Pregnancy exposure to synthetic phenols and placental DNA methylation — An epigenome-wide association study in male infants from the EDEN cohort

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CRediT authorship contribution statement

Jedynak had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Heude, Philippat, Lepeule. Cohort data collection: Heude. Acquisition, analysis, or interpretation of data: Calafat, Heude, Tost, Busato, Vaiman, Jedynak, Philippat, Lepeule, Slama. Drafting of the manuscript: Jedynak, Lepeule, Philippat. Statistical analysis: Jedynak. Obtained funding: Philippat, Slama, Lepeule. Technical or material support: Bourova-Flin, Forhan, Jakobi, Rousseaux, Schwartz, Tost. Study supervision: Philippat, Lepeule. All authors have read, commented on and approved the manuscript.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2021.118024.
Abstract

In utero exposure to environmental chemicals, such as synthetic phenols, may alter DNA methylation in different tissues, including placenta — a critical organ for fetal development. We studied associations between prenatal urinary biomarker concentrations of synthetic phenols and placental DNA methylation. Our study involved 202 mother-son pairs from the French EDEN cohort. Nine phenols were measured in spot urine samples collected between 22 and 29 gestational weeks. We performed DNA methylation analysis of the fetal side of placental tissues using the IlluminaHM450 BeadChips. We evaluated methylation changes of individual CpGs in an adjusted epigenome-wide association study (EWAS) and identified differentially methylated regions (DMRs). We performed mediation analysis to test whether placental tissue heterogeneity mediated the association between urinary phenol concentrations and DNA methylation. We identified 46 significant DMRs (≥5 CpGs) associated with triclosan (37 DMRs), 2,4-dichlorophenol (3), benzophenone-3 (3), methyl-(2) and propylparaben (1). All but 2 DMRs were positively associated with phenol concentrations. Out of the 46 identified DMRs, 7 (6 for triclosan) encompassed imprinted genes (APC, FOXG1, GNAS, GNASAS, MIR886, PEG10, SGCE), which represented a significant enrichment. Other identified DMRs encompassed genes encoding proteins responsible for cell signaling, transmembrane transport, cell adhesion, inflammatory, apoptotic and immunological response, genes encoding transcription factors, histones, tumor suppressors, genes involved in tumorigenesis and several cancer risk biomarkers. Mediation analysis suggested that placental cell heterogeneity may partly explain these associations. This is the first study describing the genome-wide modifications of placental DNA methylation associated with pregnancy exposure to synthetic phenols or their precursors. Our results suggest that cell heterogeneity might mediate the effects of triclosan exposure on placental DNA methylation. Additionally, the enrichment of imprinted genes within the DMRs suggests mechanisms by which certain exposures, mainly to triclosan, could affect fetal development.

Keywords

Placenta; DNA methylation; Prenatal exposure; Triclosan; Bisphenol A; Alu and LINE-1

1. Introduction

Pregnancy exposure to environmental chemicals has been associated with developmental disorders, birth defects and child health issues later in life (Kishi and Grandjean, 2020). These effects might be mediated by the placenta, which has a critical function in fetal development. The role of this organ in the developmental origins of health and disease (DOHaD) paradigm makes it an important target for research aiming to understand its function as a mediator between the environment and the maternal, fetal, and child health. Moreover, its epigenetic landscape may provide a “molecular archive” of exposures occurring during pregnancy (Heijmans et al., 2009).

Among the chemicals suspected to affect child health, some have been associated with epigenetic changes occurring in different tissues, including placenta (reviewed by Alvarado-Cruz et al., 2018). Herein we focused on placental DNA methylation which has been proposed as one of the potential mechanisms of action of environmental chemicals on health.
outcomes associated with in utero exposure (reviewed by Vlahos et al., 2019). We studied one family of chemicals suspected to have endocrine disrupting properties i.e., synthetic phenols (The International Panel on Chemical Pollution (IPCP) 2017). Synthetic phenols are industrial precursors to many materials and products, which are in turn used for production of polycarbonates and epoxy resins (bisphenols), ultraviolet filters used in cosmetics and food packaging (benzophenone-3 also known as oxybenzone), biocides in personal care products (triclosan), antimicrobial and preserving agents in cosmetics, pharmaceuticals, and food (parabens). Some, like dichlorophenols, are metabolites of substances used to produce herbicides and insecticides.

