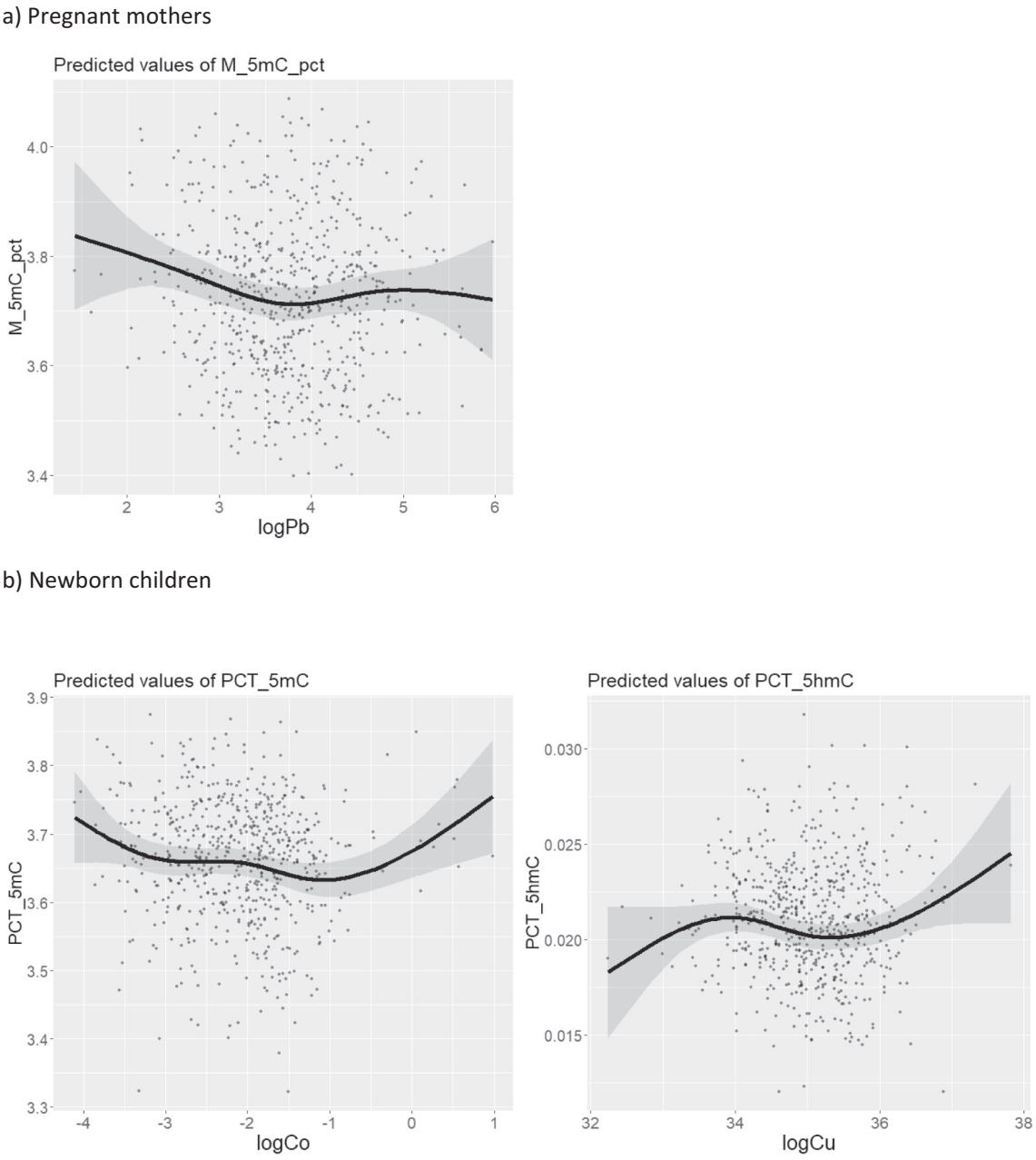


Fig. 6. Marginal effects of non-linear metal-DNA methylation associations for a) Mothers, and b) Children, modelled with natural splines with knots at 10th, 50th, and 90th percentiles. Based on multiple imputed data (m = 20). Analyses were adjusted for maternal age, maternal education, parity, maternal smoking, maternal seafood and folate intake, and child sex (only for children).

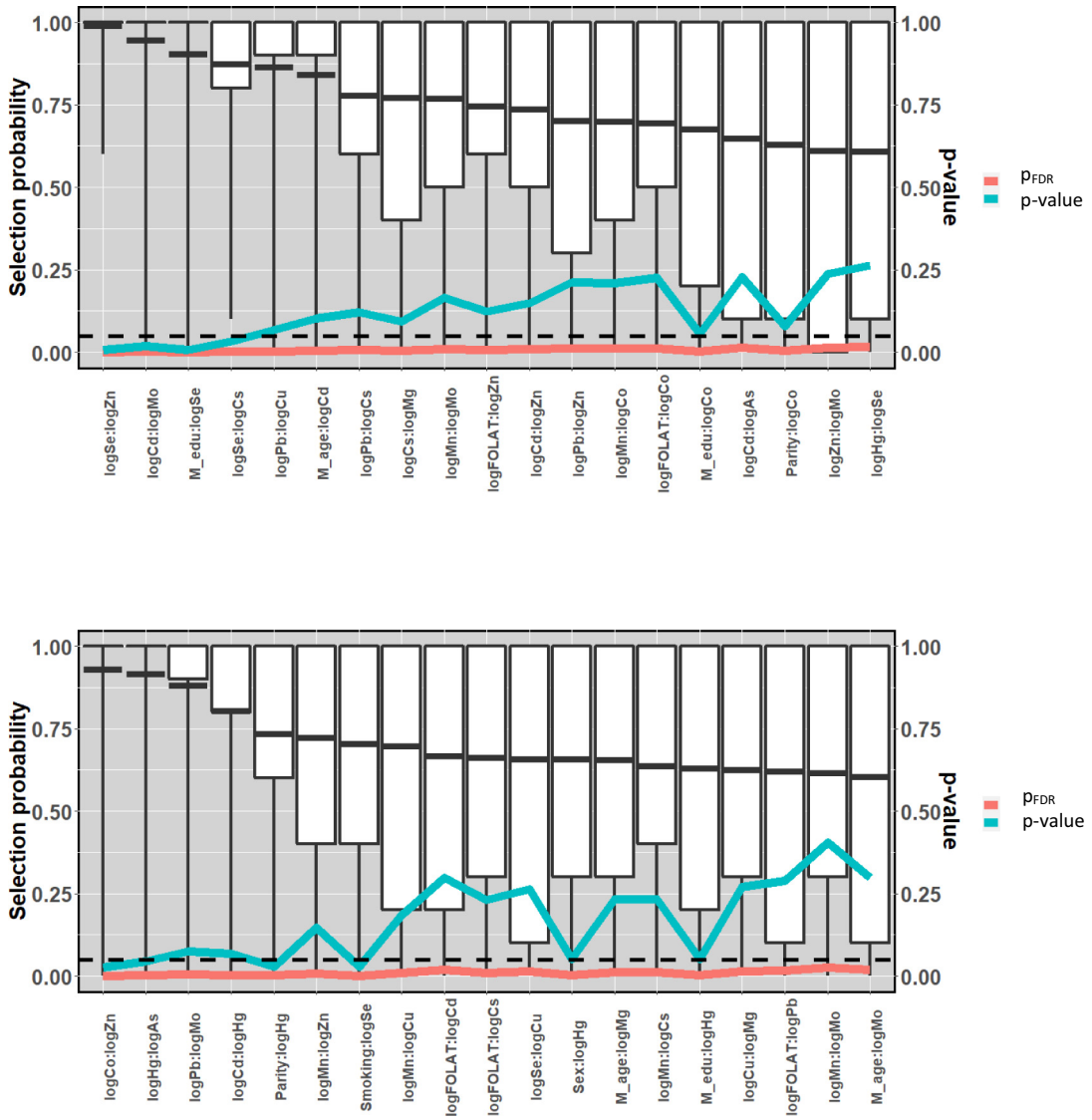


Fig. 7. Mean selection probability (boxplot) for two-way interaction terms, based on elastic net regression in 2000 datasets (20 multiple imputed datasets in each of 100 randomly drawn datasets with replacement), and calculated p-values and Benjamini and Hochberg false discovery rate thresholds, based on 500.000 elastic net runs. All analyses adjusted for minimal adjustment set of covariates. Upper figure: M_5mC (N = 652); lower figure: C_5mC (N = 625). All analyses adjusted for minimal adjustment set of covariates. Only interaction terms with a mean selection probability of 0.6 or higher are displayed. Upper figure: Maternal 5mC. Lower figure: Child 5mC.

(Figs. 7; 8b; Tables S9). However, interaction term p-values were above the FDR thresholds (p_{FDR} = 0.001–0.003) (Table S9). In line plots, we observed from the line plots that the Se-5mC association was positive among children of non-smokers, but negative among children of smokers. The Hg-5mC relationships was positive among children of less educated mothers, and negative among children of higher educated mothers. Also, girls and first-born children appeared to be driving the negative Hg-5mC relationship (Fig. 8b). Maternal Hg was more strongly, negatively associated with 5mC in the low As stratum. In the low maternal Zn concentration stratum, maternal Co was positively associated with 5mC in newborns whereas Co was inversely related to 5mC the mid and high Zn strata (Fig. 8b).

We did not identify any important prenatal metal/element or covariates two-way interactions for 5hmC levels in newborns (data not presented).

3.2. Variable importance

The variable importance plots (from random forest models) ranked metals/elements according to their importance for 5mC and 5hmC. For 5mC the 15 most important for pregnant mothers were: Se > Pb > Co ≈ As≈Pb^2 ≈ Cs ≈ Zn > Mo ≈ Hg ≈ Cd > Mat.Edu ≈ Parity≈Smoking≈Mg > Folate≈Mat.age (Fig. S2).

For newborn children, the 15 most important variables were: Sex> > M_5mC > Hg ≈ Se ≈ Co^2 ≈ Co > Mat.age≈Folate>Parity≈Mo ≈ Mat.smoking≈Mat.Seafood≈Mat.edu ≈ Pb ≈ Mg (Fig. S2).

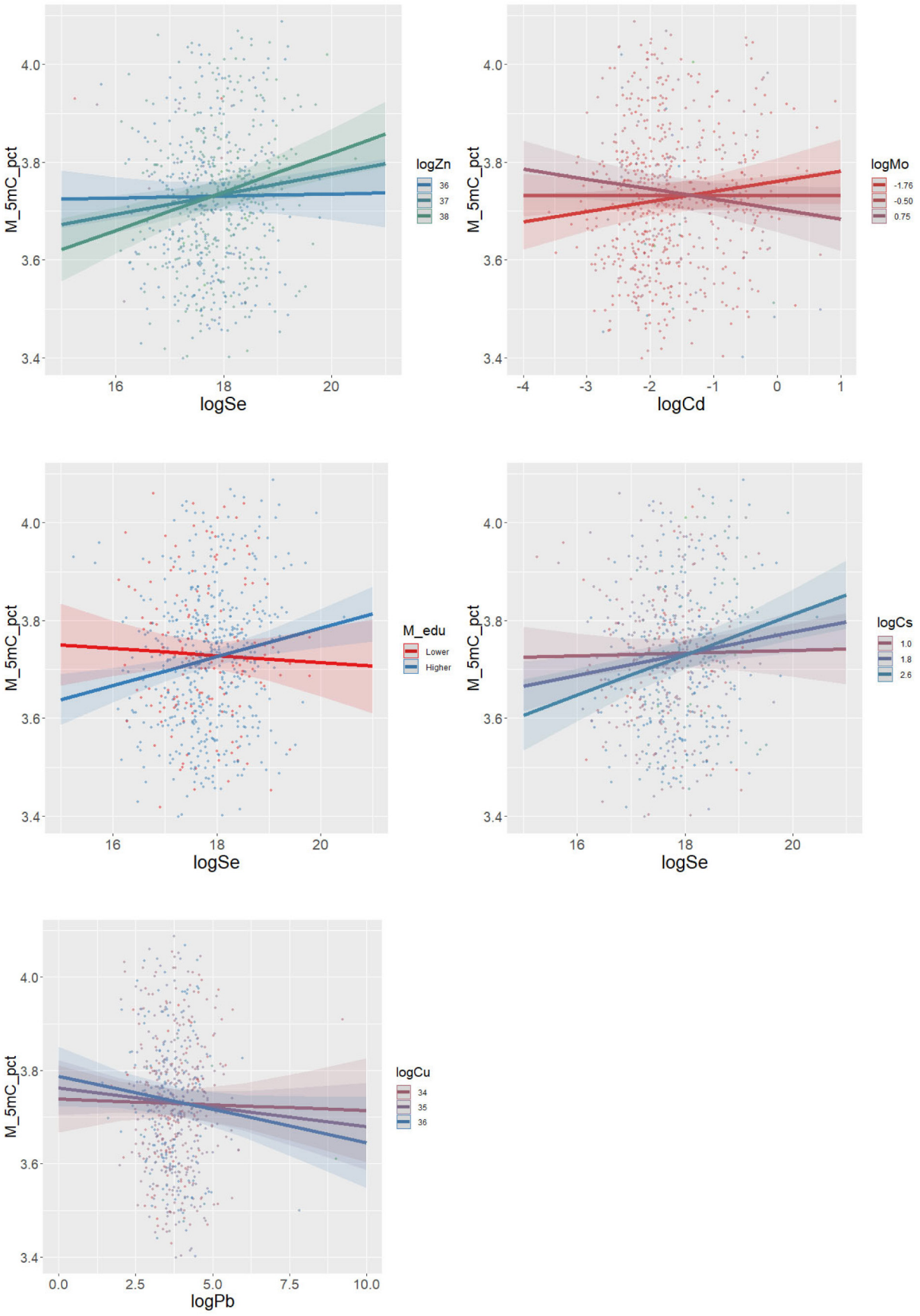
3.3. Total effect of the mixture(s)

In the quantile g-computation modelling, we did not identify any significant association between the gestational levels of MixAll, MixEss or MixToxic with 5mC or 5hmC levels in pregnant women or their newborn children (Figs. 5; S4; Tables S2b; S5; S6b; S7b).

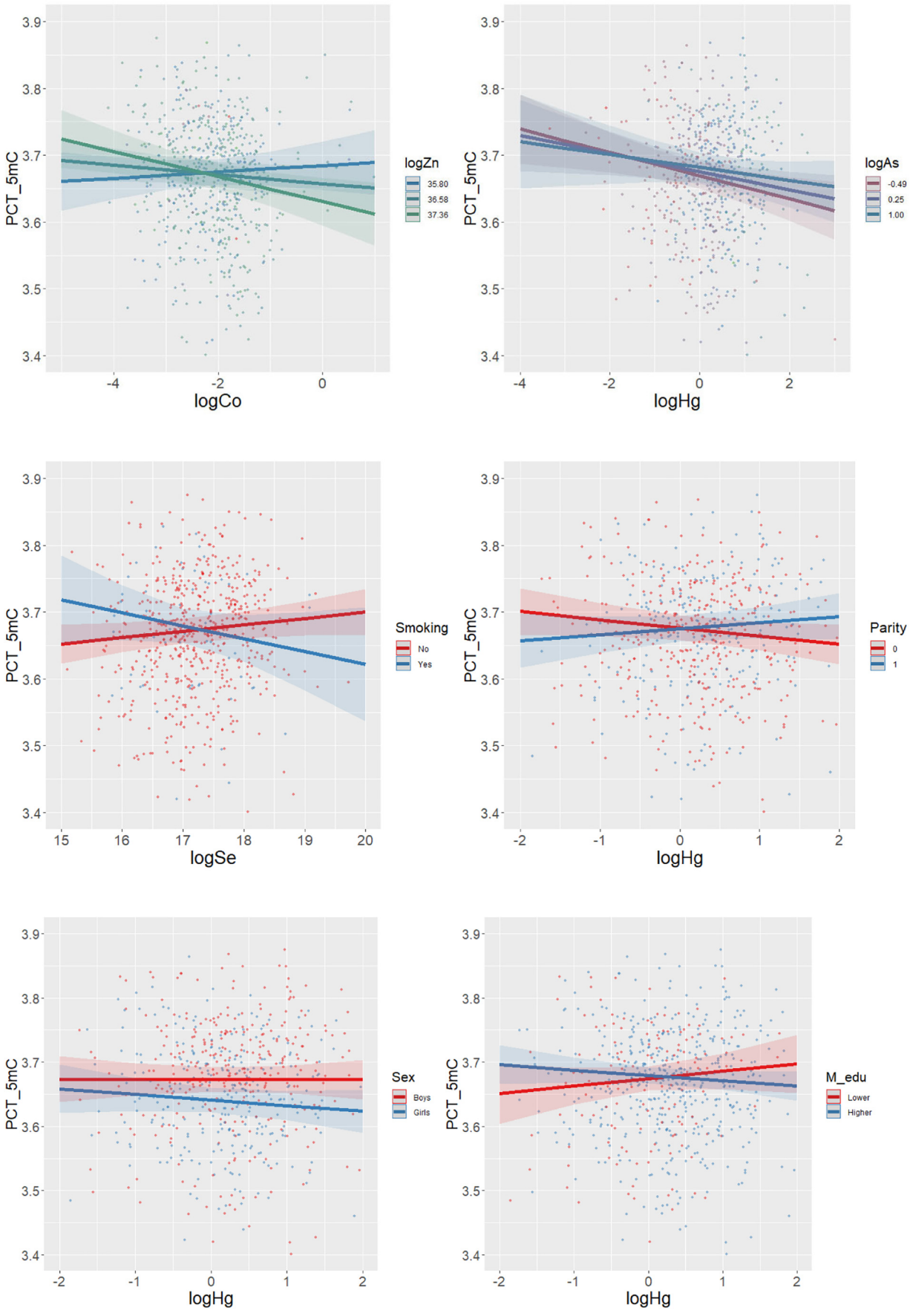
4. Discussion

By using a combination of mixture methods, this comprehensive study has addressed important knowledge gaps concerning mid-gestational levels of multiple toxic metals and essential elements measured in maternal blood mid-pregnancy and relationships with global

a


M_5mC_pct
logSe
logZn
36
37
38
logCd
logMo
-1.76
-0.50
0.75
M_edu
Lower
Higher
logCs
1.0
1.8
2.6
logPb
logCu
34
35
36

b


PCT_5mC
logCo
logZn
35.80
36.58
37.36
logHg
logAs
-0.49
0.25
1.00
logSe
Smoking
No
Yes
Parity
0
1
Sex
Boys
Girls
M_edu
Lower
Higher

DNA methylation markers (5mC and 5hmC) in both pregnant mothers and their newborn children. Using elastic net regression with stability selection, we identified the most important metals/elements in the mixture, as well as their to-way interactions (including with covariates) and modelled the individual relationships of these selected compounds with total 5mC and 5hmC in blood (newborns: cord blood). The most sensitive marker appeared to be 5mC. In pregnant mothers, results indicated associations between 5mC and Se and Pb (non-linear), while in newborns there were relationships between maternal Se, Co (non-linear) and Hg and 5mC, as well as maternal Cu (non-linear) and 5hmC levels. No relationship with 5mC or 5hmC was found for As, Cd, Mn, Cs, Mg, or Zn. We did not identify joint effects of the metals/elements of the total mixture on 5mC or 5hmC levels in either populations using quantile g-computation. Although the elastic net-based selected metal/element exposures met a priori defined limits for selection probability and significance levels, none of the results were significant when controlling for multiple comparisons with FDR thresholds. Thus, the inference and conclusion based on the findings herein must be done with caution. Still, our findings point towards potential candidates for hypothesis testing of specific metals/element combinations in further studies. In this respect, we address consistency with literature, biological plausibility and possible health implications of the main relationships identified herein.

4.1. Toxic metals and essential elements and associations with global methylation

4.1.1. Mercury

Few other epidemiologic studies have explored associations between in utero Hg exposure and global DNA methylation (Bommarito et al., 2017; Martin and Fry, 2018). Cardenas et al. (2017c) reported a negative association between Hg concentrations in red blood cells of pregnant women from second trimester and global 5hmC levels measured in newborn children and during early childhood (3–5 years), with a corresponding positive association with 5mC:5hmC ratio. They did, however, not find an association between prenatal Hg exposure and global 5mC levels in newborns as we did in the present study (Cardenas et al., 2017c). Other studies on prenatal Hg or MeHg exposure and newborn (cord blood) methylation in specific DNA regions report hypomethylation (Bakulski et al., 2015), hypermethylation (Cardenas et al., 2015b), or no association (Leung et al., 2018).

In our population of newborns the negative associations between Hg and 5mC levels seem to be driven mainly by girls. Although Cardenas et al. (2017b) did not find a significant interaction by child sex in the Hg-5hmC association, analyses stratified by sex suggested stronger (negative) associations for girls. Furthermore, sex-specific methylation pattern in relations to prenatal mercury exposure have been reported in other studies of newborns (Cardenas et al., 2017a; Nishizawa-Jotaki et al., 2020). Sex-specific associations in research on environmental exposures and epigenetics are common findings (Gabory et al., 2011; Bommarito et al., 2017), and have also been reported in animal studies (Richard Pilsner et al., 2010). This could be caused by sex hormones and their differential effects on organ development (Gabory et al., 2011). In addition, emerging evidence imply a differential methylation pattern for placentas supplying male and female for genes involved in transport and transcriptional control of immune and stress responses (Clifton, 2010; Maccani et al., 2015; Martin et al., 2017). Additionally, the sexual dimorphic DNA methylation pattern might be explained by sex-dependent transport of toxicants, nutrients and signalling molecules across the placenta as well susceptibility for toxicant associated health effects (Saif et al., 2014; Martin et al., 2017). Little is known to date, however, about mechanistic underpinnings and health implications of sex differences toxicant-DNA methylation associations.

Interestingly, the association between prenatal Hg exposure and 5mC was significantly modified by parity and maternal education in the present study, with stronger negative estimates for firstborns and children of mothers with higher education. Studies have reported positive associations between socioeconomic status (SES) and mercury levels in blood in children (Lim et al., 2015; Montazeri et al., 2019), pregnant women (Vrijheid et al., 2012; Montazeri et al., 2019) and adults (Tyrrell et al., 2013), which can be related to a generally higher seafood intake in the higher SES strata, resulting in an elevated Hg prenatal exposure compared to the lower SES strata (Caspersen et al., 2019; Montazeri et al., 2019; Papadopoulou et al., 2019). Parity is related to prenatal Hg exposure (Grandjean et al., 1992; Ramon et al., 2011; Bocca et al., 2019). Increasing age (and thus parity) can also influence DNA methylation levels and patterns (Jones et al., 2015).