Exposure to synthetic phenols (or their precursors) in the general population is widespread (Haug et al., 2018; Rolland et al., 2020) and some can cross the placental barrier (Bai et al., 2020; Philippat et al., 2013; Santamaria et al., 2020; Song et al., 2020; Towers et al., 2015) and can be detected in placental tissue (Feng et al., 2016; Song et al., 2020; Valle-Sistac et al., 2016; Vela-Soria et al., 2017). Nevertheless, the effect of prenatal exposure to phenols (or their precursors) on the DNA methylation status of human placenta has not been extensively investigated. Available studies either focused on candidate genes [imprinted maternally expressed transcript (H19) and insulin like growth factor 2 (IGF2), LaRocca et al., 2014] or assessed both phenol concentrations and DNA methylation in placental tissue coming from elective terminations (Nahar et al., 2015). Genome-wide studies investigating associations between pregnancy exposure to phenols and DNA methylation in placentas collected at delivery are lacking.

The aim of this study was to evaluate the associations of urinary concentrations of nine phenols during pregnancy on epigenome-wide DNA methylation in male placental samples collected at delivery. We further investigated whether placental cell heterogeneity could mediate the association between phenol exposure and DNA methylation levels.

2. Methods

2.1. Study design and population

The present study included a subsample of mother-son pairs out of 2002 enrolled between 2003 and 2006 in the French mother-child cohort EDEN (Etude des Déterminants pré et postnatals du développement et de la santé de l’Enfant, Heude et al., 2016). Pregnant women were recruited before their 24th week of gestation at the Nancy and Poitiers University hospitals. Exclusion criteria are described in the Supplement. Out of the 2002 study participants, 1301 had placental samples collected and placental DNA was extracted for 668 subjects (Abraham et al., 2018). Out of those, 202 women who delivered a boy had phenol metabolite concentrations assessed in urine (Philippat et al., 2014) as well as information on covariates available, and were included in the present study (Appendix Fig. 1). Inclusion of pregnancies with a male fetus only was due to phenol concentrations being assessed in the framework of a previous project focusing on the associations between these compounds and male congenital malformations (Chevrier et al., 2012). The EDEN cohort received approval from the ethics committee (CCPPRB) of Kremlin Bicêtre and from the French data privacy institution “Commission Nationale de l’Informatique et des Libertés”. Written consent was obtained from the mother for herself and for the offspring. The involvement of the Centers

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for Disease Control and Prevention (CDC) laboratory did not constitute engagement in human subjects research.

2.2. Quantification of concentrations of synthetic phenols in maternal urine

Pregnant women between 22 and 29 gestational weeks were asked to collect the first morning urine void at home, before a study visit. A spot sample was collected during the visit at the hospital by the women who did not collect the urine at home. Samples were then aliquoted and stored on dry ice at −80 °C before shipment to the National Center for Environmental Health laboratory at the CDC in Atlanta, Georgia, USA.

Quantification of maternal concentrations of creatinine and nine phenols (free plus conjugated species): bisphenol A, benzophenone-3, triclosan, 2,4- and 2,5-dichlorophenol, butyl-, ethyl-, methyl-, and propylparaben was performed at the CDC. Detailed quantification methods are described in the Supplement. The limits of detection (LODs) were calculated as three times the standard deviation as the concentration approaches zero from repeated measurements of low-level standards spiked onto human urine (Taylor, 1987). The LODs ranged from 0.2 to 2.3 ng/mL, depending on the analyte. The details of the quality control/quality assurance for the assessment of phenols are provided in the Supplement.