Our study adds to the increasing evidence that Hg exposure alters DNA methylation markers, and appear in accordance with findings from cross-sectional studies of adult populations (Hanna et al., 2012; Goodrich et al., 2013; Narváez et al., 2017), and experimental animal (Desaulniers et al., 2009; Basu et al., 2013; Carvan Iii, 2020) and wildlife studies (Richard Pilsner et al., 2010; Nilsen et al., 2016; Martín-del-Campo et al., 2019). Animal studies indicate that Hg affects DNA methylation across tissues (mainly hypomethylation), including brain tissue (Pilsner et al., 2010). Hg (especially MeHg) is a well-documented developmental neurotoxicant, and MeHg exposure during foetal development is associated with later neurocognitive deficits, behavioural problems and increased risk of ADHD and ASD in children (Vrijheid et al., 2016; Barker et al., 2018). Because of the importance of DNA methylation processes during brain development, it is hypothesised that toxicant-induced alteration in DNA methylation regulation and patterns during early developmental stages might be a mechanistic link to the well-documented developmental neurotoxicity of Hg (Tran and Miyake, 2017). Two recent studies also report that alteration in DNA methylation pattern in cord blood of newborns appear to mediate the relationship between prenatal Hg exposure and adverse neurobehavioral outcomes or lowered cognitive functions in children (Maccani et al., 2015; Cardenas et al., 2017a). Furthermore, Bose et al. (2012) showed that in vitro exposure of neuronal stem cells to MeHg induced hypomethylation that was transferred to the next daughter cells, demonstrating MeHg-induced programming of nerve cells. An experimental study of zebrafish exposed to MeHg showed transgenerational effects with alterations in DNA methylation pattern and adverse neurobehavior in the unexposed 2nd generation (Carvan III et al., 2017).

4.1.2. Arsenic

Pilsner et al. (2012) reported a positive association between maternal urinary As concentration and global DNA methylation in newborn children. We did not observe any association between maternal As levels and 5mC in pregnant mothers or newborns, although for newborns As had a high selection probability and a *p*-value just above the a priori threshold. Furthermore, the results implied an interaction between Hg and As in newborns; Hg was more strongly, negatively associated with 5mC at lower As strata. A previous study also identified an interaction between Hg and As on gene-specific methylation in newborn children (Cardenas et al., 2015b). The mechanism behind this interaction is not clear, however, it might be that As at higher exposure levels acts on mechanisms that antagonizes the negative effect of Hg on DNA methylation.

Fig. 8. a: Plots of interaction terms with selection probability >0.6 and p-value<0.05, 5mC in pregnant mothers. Legend values for continuous variables represent mean and +/− 1SD. Adjusted for maternal age, folate intake during pregnancy, parity, maternal education, smoking during pregnancy, and seafood intake during pregnancy. $N = 652$. **b:** Plots of interaction terms with selection probability >0.6 and p-value<0.05, 5mC, newborn children. Legend values for continuous variables represent mean and +/− 1SD. Adjusted for maternal age, folate intake during pregnancy, parity, maternal education, smoking during pregnancy, sex, and seafood intake during pregnancy. $N = 625$.

Previous studies have shown both hypo- and hypermethylation in association with As exposure in both child and adult populations (Pilsner et al., 2007; Reichard and Puga, 2010; Pilsner et al., 2012; Ray et al., 2014; Cardenas et al., 2015b). In the Norwegian population, total As (and total Hg) consist of mainly organic species originating from fish and seafood (Julshamn et al., 2012; Birgisdottir et al., 2013; Caspersen et al., 2019). While organic methylated Hg (MeHg) form is considered as more toxic than inorganic Hg (Tchounwou et al., 2012), the organic As forms (e.g. arsenobetaines) from fish is considered less toxic than inorganic As (ATSDR, 2007; Molin et al., 2015). This may explain some of the inconsistent findings across studies (Pilsner et al., 2007, Reichard and Puga, 2010, Pilsner et al., 2012, Ray et al., 2014, Cardenas et al., 2015b), including the lack of clear findings for As in the present study, with regards to DNA methylation outcomes.

4.1.3. Lead

In pregnant mothers, we observed a significant non-monotonic (U-shaped) association between Pb and global 5mC methylation levels. Non-linear or non-monotonic dose-response relationships are not uncommon findings in toxicological research and may reflect different biological mechanisms acting at low and high exposures (Calabrese and Baldwin, 2001; Varret et al., 2018). An experimental rodent study reported a non-linear dose-response relationship between lead exposure and DNA methylation markers in the hippocampal area of the brain (Singh et al., 2018), otherwise this phenomena is relatively undescribed within environmental epigenomics research. Contrary to the finding in pregnant mothers, we observed no associations with prenatal Pb and global 5mC or 5hmC levels in newborn children, despite reports of association between in utero Pb exposure (measured in maternal bone) and offspring DNA methylation markers in both human (Pilsner et al., 2009) and experimental studies (Singh et al., 2018). Nonetheless, in this study, 5mC levels appear more susceptible to alterations by Pb-exposure in pregnant women than in newborns.

In pregnancy, an increase in maternal blood Pb levels can occur as a consequence of bone resorption in order to supply the fetus with calcium (Gulson et al., 2003; Téllez-Rojo et al., 2004). Concurrently, this leads to a release of long-term accumulated Pb stored in bone tissue into the bloodstream (Gulson et al., 2003, Téllez-Rojo et al., 2004). One study of maternal bone lead content and newborn (cord blood) 5mC levels in selected CpGs in long-interspersed element-1 (LINE-1) and *Alu* repeats, both used as genome wide DNA methylation surrogates (Pilsner et al., 2009), report negative relationships. Other studies generally report negative associations between Pb exposure and global DNA methylation measures (Ruiz-Hernandez et al., 2015).

Both high and low Pb exposure in pregnancy has been associated with stillbirth, spontaneous abortions, preterm birth, low birthweight and hypertension (Rahman et al., 2016). In adult, non-pregnant populations, lead exposure is associated with a wide range of adverse health outcomes such as breast cancer, kidney dysfunctions and possibly neurocognitive effects and psychiatric symptoms (Kosnett et al., 2007; Shih et al., 2007; Alatise and Schrauzer, 2010). Thus, pregnancy could represent sensitive window of lead exposure for women and later lead-associated health effect mediated by alterations in DNA methylation.

4.1.4. Selenium

Se is an essential element important in one-carbon metabolism and thus for methylation processes of DNA (Speckmann and Grune, 2015). Se within a nutritionally relevant range may also act as functional antagonist to the toxic effects of Hg and As (Gailer, 2007; Ralston and Raymond, 2010).

This element was positively associated with global 5mC levels in both pregnant mothers and newborns. Among all exposures and covariates, Se had the highest variable importance for 5mC levels in mothers and was the third most important for newborn 5mC levels. Se importance in both pregnant mothers and their newborns could point to a general importance across life-stages.

A previous review concluded that in vitro, animal and human studies suggest an inverse association between Se and global DNA methylation (Speckmann and Grune, 2015). For example, inverse relationships between Se and global DNA methylation was reported in an adult population (Pilsner et al., 2011). Newer findings, however, imply that positive associations indeed may be present (Zhang et al., 2019). Se status correlated positively with LINE-1 methylation in adult women (Tapp et al., 2013). Se deficiency has also been associated with reduced methylation in several target-genes (Faulk, 2019), in line with our findings in pregnant mothers and newborn children. In an experimental study, global DNA methylation indicated by LINE-1 methylation increased in mice with adequate or supra-nutritional Se intake in comparison to those with a suboptimal intake (Speckmann et al., 2017).

In newborns, the positive Se-5mC association seemed to be driven by children of mothers who reported that they did not smoke during pregnancy. Among the children whose mothers smoked, there was a negative association. Smoking can influence antioxidant enzyme activities, and thus the metabolism of trace elements such as Se (Kocyigit et al., 2001), and some studies report lower levels of Se in smokers compared to non-smokers (Bashar and Mitra, 2004; Kocyigit et al., 2001). In addition, smoking during pregnancy has also been suggested to affect placental DNA methylation (van Otterdijk et al., 2017). Another study on DNA methylation measured in the placenta, found associations with Se, but did not detect interaction between smoking and Se (Tian et al., 2020).

Se, as other essential elements, have a narrow range of optimal function with beneficial health outcomes, whereas levels below or above this range may have adverse effects (Roman et al., 2014; Tian et al., 2020). Se deficiency has been associated with detrimental health in adult population, including cancer and thyroid diseases (Rayman, 2012). Based on in vitro, rodent and human studies, it seems that high levels of Se can inhibit DNA methyltransferase expression in relation to cancer and tumor genes (Jablonska and Reszka, 2017). Excess Se have been implicated as a risk factor for several diseases in adult populations, for example hyperglycemia and amyotrophic lateral sclerosis (Tian et al., 2020). For children, studies have shown associations between prenatal Se levels and perinatal and neurodevelopmental outcomes (Sun et al., 2014; Tian et al., 2020). Tian et al. (2020) reported lower muscle tone in newborns with increasing placental Se concentrations, which appeared to be mediated by placental hypermethylation of a specific gene.

4.1.5. Cobalt

Co is important for DNA methylation homeostasis as a central ion in vitamin B12, a critical co-factor in one-carbon metabolism supplying methyl-donors for DNA methylation (McKay et al., 2012). This may explain why we identified association between gestational Co and 5mC levels in newborn children. However, the same relationship was not found for their mothers during pregnancy. Studies investigating the role of Co in global DNA methylation in humans are very limited or lacking. One study investigated effects of elevated cobalt and chromium exposure from metal-on-metal prosthetic hip replacement in adults, and found no differences in methylation levels in a epigenome-wide-association study (Steinberg et al., 2017). In a study of maternal smoking during pregnancy, which depletes maternal and foetal Co levels and thus lowers vitamin B12, Co affected one-carbon metabolism, DNA methylation and functional gene-expression of target-genes in the foetal liver (Drake et al., 2015). In our study, however, the Co results were not altered when we restricted analyses to children of non-smoking mothers during pregnancy.

4.1.6. Copper

Copper is an essential element present in all organs and cells. It is important in numerous biological processes and necessary for a normal foetal and child development (Zoroddu et al., 2019). The main source of Cu is dietary, and during pregnancy Cu will be transported from maternal blood to foetus over the placenta and accumulate in the foetal

liver to serve as a source for months after birth when Cu supply is more scarce (Gambling et al., 2003; Zoroddu et al., 2019). Cu is highly reactive and can in surplus concentrations cause oxidative, toxic effects within cells and tissues via the formation of free radicals (Zoroddu et al., 2019). No previous epidemiologic study has investigated the effects of Cu on the epigenome. Nonetheless, experimental in vivo and in vitro studies have shown that increased Cu exposures alters the epigenome, including DNA methylation (Zhou et al., 2001; Medici et al., 2014; Yagci et al., 2019). In a study of zebra-fish embryos, increased Cu exposure upregulated DNA methyltransferase genes (Dorts et al., 2016), indicating a possible mode of action. Although much less studied as a developmental neurotoxicant than toxic metals, a recent study showed that elevated prenatal Cu concentrations was associated with lower neuropsychological scores at child age 12 months and five years (Amoros et al., 2019).

4.1.7. The mixture

No previous epidemiological study has investigated the impact of chronic low-dose exposure to multiple toxic metals and essential elements on global DNA methylation using mixture approaches. Also, few experimental studies have investigated this. Exposure of rats to a mixture of organic contaminants and MeHg, negatively affected gene expression of several DNA methyltransferases and the abundance of the methyl donor S-adenosylmethionine, and lowered global DNA methylation levels (Desaulniers et al., 2009). Rats exposed to soil containing higher concentrations of Cd and Pb showed elevated genome-wide methylation status and DNA methyltransferase mRNA levels (Nakayama et al., 2019). In vitro exposure of mice cells to 25 of the environmental chemicals that normally exposed the human foetus, Hg and Se were among five chemicals shown to cause epigenetic changes, and Hg and Se also affected DNA methylation at specific gene loci (Arai et al., 2011). Additionally, Hg was among the chemicals that showed potential for impairing embryo formation (Arai et al., 2011).

The exact mechanisms for disruption of DNA methylation pathways by toxic metal exposure are not yet established (Skaar et al., 2016; Martin and Fry, 2018). The toxic metals included herein may share similar modes of action which are proposed to involve production of reactive oxygen species which can inhibit methyl-CpG binding proteins, as well as alteration of DNA methyltransferases function or affecting of homocysteine that are important methylation donors (Valko et al., 2005). On the other hand, some essential elements (e.g. Fe, Zn, Mn) and other nutritional factors (e.g. Se, folate, B12, and B6) are important in DNA methylation pathways as well as for the uptake, transport or detoxification of toxic metals (Gambling et al., 2003; McKay et al., 2012; Medici et al., 2014; Lau et al., 2017; Zoroddu et al., 2019). Thus, it is likely that the mixture of metals/elements included herein may interact additively, or even synergistically or antagonistically, and that the relationships with DNA methylation is modified by nutrition factors (e.g. folate intake), resulting in mixture-specific effects on DNA methylation that cannot be deduced from relationships of individual metals/elements. We did indeed observe several indications of synergetic two-way interaction between several metals/elements; for example between cobalt and zinc, where elevated zinc seemed to increase the negative effect of cobalt on infant 5mC levels and the positive effect of Se on 5mC in mothers, while As appeared to antagonize the negative association between Hg and 5mC levels in newborns.

Even though Se was not identified in important two-way interaction with other metals/elements, its positive influence on 5mC levels in both mother and newborn could reflect that Se has an important role in modulating toxic metals' negative impacts on 5mC methylation (e.g. Hg). This may in part explain why we did not identify any effect of the total mixtures of toxic metals or essential elements on DNA methylation outcomes in the present study. Maternal levels of essential elements, such as Se, in their optimal physiological ranges, or other micronutrients during pregnancy and foetal development could thus be important in antagonising metals' toxic effects on DNA methylation homeostasis. The total mixture, nonetheless, may have a stronger impact in other populations exposed to higher levels of toxic metals (e.g. Hg) and/or with inadequate intake of essential element (e.g. Se) or other micronutrients (e.g. folate).

4.1.8. DNA methylation levels

Our results indicated very weak or no correlation between maternal and newborn levels of 5mC and 5hmC. This shows that the sampled cord blood originates from the child, with negligible interference from maternal blood, and could support that the de-methylation and re-methylation that occurs immediately after fertilization leads to a child-specific DNA methylation pattern (Zeng and Chen, 2019), although these differences might also be attributed age-related DNA methylation levels (Jones et al., 2015). Together with life-stage associated differences in susceptibility to specific toxic metals or requirements for essential elements, this may have contributed to the observed differences in metal/element exposures and DNA methylation levels in maternal and newborn blood, as well as study designs (cross-sectional in pregnant women versus prospective in infant). Still, our results offer some insight into possible general and differential life-stage associated sensitive windows of exposure to specific toxic metals or variations in essential element levels with respect to DNA methylation homeostasis.

4.2. Strengths and limitations

The present study has some limitations. The samples are obtained from the preschool ADHD sub-study and are not necessarily representative of MoBa or the general Norwegian population of pregnant women and their newborns, due to over-selection on high ADHD scores in the child. In the ADHD-study, 17% of the girls and 20% of the boys met symptom criteria for ADHD diagnosis according to the parent interview (Overgaard et al., 2018, 2019). In MoBa, there is an under-representation of mothers with low socioeconomic status and young mothers, and an over-representation of multivitamin and folic acid supplement users (Nilsen et al., 2009). Studies report that factors such as SES and nutrition are correlated with DNA methylation (Lam et al., 2012; Tapp et al., 2013). Generalizing the present findings should therefore be done with caution. In addition, parts of the study was cross-sectional (pregnant women), as are most studies on epigenetics to date (Breton et al., 2017), which limits inference of causality. Due to only one sampling point for DNA from pregnant women and newborns, it was not possible to assess the stability of the associations by taking into account the intra-individual change in DNA methylation over time (Wu et al., 2012a). A recent study showed that prenatal Hg exposure was consistently associated with 5hmC levels measured at birth (cord blood) and in blood in early childhood, but did not persist into mid-childhood (Cardenas et al., 2017b). Moreover, the relevance of methylation levels of circulating DNA as representative for DNA methylation changes in other tissues is not clear. Additionally, since the isolated leukocyte DNA is a mixture of DNA from numerous cell types, we cannot rule out that the metal exposures has resulted in shifts in white blood cell distributions, which potentially could affect outcome measurements. Also, we did not have a measure of iron status (e.g. plasma ferritin), which can influence absorption and toxicity of metals, especially divalent metals such as Cd, Pb, Mn, and Co (Meltzer et al., 2016). Although some of the exposures measured have long half-lives, and therefore can be expected to be relatively stable throughout pregnancy, other exposure concentrations, especially those of essential elements, can vary more (Aylward et al., 2014). Ideally, we should have had multiple measures of metal and element concentrations, but that was unfortunately not available in the present study. Last, we did not investigate gene-specific methylation or functional gene expression, hence inference on potential health effects of the metal/element-DNA methylation associations were not possible.

Nevertheless, this study has many strengths. A major advantage was the sample size, being one of the largest to date in this research area and among the first to investigate toxicant-associated global methylation levels in both pregnant mothers and their neonates. A novel approach in

this research field is the assessment of the impact of multiple toxic metals and essential elements on global DNA methylation outcomes using several mixture approaches investigating single metals/elements and their two-way interactions (elastic net regression with stability selection), and total mixture effects of (quantile g-computation) as well as controlling for multiple testing with FDR thresholds. Furthermore, our study was nested within a well-characterized prospective birth cohort with extensive questionnaire data that enabled us to obtain a wide range of relevant information on covariates. A last but important strength is that we analysed global DNA methylation using LC–MS/MS, which is considered a better and more precise method of global DNA estimation than other proxy measures (e.g. LINE-1 or *Alu* repeats methylation) (Price et al., 2012; Wu et al., 2012a). However, comparison with studies using other estimation techniques, such as pyrosequencing of repeat elements (Alu, LINE1) or luminometric methylation assays, is not straightforward as they do not measure global DNA methylation as the same biological construct (Price et al., 2012, Wu et al., 2012a). According to Price et al. (2012), total 5mC content (by HPLC-MS/MS) is the true measure of global methylation, and results should only be compared across studies when methylation has been assayed using the same technique and in the similar tissues. The disadvantage using these global techniques is that no information is obtained on gene specific methylation patterns, making it impossible to associate outcomes to specific modes of actions. Other factors complicating comparison include exposure assessment with regards to timing, target matrix (e.g. urine, bone or blood), as well as genetic or nutritional factors (Pilsner et al., 2007; Price et al., 2012; Basu et al., 2013; Breton et al., 2017). What seems clear, however, is that any alteration, even small, in global DNA methylation levels may produce genomic instability over time that may manifest phenotypically as adverse health effects in later life-stages (Wu et al., 2012b; Zhao et al., 2012; Breton et al., 2017; Martin and Fry, 2018). Although the biological importance of small changes in DNA methylation is difficult to interpret in terms of potential adverse health effects, the selected toxic metals and essential elements in the present study were more important predictors for global DNA methylation than other known environmental factors such as maternal smoking, age or folate intake (Boeke et al., 2012; McKay et al., 2012; Joubert et al., 2016). Thus, our findings are important candidates for further research, especially for foetal development with possible implications for neurodevelopmental outcomes, as well as other child or adult onset diseases (Skaar et al., 2016; Banik et al., 2017; Tran and Miyake, 2017).

5. Conclusion

Investigating the associations between gestational levels of 12 toxic metals and essential elements, Hg, Se, Co, and Cu had the strongest associations with global cord blood DNA methylation in infants. Se and Pb had the strongest associations with global DNA methylation in whole blood in mothers. Most associations were related to 5mC as outcome. Several potential two-way interactions were identified, both between metals/elements, and between metals/elements and covariates. However, none of the associations (including both main effects and interactions) survived correction for multiple comparisons. The results of the present study, therefore, must be interpreted with caution, and Se, Co, Pb, and Cu may be considered as possible prenatal risk factor candidates for DNA methylation alteration. Future studies with larger sample sizes are needed to further explore the identified main effects and two-way interactions, in addition to other environmental exposures.

CRediT authorship contribution statement

Kjell Vegard F. Weyde: Methodology, Formal analysis, Writing – original draft. **Ann-Karin Olsen:** Writing – review & editing. **Nur Duale:** Writing – review & editing. **Jorke H. Kamstra:** Writing – review & editing. **Thea S. Skogheim:** Writing – review & editing. **Ida H. Caspersen:** Writing – review & editing. **Stephanie M. Engel:** Methodology, Writing – review & editing. **Guido Biele:** Methodology, Writing – review & editing. **Yankai Xia:** Writing – review & editing. **Helle M. Meltzer:** Writing – review & editing. **Heidi Aase:** Conceptualization, Writing – review & editing. **Gro D. Villanger:** Conceptualization, Methodology, Writing – original draft, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This study was funded by The Research Council of Norway's "NeuroTox" project (grant no. 267984/E50). The Norwegian Mother, Father and Child Cohort Study (MoBa) is supported by the Norwegian Ministry of Health and Care Services and the Ministry of Education and Research, NIH/NINDS (grants no. 1 UO1 NS 047537-01 and 2 UO1 NS 047537-06A1). We are grateful to all the participating families in Norway who take part in this ongoing cohort study. Thanks to the Research Council of Norway through its Centres of Excellence funding scheme, project number223268/F50 CERAD.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.147621.

References

Ahmed, I., Hartikainen, A.-L., Järvelin, M.-R., Richardson, S., 2011. False discovery rate estimation for stability selection: application to genome-wide association studies. Stat. Appl. Genet. Mol. Biol. 10.

Alatise, O.I., Schrauzer, G.N., 2010. Lead exposure: a contributing cause of the current breast cancer epidemic in Nigerian women. Biol. Trace Elem. Res. 136, 127–139.

Alexander, J., Oskarsson, A., 2019. Toxic metals. Pages 67–73 Chemical Hazards in Foods of Animal Origin. Publishers, Wageningen Academic.

Alvarado-Cruz, I., Alegria-Torres, J.A., Montes-Castro, N., Jimenez-Garza, O., Quintanilla-Vega, B., 2018. Environmental epigenetic changes, as risk factors for the development of diseases in children: a systematic review. Ann Glob Health 84, 212–224.

Amoros, R., Murcia, M., Gonzalez, L., Soler-Blasco, R., Rebagliato, M., Iniguez, C., Carrasco, P., Vioque, J., Broberg, K., Levi, M., Lopez-Espinosa, M.J., Ballester, F., Llop, S., 2019. Maternal copper status and neuropsychological development in infants and preschool children. Int. J. Hyg. Environ. Health 222, 503–512.

Arai, Y., Ohgane, J., Yagi, S., Ito, R., Iwasaki, Y., Saito, K., Akutsu, K., Takatori, S., Ishii, R., Hayashi, R., Izumi, S.I., Sugino, N., Kondo, F., Horie, M., Nakazawa, H., Makino, T., Shiota, K., 2011. Epigenetic assessment of environmental chemicals detected in maternal peripheral and cord blood samples. J. Reprod. Dev. 57, 507–517.

ATSDR, 2007. Toxicological profile for Arsenic. Atlanta, Georgia, USA.

Aylward, L., Hays, S., Kirman, C., Marchitti, S., Kenneke, J., English, C., Mattison, D., Becker, R., 2014. Relationships of chemical concentrations in maternal and cord blood: a review of available data. Journal of Toxicology and Environmental Health, Part B 17, 175–203.

Bakulski, K.M., Lee, H., Feinberg, J.I., Wells, E.M., Brown, S., Herbstman, J.B., Witter, F.R., Halden, R.U., Caldwell, K., Mortensen, M.E., Jaffe, A.E., Moye Jr., J., Caulfield, L.E., Pan, Y., Goldman, L.R., Feinberg, A.P., Fallin, M.D., 2015. Prenatal mercury concentration is associated with changes in DNA methylation at TCEANC2 in newborns. Int. J. Epidemiol. 44, 1249–1262.

Banik, A., Kandilya, D., Ramya, S., Stunkel, W., Chong, Y.S., Dheen, S.T., 2017. Maternal factors that induce epigenetic changes contribute to neurological disorders in offspring. Genes (Basel) 8.

Barker, E.D., Walton, E., Cecil, C.A.M., 2018. Annual research review: DNA methylation as a mediator in the association between risk exposure and child and adolescent psychopathology. J. Child Psychol. Psychiatry 59, 303–322.

Bashar, S.K., Mitra, A.K., 2004. Effect of smoking on vitamin A, vitamin E, and other trace elements in patients with cardiovascular disease in Bangladesh: a cross-sectional study. Nutr. J. 3 (1), 1–5.

Basu, N., Head, J., Nam, D.H., Pilsner, J.R., Carvan, M.J., Chan, H.M., Goetz, F.W., Murphy, C.A., Rouvinen-Watt, K., Scheuhammer, A.M., 2013. Effects of methylmercury on epigenetic markers in three model species: mink, chicken and yellow perch. Comp. Biochem. Physiol. Toxicol. Pharmacol. 157, 322–327.

Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate - a practical and powerful approach to multiple testing. J. Royal Statist. Soc. Series B 57, 289–300.

Bennett, D., Bellinger, D.C., Birnbaum, L.S., DABT, A.T.S., Bradman, A., Chen, A., Cory-Slechta, D.A., Engel, S.M., Fallin, M.D., 2016. Project TENDR: targeting environmental

neuro-developmental risks the TENDR consensus statement. Environ. Health Perspect. 124, A118–A122.
Birgisdottir, B.E., Knutsen, H.K., Haugen, M., Gjelstad, I.M., Jenssen, M.T.S., Ellingsen, D.G., Thomassen, Y., Alexander, J., Meltzer, H.M., Brantsæter, A.L., 2013. Essential and toxic element concentrations in blood and urine and their associations with diet: results from a Norwegian population study including high-consumers of seafood and game. Sci. Total Environ. 463-464, 836–844.
Bitto, A., Pizzino, G., Irrera, N., Galfo, F., Squadrito, F., 2014. Epigenetic modifications due to heavy metals exposure in children living in polluted areas. Current Genomics 15, 464–468.
Bocca, B., F. Ruggieri, A. Pino, J. Rovira, G. Calamandrei, M. Martínez, J. L. Domingo, A. Alimonti, and M. Schuhmacher. 2019. Human biomonitoring to evaluate exposure to toxic and essential trace elements during pregnancy. Part A. Concentrations in maternal blood, urine and cord blood. Environmental Research **177**:108599.
Boeke, C.E., Baccarelli, A., Kleinman, K.P., Burris, H.H., Litonjua, A.A., Rifas-Shiman, S.L., Tarantini, L., Gillman, M., 2012. Gestational intake of methyl donors and global LINE-1 DNA methylation in maternal and cord blood: prospective results from a folate-replete population. Epigenetics 7, 253–260.
Bollati, V., Baccarelli, A., 2010. Environmental epigenetics. Heredity 105, 105–112.
Bollen, K.A., Jackman, R.W., 1990. Regression Diagnostics: An Expository Treatment of Outliers and Influential Cases. Sage Publications.
Bommarito, P.A., Martin, E., Fry, R.C., 2017. Effects of prenatal exposure to endocrine disruptors and toxic metals on the fetal epigenome. Epigenomics 9, 333–335.
Bose, R., Onishchenko, N., Edoff, K., Lang, A.M.J., Ceccatelli, S., 2012. Inherited effects of low-dose exposure to methylmercury in neural stem cells. Toxicol. Sci. 130, 383–390.
Brantsaeter, A.L., Haugen, M., Alexander, J., Meltzer, H.M., 2008. Validity of a new food frequency questionnaire for pregnant women in the Norwegian Mother and Child Cohort Study (MoBa). Matern.Child Nutr. 4, 28–43.
Breiman, L., 2001. Random forests. Mach. Learn. 45, 5–32.
Breton, C.V., Marsit, C.J., Faustman, E., Nadeau, K., Goodrich, J.M., Dolinoy, D.C., Herbstman, J., Holland, N., LaSalle, J.M., Schmidt, R., Yousefi, P., Perera, F., Joubert, B.R., Wiemels, J., Taylor, M., Yang, I.V., Chen, R., Hew, K.M., Freeland, D.M., Miller, R., Murphy, S.K., 2017. Small-magnitude effect sizes in epigenetic end points are important in children's environmental health studies: the children's environmental health and disease prevention research center's epigenetics working group. Environ. Health Perspect. 125, 511–526.
Calabrese, E.J., Baldwin, L.A., 2001. U-shaped dose-responses in biology, toxicology, and public health. Annu. Rev. Public Health 22, 15–33.
Cardenas, A., Koestler, D.C., Houseman, E.A., Jackson, B.P., Kile, M.L., Karagas, M.R., Marsit, C.J., 2015a. Differential DNA methylation in umbilical cord blood of infants exposed to mercury and arsenic in utero. Epigenetics 10, 508–515.
Cardenas, A., Koestler, D.C., Houseman, E.A., Jackson, B.P., Kile, M.L., Karagas, M.R., Marsit, C.J., 2015b. Differential DNA methylation in umbilical cord blood of infants exposed to mercury and arsenic in utero. Epigenetics 10, 508–515.
Cardenas, A., Rifas-Shiman, S.L., Agha, G., Hivert, M.-F., Litonjua, A.A., DeMeo, D.L., Lin, X., Amarasiriwardena, C.J., Oken, E., Gillman, M.W., Baccarelli, A.A., 2017a. Persistent DNA methylation changes associated with prenatal mercury exposure and cognitive performance during childhood. Sci. Rep. 7, 288.
Cardenas, A., Rifas-Shiman, S.L., Godderis, L., Duca, R.-C., Navas-Acien, A., Litonjua, A.A., DeMeo, D.L., Brennan, K.J., Amarasiriwardena, C.J., Hivert, M.-F., 2017b. Prenatal exposure to mercury: associations with global DNA methylation and hydroxymethylation in cord blood and in childhood. Environ. Health Perspect. 125, 087022.
Cardenas, A., Rifas-Shiman, S.L., Godderis, L., Duca, R.C., Navas-Acien, A., Litonjua, A.A., DeMeo, D.L., Brennan, K.J., Amarasiriwardena, C.J., Hivert, M.F., Gillman, M.W., Oken, E., Baccarelli, A.A., 2017c. Prenatal exposure to mercury: associations with global DNA methylation and hydroxymethylation in cord blood and in childhood. Environ. Health Perspect. 125, 087022.
Carvan Iii, M. J. 2020. Chapter 28 - Methylmercury induces transgenerationally transmissible epigenetic changes influencing zebrafish behavior. Pages 493-510 *in* R. T. Gerlai, editor. Behavioral and Neural Genetics of Zebrafish. Academic Press.
Carvan III, M.J., Kalluvila, T.A., Klingler, R.H., Larson, J.K., Pickens, M., Mora-Zamorano, F.X., Connaughton, V.P., Sadler-Riggleman, I., Beck, D., Skinner, M.K., 2017. Mercury-induced epigenetic transgenerational inheritance of abnormal neurobehavior is correlated with sperm epimutations in zebrafish. PLoS One 12, e0176155.
Caspersen, I.H., Thomsen, C., Haug, L.S., Knutsen, H.K., Brantsaeter, A.L., Papadopoulou, E., Erlund, I., Lundh, T., Alexander, J., Meltzer, H.M., 2019. Patterns and dietary determinants of essential and toxic elements in blood measured in mid-pregnancy: the Norwegian Environmental Biobank. Sci. Total Environ. 671, 299–308.
Chasapis, C.T., Loutsidou, A.C., Spiliopoulou, C.A., Stefanidou, M.E., 2012. Zinc and human health: an update. Arch. Toxicol. 86, 521–534.
Chen, Z., Myers, R., Wei, T., Bind, E., Kassim, P., Wang, G., Ji, Y., Hong, X., Caruso, D., Bartell, T., Gong, Y., Strickland, P., Navas-Acien, A., Guallar, E., Wang, X., 2014. Placental transfer and concentrations of cadmium, mercury, lead, and selenium in mothers, newborns, and young children. Journal of Exposure Science & Environmental Epidemiology 24, 537–544.
Cheng, T.F., Choudhuri, S., Muldoon-Jacobs, K., 2012. Epigenetic targets of some toxicologically relevant metals: a review of the literature. J. Appl. Toxicol. 32, 643–653.
Clifton, V.L., 2010. Review: sex and the human placenta: mediating differential strategies of fetal growth and survival. Placenta 31 (Suppl), S33–S39.
Colquitt, B.M., Allen, W.E., Barnea, G., Lomvardas, S., 2013. Alteration of genic 5-hydroxymethylcytosine patterning in olfactory neurons correlates with changes in gene expression and cell identity. Proc. Natl. Acad. Sci. 110, 14682–14687.
Dao, T., Cheng, R.Y., Revelo, M.P., Mitzner, W., Tang, W., 2014. Hydroxymethylation as a novel environmental biosensor. Curr Environ Health Rep 1, 1–10.
Desaulniers, D., Xiao, G.-h., Lian, H., Feng, Y.-L., Zhu, J., Nakai, J., Bowers, W.J., 2009. Effects of mixtures of polychlorinated biphenyls, methylmercury, and organochlorine pesticides on hepatic DNA methylation in prepubertal female Sprague-Dawley rats. Int. J. Toxicol. 28, 294–307.
Dorts, J., Falisse, E., Schoofs, E., Flamion, E., Kestemont, P., Silvestre, F., 2016. DNA methyltransferases and stress-related genes expression in zebrafish larvae after exposure to heat and copper during reprogramming of DNA methylation. Sci. Rep. 6, 34254.
Drake, A.J., O'Shaughnessy, P.J., Bhattacharya, S., Monteiro, A., Kerrigan, D., Goetz, S., Raab, A., Rhind, S.M., Sinclair, K.D., Meharg, A.A., Feldmann, J., Fowler, P.A., 2015. In utero exposure to cigarette chemicals induces sex-specific disruption of one-carbon metabolism and DNA methylation in the human fetal liver. BMC Med. 13, 18.
Egger, G., Liang, G., Aparicio, A., Jones, P.A., 2004. Epigenetics in human disease and prospects for epigenetic therapy. Nature 429, 457–463.
Faulk, C. 2019. Chapter 2-2 - implications of DNA methylation in toxicology. Pages 153-171 *in* S. D. McCullough and D. C. Dolinoy, editors. Toxicoepigenetics. Academic Press.
Gabory, A., Attig, L., Junien, C., 2011. Developmental programming and epigenetics. Am. J. Clin. Nutr. 94, 1943S–1952S.
Gailer, J., 2007. Arsenic–selenium and mercury–selenium bonds in biology. Coord. Chem. Rev. 251, 234–254.
Gambling, L., Danzeisen, R., Fosset, C., Andersen, H.S., Dunford, S., Srai, S.K.S., McArdle, H.J., 2003. Iron and copper interactions in development and the effect on pregnancy outcome. J. Nutr. 133, 1554S–1556S.
Gibney, E.R., Nolan, C.M., 2010. Epigenetics and gene expression. Heredity (Edinb) 105, 4–13.
Gibson, E.A., Goldsmith, J., Kioumourtzoglou, M.-A., 2019. Complex mixtures, complex analyses: an emphasis on interpretable results. Curr Environ Health Rep 6, 53–61.
Glasdam, S.-M., S. Glasdam, and G. H. Peters. 2016. The importance of magnesium in the human body: a systematic literature review. Pages 169-193 Advances in Clinical Chemistry. Elsevier.
Godfrey, K.M., Lillycrop, K.A., Burdge, G.C., Gluckman, P.D., Hanson, M.A., 2007. Epigenetic mechanisms and the mismatch concept of the developmental origins of health and disease. Pediatr. Res. 61, 5–10.
Goodrich, J.M., Basu, N., Franzblau, A., Dolinoy, D.C., 2013. Mercury biomarkers and DNA methylation among Michigan dental professionals. Environ. Mol. Mutagen. 54, 195–203.
Gorini, F., Muratori, F., Morales, M.A., 2014. The role of heavy metal pollution in neurobehavioral disorders: a focus on autism. Review Journal of Autism and Developmental Disorders 1, 354–372.
Grandjean, P., Weihe, P., Jørgensen, P.J., Clarkson, T., Cernichiari, E., Viderø, T., 1992. Impact of maternal seafood diet on fetal exposure to mercury, selenium, and lead. Archives of Environmental Health: An International Journal 47, 185–195.
Greenberg, M.V.C., Bourc'his, D., 2019. The diverse roles of DNA methylation in mammalian development and disease. Nat. Rev. Mol. Cell Biol. 20, 590–607.
Greenland, S., Pearl, J., Robins, J.M., 1999. Causal diagrams for epidemiologic research. Epidemiology 10, 37–48.
Gulson, B.L., Mizon, K.J., Korsch, M.J., Palmer, J.M., Donnelly, J.B., 2003. Mobilization of lead from human bone tissue during pregnancy and lactation–a summary of long-term research. Sci. Total Environ. 303, 79–104.
Hanna, C.W., Bloom, M.S., Robinson, W.P., Kim, D., Parsons, P.J., vom Saal, F.S., Taylor, J.A., Steuerwald, A.J., Fujimoto, V.Y., 2012. DNA methylation changes in whole blood is associated with exposure to the environmental contaminants, mercury, lead, cadmium and bisphenol A, in women undergoing ovarian stimulation for IVF. Hum. Reprod. 27, 1401–1410.
Harrell Jr., F.E., 2015. Regression Modeling Strategies: With Applications to Linear Models, Logistic and Ordinal Regression, and Survival Analysis. Springer.
Haug, L.S., Sakhi, A.K., Cequier, E., Casas, M., Maitre, L., Basagana, X., Andrusaityte, S., Chalkiadaki, G., Chatzi, L., Coen, M., de Bont, J., Dedele, A., Ferrand, J., Grazuleviciene, R., Gonzalez, J.R., Gutzkow, K.B., Keun, H., McEachan, R., Meltzer, H.M., Petraviciene, I., Robinson, O., Saulnier, P.J., Slama, R., Sunyer, J., Urquiza, J., Vafeiadi, M., Wright, J., Vrijheid, M., Thomsen, C., 2018. In-utero and childhood chemical exposome in six European mother-child cohorts. Environ. Int. 121, 751–763.
Haugen, M., Brantsæter, A.L., Alexander, J., Meltzer, H.M., 2008. Dietary supplements contribute substantially to the total nutrient intake in pregnant Norwegian women. Ann. Nutr. Metab. 52, 272–280.
Head, J.A., 2014. Patterns of DNA methylation in animals: an ecotoxicological perspective. Integr. Comp. Biol. 54, 77–86.
Honaker, J., King, G., Blackwell, M., 2011. Amelia II: a program for missing data. J. Stat. Softw. 45, 1–47.
Jablonska, E., Reszka, E., 2017. Selenium and epigenetics in cancer: focus on DNA methylation. Adv. Cancer Res. 136, 193–234.
Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B.B., Beeregowda, K.N., 2014. Toxicity, mechanism and health effects of some heavy metals. Interdiscip. Toxicol. 7, 60–72.
Järup, L., 2003. Hazards of heavy metal contamination. Br. Med. Bull. 68, 167–182.
Jirtle, R.L., Skinner, M.K., 2007. Environmental epigenomics and disease susceptibility. Nat. Rev. Genet. 8, 253–262.
Johnson, D.R., Young, R., 2011. Toward best practices in analyzing datasets with missing data: comparisons and recommendations. J. Marriage Fam. 73 (5), 926–945.
Jones, M.J., Goodman, S.J., Kobor, M.S., 2015. DNA methylation and healthy human aging. Aging Cell 14, 924–932.
Jones, P.A., 2012. Functions of DNA methylation: islands, start sites, gene bodies and beyond. Nat. Rev. Genet. 13, 484.
Joubert, B.R., Felix, J.F., Yousefi, P., Bakulski, K.M., Just, A.C., Breton, C., Reese, S.E., Markunas, C.A., Richmond, R.C., Xu, C.J., Kupers, L.K., Oh, S.S., Hoyo, C., Gruzieva, O., Soderhal, C., Salas, L.A., Baiz, N., Zhang, H.M., Lepeule, J., Ruiz, C., Ligthart, S., Wang, T.Y., Taylor, J.A., Duijts, L., Sharp, G.C., Jankipersadsing, S.A., Nilsen, R.M., Vaez, A., Fallin, M.D., Hu, D.L., Litonjua, A.A., Fuemmeler, B.F., Huen, K., Kere, J., Kull, I., Munthe-Kaas, M.C., Gehring, U., Bustamante, M., Saurel-Coubizolles, M.J., Quraishi, B.M., Ren, J., Tost, J., Gonzalez, J.R., Peters, M.J., Haberg, S.E., Xu, Z.L., van Meurs, J.B.,

Gaunt, T.R., Kerkhof, M., Corpeleijn, E., Feinberg, A.P., Eng, C., Baccarelli, A.A., Neelon, S.E.B., Bradman, A., Merid, S.K., Bergstrom, A., Herceg, Z., Hernandez-Vargas, H., Brunekreef, B., Pinart, M., Heude, B., Ewart, S., Yao, J., Lemonnier, N., Franco, O.H., Wu, M.C., Hofman, A., McArdle, W., Van der Vlies, P., Falahi, F., Gillman, M.W., Barcellos, L.F., Kumar, A., Wickman, M., Guerra, S., Charles, M.A., Holloway, J., Auffray, C., Tiemeier, H.W., Smith, G.D., Postma, D., Hivert, M.F., Eskenazi, B., Vrijheid, M., Arshad, H., Anto, J.M., Dehghan, A., Karmaus, W., Annesi-Maesano, I., Sunyer, J., Ghantous, A., Pershagen, G., Hollands, N., Murphys, S.K., DeMeo, D.L., Burchard, E.G., Ladd-Acosta, C., Snieder, H., Nystad, W., Koppelman, G.H., Relton, C.L., Jaddoe, V.W.V., Wilcox, A., Melen, E., London, S.J., 2016. DNA methylation in newborns and maternal smoking in pregnancy: genome-wide consortium meta-analysis. Am. J. Hum. Genet. 98, 680–696.

Julshamn, K., Nilsen, B.M., Frantzen, S., Valdersnes, S., Maage, A., Nedreaas, K., Sloth, J.J., 2012. Total and inorganic arsenic in fish samples from Norwegian waters. Food Additives and Contaminants: Part B 5, 229–235.

Kamstra, J.H., Sales, L.B., Alestrom, P., Legler, J., 2017. Differential DNA methylation at conserved non-genic elements and evidence for transgenerational inheritance following developmental exposure to mono(2-ethylhexyl) phthalate and 5-azacytidine in zebrafish. Epigenetics Chromatin 10, 20.

Kantidze, O.L., Razin, S.V., 2017. 5-Hydroxymethylcytosine in DNA repair: a new player or a red herring? Cell Cycle 16, 1499–1501.

Keil, A., Buckley, J., O'Brien, K., Ferguson, K., Zhao, S., White, A., 2019. A quantile-based g-computation approach to addressing the effects of exposure mixtures. Environmental Epidemiology 3, 44.

King, J.C., 2000. Physiology of pregnancy and nutrient metabolism. Am. J. Clin. Nutr. 71, 1218S–1225S.

Kocyigit, A., et al., 2001. Effects of tobacco smoking on plasma selenium, zinc, copper and iron concentrations and related antioxidative enzyme activities. Clin. Biochem. 34 (8), 629–633.

Kosnett, M.J., Wedeen, R.P., Rothenberg, S.J., Hipkins, K.L., Materna, B.L., Schwartz, B.S., Hu, H., Woolf, A., 2007. Recommendations for medical management of adult lead exposure. Environ. Health Perspect. 115, 463–471.

Lam, L.L., Emberly, E., Fraser, H.B., Neumann, S.M., Chen, E., Miller, G.E., Kobor, M.S., 2012. Factors underlying variable DNA methylation in a human community cohort. Proc. Natl. Acad. Sci. 109, 17253–17260.

Latham, K.E., Sapienza, C., Engel, N., 2012. The epigenetic lorax: gene-environment interactions in human health. Epigenomics 4, 383–402.

Lau, A.T.Y., Tan, H.W., Xu, Y.-M., 2017. Epigenetic effects of dietary trace elements. Current Pharmacology Reports 3, 232–241.

Leung, Y.-K., Ouyang, B., Niu, L., Xie, C., Ying, J., Medvedovic, M., Chen, A., Weihe, P., Valvi, D., Grandjean, P., Ho, S.-M., 2018. Identification of sex-specific DNA methylation changes driven by specific chemicals in cord blood in a Faroese birth cohort. Epigenetics 13, 290–300.

Lim, S., Ha, M., Hwang, S.-S., Son, M., Kwon, H.-J., 2015. Disparities in children's blood Lead and mercury levels according to community and individual socioeconomic positions. Int. J. Environ. Res. Public Health 12, 6232–6248.

Maccani, J.Z., Koestler, D.C., Lester, B., Houseman, E.A., Armstrong, D.A., Kelsey, K.T., Marsit, C.J., 2015. Placental DNA methylation related to both infant toenail mercury and adverse neurobehavioral outcomes. Environ. Health Perspect. 123, 723–729.

Magnus, P., Irgens, L.M., Haug, K., Nystad, W., Skjaerven, R., Stoltenberg, C., G. MoBa Study, 2006. Cohort profile: the Norwegian Mother and Child Cohort Study (MoBa). Int. J. Epidemiol. 35, 1146–1150.

Magnus, P., Birke, C., Vejrup, K., Haugan, A., Alsaker, E., Daltveit, A.K., Handal, M., Haugen, M., Hoiseth, G., Knudsen, G.P., Paltiel, L., Schreuder, P., Tambs, K., Vold, L., Stoltenberg, C., 2016. Cohort profile update: the Norwegian Mother and Child Cohort Study (MoBa). Int. J. Epidemiol. 45, 382–388.

Martin, E., Smeester, L., Bommarito, P.A., Grace, M.R., Boggess, K., Kuban, K., Karagas, M.R., Marsit, C.J., O'Shea, T.M., Fry, R.C., 2017. Sexual epigenetic dimorphism in the human placenta: implications for susceptibility during the prenatal period. Epigenomics 9, 267–278.

Martin, E.M., Fry, R.C., 2018. Environmental influences on the epigenome: exposure- associated DNA methylation in human populations. Annu. Rev. Public Health 39, 309–333.

Martín-del-Campo, R., Bárcenas-Ibarra, A., Lund, G., Rodríguez-Ríos, D., Yong-Villalobos, L., García-Hernández, J., García-Gasca, A., 2019. Mercury concentration, DNA methylation, and mitochondrial DNA damage in olive Ridley Sea turtle embryos with Schistosomus Reflexus syndrome. Vet. Pathol. 56, 940–949.

McKay, J.A., Groom, A., Potter, C., Coneyworth, L.J., Ford, D., Mathers, J.C., Relton, C.L., 2012. Genetic and non-genetic influences during pregnancy on infant global and site specific DNA methylation: role for folate gene variants and vitamin B12. PLoS One 7, e33290.

Medici, V., Shibata, N.M., Kharbanda, K.K., Islam, M.S., Keen, C.L., Kim, K., Tillman, B., French, S.W., Halsted, C.H., LaSalle, J.M., 2014. Maternal choline modifies fetal liver copper, gene expression, DNA methylation, and neonatal growth in the tx-j mouse model of Wilson disease. Epigenetics 9, 286–296.

Meinshausen, N., Bühlmann, P., 2010. Stability selection. Journal of the Royal Statistical Society: Series B (Statistical Methodology) 72, 417–473.

Meltzer, H.M., Brantsaeter, A.L., Ydersbond, T.A., Alexander, J., Haugen, M., 2008. Methodological challenges when monitoring the diet of pregnant women in a large study: experiences from the Norwegian Mother and Child Cohort Study (MoBa). Matern. Child Nutr. 4, 14–27.

Meltzer, H.M., Alexander, J., Brantsæter, A.L., Borch-Iohnsen, B., Ellingsen, D.G., Thomassen, Y., Holmen, J., Ydersbond, T.A., 2016. The impact of iron status and smoking on blood divalent metal concentrations in Norwegian women in the HUNT2 Study. J. Trace Elem. Med. Biol. 38, 165–173.

Molin, M., Ulven, S.M., Meltzer, H.M., Alexander, J., 2015. Arsenic in the human food chain, biotransformation and toxicology–review focusing on seafood arsenic. J. Trace Elem. Med. Biol. 31, 249–259.

Montazeri, P., Thomsen, C., Casas, M., de Bont, J., Haug, L.S., Maitre, L., Papadopoulou, E., Sakhi, A.K., Slama, R., Saulnier, P.J., Urquiza, J., Grazuleviciene, R., Andrusaityte, S., McEachan, R., Wright, J., Chatzi, L., Basagana, X., Vrijheid, M., 2019. Socioeconomic position and exposure to multiple environmental chemical contaminants in six European mother-child cohorts. Int. J. Hyg. Environ. Health 222, 864–872.

Nakayama, S. M. M., H. Nakata, Y. Ikenaka, J. Yabe, B. Oroszlany, Y. B. Yohannes, N. Bortey-Sam, K. Muzandu, K. Choongo, T. Kuritani, M. Nakagawa, and M. Ishizuka. 2019. One year exposure to Cd- and Pb-contaminated soil causes metal accumulation and alteration of global DNA methylation in rats. Environmental Pollution (Barking, Essex: 1987) **252**:1267-1276.

Narváez, D.M., Groot, H., Diaz, S.M., Palma, R.M., Muñoz, N., Cros, M.-P., Hernández-Vargas, H., 2017. Oxidative stress and repetitive element methylation changes in artisanal gold miners occupationally exposed to mercury. Heliyon 3, e00400.

Needham, L.L., Grandjean, P., Heinzow, B., Jørgensen, P.J., Nielsen, F., Patterson, D.G., Sjödin, A., Turner, W.E., Weihe, P., 2011. Partition of environmental chemicals between maternal and fetal blood and tissues. Environ. Sci. Technol. 45, 1121–1126.

Niehoff, N.M., Keil, A.P., O'Brien, K.M., Jackson, B.P., Karagas, M.R., Weinberg, C.R., White, A.J., 2020. Metals and trace elements in relation to body mass index in a prospective study of US women. Environ. Res. 184, 109396.

Nilsen, F.M., Parrott, B.B., Bowden, J.A., Kassim, B.L., Somerville, S.E., Bryan, T.A., Bryan, C.E., Lange, T.R., Delaney, J.P., Brunell, A.M., 2016. Global DNA methylation loss associated with mercury contamination and aging in the American alligator (Alligator mississippiensis). Sci. Total Environ. 545, 389–397.

Nilsen, R.M., Vollset, S.E., Gjessing, H.K., Skjaerven, R., Melve, K.K., Schreuder, P., Alsaker, E.R., Haug, K., Daltveit, A.K., Magnus, P., 2009. Self-selection and bias in a large prospective pregnancy cohort in Norway. Paediatr. Perinat. Epidemiol. 23, 597–608.

Nishizawa-Jotaki, S., Sakurai, K., Eguchi, A., Tanabe, H., Watanabe, M., Mori, C., 2020. Association between mercury in cord serum and sex-specific DNA methylation in cord tissues. J. Dev. Orig. Health Dis. 1–8.

Overgaard, K.R., Oerbeck, B., Friis, S., Pripp, A.H., Biele, G., Aase, H., Zeiner, P., 2018. Attention-deficit/hyperactivity disorder in preschoolers: the accuracy of a short screener. J. Am. Acad. Child Adolesc. Psychiatry 57, 428–435.

Overgaard, K.R., et al., 2019. Screening with an ADHD-specific rating scale in preschoolers: a cross-cultural comparison of the Early Childhood Inventory-4. Psychol. Assess. 31 (8), 985.

Paltiel, L., Haugan, A., Skjerden, T., Harbak, K., Baekken, S., Stensrud, N.K., Knudsen, G.P., Magnus, P., 2014. The biobank of the Norwegian mother and child cohort study - present status. Norsk Epidemiologi 24, 29–35.

Papadopoulou, E., Haug, L.S., Sakhi, A.K., Andrusaityte, S., Basagaña, X., Brantsaeter, A.L., Casas, M., Fernández-Barrés, S., Grazuleviciene, R., Knutsen, H.K., Maitre, L., Meltzer, H.M., McEachan, R.R.C., Roumeliotaki, T., Slama, R., Vafeiadi, M., Wright, J., Vrijheid, M., Thomsen, C., Chatzi, L., 2019. Diet as a source of exposure to environmental contaminants for pregnant women and children from six European countries. Environ. Health Perspect. 127, 107005.

Perera, F., and J. Herbstman. 2011a. Prenatal environmental exposures, epigenetics, and disease. Reproductive Toxicology (Elmsford, N.Y.) **31**:363-373.

Perera, F., Herbstman, J., 2011b. Prenatal environmental exposures, epigenetics, and disease. Reprod. Toxicol. 31, 363–373.

Pilsner, J.R., Liu, X., Ahsan, H., Ilievski, V., Slavkovich, V., Levy, D., Factor-Litvak, P., Graziano, J.H., Gamble, M.V., 2007. Genomic methylation of peripheral blood leukocyte DNA: influences of arsenic and folate in Bangladeshi adults. Am. J. Clin. Nutr. 86, 1179–1186.

Pilsner, J.R., Hu, H., Ettinger, A., Sánchez, B.N., Wright, R.O., Cantonwine, D., Lazarus, A., Lamadrid-Figueroa, H., Mercado-García, A., Téllez-Rojo, M.M., Hernández-Avila, M., 2009. Influence of prenatal Lead exposure on genomic methylation of cord blood DNA. Environ. Health Perspect. 117, 1466–1471.

Pilsner, J.R., Lazarus, A.L., Nam, D.H., Letcher, R.J., Sonne, C., Dietz, R., Basu, N., 2010. Mercury-associated DNA hypomethylation in polar bear brains via the LUminometric Methylation Assay: a sensitive method to study epigenetics in wildlife. Mol. Ecol. 19, 307–314.

Pilsner, J.R., Hall, M.N., Liu, X., Ahsan, H., Ilievski, V., Slavkovich, V., Levy, D., Factor-Litvak, P., Graziano, J.H., Gamble, M.V., 2011. Associations of plasma selenium with arsenic and genomic methylation of leukocyte DNA in Bangladesh. Environ. Health Perspect. 119, 113–118.

Pilsner, J.R., Hall, M.N., Liu, X., Ilievski, V., Slavkovich, V., Levy, D., Factor-Litvak, P., Yunus, M., Rahman, M., Graziano, J.H., Gamble, M.V., 2012. Influence of prenatal arsenic exposure and newborn sex on global methylation of cord blood DNA. PLoS One 7, e37147.

Price, E.M., Cotton, A.M., Penaherrera, M.S., McFadden, D.E., Kobor, M.S., Robinson, W., 2012. Different measures of "genome-wide" DNA methylation exhibit unique properties in placental and somatic tissues. Epigenetics 7, 652–663.

R Core Team, 2017. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Rahman, A., Kumarathasan, P., Gomes, J., 2016. Infant and mother related outcomes from exposure to metals with endocrine disrupting properties during pregnancy. Sci. Total Environ. 569-570, 1022–1031.

Ralston, N.V., Raymond, L.J., 2010. Dietary selenium's protective effects against methylmercury toxicity. Toxicology 278, 112–123.

Ramon, R., Murcia, M., Aguinagalde, X., Amurrio, A., Llop, S., Ibarluzea, J., Lertxundi, A., Alvarez-Pedrerol, M., Casas, M., Vioque, J., Sunyer, J., Tardon, A., Martinez-Arguelles, B., Ballester, F., 2011. Prenatal mercury exposure in a multicenter cohort study in Spain. Environ. Int. 37, 597–604.

Ray, P.D., Yosim, A., Fry, R.C., 2014. Incorporating epigenetic data into the risk assessment process for the toxic metals arsenic, cadmium, chromium, lead, and mercury: strategies and challenges. Front. Genet. 5.
Rayman, M.P., 2012. Selenium and human health. Lancet 379 (9822), 1256–1268.
Reichard, J.F., Puga, A., 2010. Effects of arsenic exposure on DNA methylation and epigenetic gene regulation. Epigenomics 2, 87–104.
Reynolds, L.P., Caton, J.S., Redmer, D.A., Grazul-Bilska, A.T., Vonnahme, K.A., Borowicz, P.P., Luther, J.S., Wallace, J.M., Wu, G., Spencer, T.E., 2006. Evidence for altered placental blood flow and vascularity in compromised pregnancies. J. Physiol. 572, 51–58.
Richard Pilsner, J., Lazarus, A.L., Nam, D.-H., Letcher, R.J., Sonne, C., Dietz, R., Basu, N., 2010. Mercury-associated DNA hypomethylation in polar bear brains via the LUminometric Methylation Assay: a sensitive method to study epigenetics in wildlife. Mol. Ecol. 19, 307–314.
Robertson, K.D., 2005. DNA methylation and human disease. Nat. Rev. Genet. 6, 597–610.
Roman, M., Jitaru, P., Barbante, C., 2014. Selenium biochemistry and its role for human health. Metallomics 6, 25–54.
Rubin, D.B., 1987. Multiple Imputation for Nonresponse in Surveys. John Wiley & Sons.
Ruiz-Hernandez, A., Kuo, C.C., Rentero-Garrido, P., Tang, W.Y., Redon, J., Ordovas, J.M., Navas-Acien, A., Tellez-Plaza, M., 2015. Environmental chemicals and DNA methylation in adults: a systematic review of the epidemiologic evidence. Clin. Epigenetics 7, 55.
Rusiecki, J., Baccarelli, A., Bollati, V., Moore, L., Bonefeld-Jorgensen, E., 2007. DNA methylation among Greenlandic Inuit with varying levels of exposures to specific persistent organic pollutants. Epidemiology 18, S151–S152.
Saif, Z., Hodyl, N.A., Hobbs, E., Tuck, A.R., Butler, M.S., Osei-Kumah, A., Clifton, V.L., 2014. The human placenta expresses multiple glucocorticoid receptor isoforms that are altered by fetal sex, growth restriction and maternal asthma. Placenta 35, 260–268.
Saunders, C.M., Rehbinder, E.M., Carlsen, K.C.L., Gudbrandsgard, M., Carlsen, K.H., Haugen, G., Hedlin, G., Jonassen, C.M., Sjøborg, K.D., Landrø, L., Nordlund, B., Rudi, K., H, O.S., Söderhäll, C., Staff, A.C., Vettukattil, R., Carlsen, M.H., 2019. Food and nutrient intake and adherence to dietary recommendations during pregnancy: a Nordic mother-child population-based cohort. Food Nutr Res 63.
Shih, R.A., Hu, H., Weisskopf, M.G., Schwartz, B.S., 2007. Cumulative Lead dose and cognitive function in adults: a review of studies that measured both blood Lead and bone lead. Environ. Health Perspect. 115, 483–492.
Shrier, I., Platt, R.W., 2008. Reducing bias through directed acyclic graphs. BMC Med. Res. Methodol. 8, 70.
Singh, G., Singh, V., Wang, Z.-X., Voisin, G., Lefebvre, F., Navenot, J., Evans, B., Verma, M., Anderson, D., Schneider, J., 2018. Effects of developmental lead exposure on the hippocampal methylome: influences of sex and timing and level of exposure. Toxicol. Lett. 290, 63–72.
Skaar, D.A., Murphy, S.K., Hoyo, C., 2016. Effects of environmentally acquired heavy metals and nutrients on the epigenome and phenotype. In: Hughes, C.L., Waters, M.D. (Eds.), Translational Toxicology: Defining a New Therapeutic Discipline. Springer International Publishing, Cham, pp. 139–169.
Skinner, M.K., Manikkam, M., Guerrero-Bosagna, C., 2011. Epigenetic transgenerational actions of endocrine disruptors. Reprod. Toxicol. 31, 337–343.
Smith, Z.D., Meissner, A., 2013. DNA methylation: roles in mammalian development. Nat. Rev. Genet. 14, 204–220.
Speckmann, B., Grune, T., 2015. Epigenetic effects of selenium and their implications for health. Epigenetics 10, 179–190.
Speckmann, B., Schulz, S., Hiller, F., Hesse, D., Schumacher, F., Kleuser, B., Geisel, J., Obeid, R., Grune, T., Kipp, A.P., 2017. Selenium increases hepatic DNA methylation and modulates one-carbon metabolism in the liver of mice. J. Nutr. Biochem. 48, 112–119.
Spiers, H., Hannon, E., Schalkwyk, L.C., Bray, N.J., Mill, J., 2017. 5-hydroxymethylcytosine is highly dynamic across human fetal brain development. BMC Genomics 18, 738.
StataCorp. 2017. Stata version 15.0. Texas, USA.
Steinberg, J., Shah, K.M., Gartland, A., Zeggini, E., Wilkinson, J.M., 2017. Effects of chronic cobalt and chromium exposure after metal-on-metal hip resurfacing: an epigenome-wide association pilot study. J. Orthop. Res. 35, 2323–2328.
Streiner, D.L., 2015. Best (but oft-forgotten) practices: the multiple problems of multiplicity—whether and how to correct for many statistical tests. Am. J. Clin. Nutr. 102, 721–728.
Sun, H., Chen, W., Wang, D., Jin, Y., Chen, X., Xu, Y., 2014. The effects of prenatal exposure to low-level cadmium, lead and selenium on birth outcomes. Chemosphere 108, 33–39.
Szyf, M., 2016. The elusive role of 5′-hydroxymethylcytosine. Epigenomics 8, 1539–1551.
Tapp, H.S., Commane, D.M., Bradburn, D.M., Arasaradnam, R., Mathers, J.C., Johnson, I.T., Belshaw, N.J., 2013. Nutritional factors and gender influence age-related DNA methylation in the human rectal mucosa. Aging Cell 12, 148–155.
Tchounwou, P. B., C. G. Yedjou, A. K. Patlolla, and D. J. Sutton. 2012. Heavy metal toxicity and the environment. Pages 133-164 Molecular, Clinical and Environmental Toxicology. Springer.
Téllez-Rojo, M.M., Hernández-Avila, M., Lamadrid-Figueroa, H., Smith, D., Hernández-Cadena, L., Mercado, A., Aro, A., Schwartz, J., Hu, H., 2004. Impact of bone lead and bone resorption on plasma and whole blood lead levels during pregnancy. Am. J. Epidemiol. 160, 668–678.
Textor, J., J. Hardt, and S. Knüppel. 2011. DAGitty. A graphical tool for analyzing causal diagrams. Epidemiology **22**:745.
Tian, F. Y., T. M. Everson, B. Lester, T. Punshon, B. P. Jackson, K. Hao, C. Lesseur, J. Chen, M. R. Karagas, and C. J. Marsit. 2020. Selenium-associated DNA methylation modifications in placenta and neurobehavioral development of newborns: an epigenome-wide study of two U.S. birth cohorts. Environment International **137**:105508.
Tran, N.Q.V., Miyake, K., 2017. Neurodevelopmental disorders and environmental toxicants: epigenetics as an underlying mechanism. Int J Genomics 2017, 7526592.
Tyrrell, J., Melzer, D., Henley, W., Galloway, T.S., Osborne, N.J., 2013. Associations between socioeconomic status and environmental toxicant concentrations in adults in the USA: NHANES 2001–2010. Environ. Int. 59, 328–335.
Valko, M., Morris, H., Cronin, M.T.D., 2005. Metals, toxicity and oxidative stress. Curr. Med. Chem. 12, 1161–1208.
van Otterdijk, S.D., et al., 2017. Locus-specific DNA methylation in the placenta is associated with levels of pro-inflammatory proteins in cord blood and they are both independently affected by maternal smoking during pregnancy. Epigenetics 12 (10), 875–885.
Varret, C., Beronius, A., Bodin, L., Bokkers, B.G.H., Boon, P.E., Burger, M., De Wit-Bos, L., Fischer, A., Hanberg, A., Litens-Karlsson, S., Slob, W., Wolterink, G., Zilliacus, J., Beausoleil, C., Rousselle, C., 2018. Evaluating the evidence for non-monotonic dose-response relationships: a systematic literature review and (re-)analysis of in vivo toxicity data in the area of food safety. Toxicol. Appl. Pharmacol. 339, 10–23.
Vrijheid, M., Martinez, D., Aguilera, I., Ballester, F., Basterrechea, M., Esplugues, A., Guxens, M., Larrañaga, M., Lertxundi, A., Mendez, M., Murcia, M., Marina, L.S., Villanueva, C.M., Sunyer, J., 2012. Socioeconomic status and exposure to multiple environmental pollutants during pregnancy: evidence for environmental inequity? J. Epidemiol. Community Health 66, 106–113.
Vrijheid, M., Casas, M., Gascon, M., Valvi, D., Nieuwenhuijsen, M., 2016. Environmental pollutants and child health-a review of recent concerns. Int. J. Hyg. Environ. Health 219, 331–342.
Wielsøe, M., Tarantini, L., Bollati, V., Long, M., Bonefeld-Jørgensen, E.C., 2019. DNA methylation level in blood and relations to breast cancer, risk factors and environmental exposure in Greenlandic Inuit women (Basic & Clinical Pharmacology & Toxicology n/a).
Wright, R.O., Schwartz, J., Wright, R.J., Bollati, V., Tarantini, L., Park, S.K., Hu, H., Sparrow, D., Vokonas, P., Baccarelli, A., 2010. Biomarkers of Lead exposure and DNA methylation within retrotransposons. Environ. Health Perspect. 118, 790–795.
Wu, H.C., Wang, Q., Yang, H.I., Tsai, W.Y., Chen, C.J., Santella, R.M., 2012b. Global DNA methylation levels in white blood cells as a biomarker for hepatocellular carcinoma risk: a nested case-control study. Carcinogenesis 33, 1340–1345.
Wu, H.-C., Wang, Q., Delgado-Cruzata, L., Santella, R.M., Terry, M.B., 2012a. Genomic methylation changes over time in peripheral blood mononuclear cell DNA: differences by assay type and baseline values. Cancer epidemiology, Biomarkers & Prevention: a Publication of the American Association for Cancer Research, Cosponsored by the American Society of Preventive Oncology 21, 1314–1318.
Yagci, S., Yildirim, E., Yildirim, N., Shams, M., Agar, G., 2019. Nitric oxide alleviates the effects of copper-induced DNA methylation, genomic instability, LTR retrotransposon polymorphism and enzyme activity in lettuce. Plant Physiology Reports 24, 289–295.
Zeng, Y., Chen, T., 2019. DNA methylation reprogramming during mammalian development. Genes 10, 257.
Zhang, Q., Zheng, S., Wang, S., Jiang, Z., Xu, S., 2019. The effects of low selenium on DNA methylation in the tissues of chickens. Biol. Trace Elem. Res. 191, 474–484.
Zhao, J., Goldberg, J., Bremner, J.D., Vaccarino, V., 2012. Global DNA methylation is associated with insulin resistance: a monozygotic twin study. Diabetes 61, 542–546.
Zhou, X., Zhu, G., Mwalilino, J., Sun, J., 2001. Influence of Cu, Zn, Pb, Cd and their heavy metalion mixture on the DNA methylation level of the fish (Carassius auratus). Zhongguo Huanjing Kexue 21, 549–552.
Zoroddu, M.A., Aaseth, J., Crisponi, G., Medici, S., Peana, M., Nurchi, V.M., 2019. The essential metals for humans: a brief overview. J. Inorg. Biochem. 195, 120–129.
Zou, H., Hastie, T., 2005. Regularization and variable selection via the elastic net. Journal of the Royal Statistical Society: Series B (Statistical Methodology) 67, 301–320.