2.3. Phenol concentrations, imputation and standardization

We used the instrumental reading values for phenol concentrations (μg/L) below the LOD. To allow log-transformation, instrumental reading values equal to zero (i.e., indicating no signal) were replaced by the phenol-specific lowest instrumental reading value obtained for these study samples divided by √2, similarly to strategies used before (Hornung and Reed, 1990). Phenol concentrations were then standardized on sampling conditions as described in the Supplement. To limit the impact of extreme values, standardized phenol concentrations were log₂-transformed.

2.4. Placental tissue collection and DNA extraction

Detailed description of tissue collection and DNA extraction is provided in the Supplement. Briefly, placental tissue from the fetal side was sampled at delivery using a standardized procedure. DNA from placental samples was extracted using the QIASymphony instrument (QIAGEN GmbH, Germany) following the manufacturer’s protocol.

2.5. Placental DNA methylation assessment and quality control

Whole-genome DNA methylation was measured at >485,000 CpGs using the Infinium HumanMethylation 450 BeadChips (Illumina, San Diego, CA, USA) following standard manufacturer’s protocols. After quality control, normalization and filtering of outliers (for details see Supplement) 379,904 methylation sites remained for further analyses (Fig. 1). For global DNA methylation, pyrosequencing was used to measure each individual’s methylation levels of four CpG sites of repetitive Alu elements (Alu) and long interspersed nucleotide elements 1 (LINE-1, Yang, 2004). One participant missed data on Alu and LINE-1 methylation, so the final sample size was n = 201.

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2.6. **Statistical analyses**

### 2.6.1. **Adjustment factors**—Adjustment factors were selected *a priori* and included variables likely to be common causes of both the phenol concentrations and the DNA methylation levels, without being likely consequences thereof, and factors that were possible predictors of the DNA methylation only. That included: recruitment center (Nancy/ Poitiers), maternal active smoking in the three months preceding pregnancy and during pregnancy (did not smoke/ smoked before pregnancy/ smoked before and during pregnancy/ other), maternal age (continuous), parity (nulliparous/ one child), maternal education level (<two years after high school/ high school + two years/ ≥high school + three years), and season of conception (January–March/ April–June/ July–September/ October–December). All analyses were additionally adjusted for technical factors related to DNA methylation measurements [batch, plate and chip for the epigenome-wide association study (EWAS) and global analysis of methylation profiles (GAMP); batch and plate for the study of repetitive *Alu* and LINE-1 elements].

### 2.6.2. **Associations between phenol urinary concentrations, CpG-specific, and global DNA methylation**—To investigate associations between phenol urinary concentrations and DNA methylation changes at the individual CpG sites level, we performed an adjusted EWAS with methylation levels of each CpG site as the response variable and standardized log₂-transformed maternal phenol concentration as a predictor of the robust linear regression (*MASS* R package, Venables and Ripley, 2002). Each chemical was tested in a separate model and p-values were calculated using the Wald test from the *survey* R package (Lumley, 2004) and corrected for false discovery rate (FDR) regarding the number of CpGs tested for each phenol (Benjamini and Hochberg, 1995). FDR corrected p-values <0.05 were considered as statistically significant.

Regional Manhattan plots were generated using the *comMETR* package (Martin et al., 2015). Genomic inflation factor (λ) and Q-Q plots were generated using the *QCEWAS* R package (Van der Most et al., 2017). Bayesian inflation factor (BIF) was calculated using the *bacon* R/Bioconductor package (Iterson et al., 2017). Gene annotations were based on information from Illumina’s v1.2 annotation available in the *IlluminaHumanMethylation450kanno.ilmn12.hg19* R/Bioconductor package for the hg19 reference genome (Hansen, 2016) and from the University of California, Santa Cruz (UCSC, https://genome.ucsc.edu). To test the associations between phenol concentrations and global placental DNA methylation we fitted one adjusted robust linear regression per phenol with the median methylation level of each repetitive element (*Alu* or LINE-1) as the dependent variable.

Non-linearity of the associations between exposure and DNA methylation was tested by modelling each phenol concentration as a restricted cubic spline with four knots using the *rms* R package (Harrell, 2021), correcting for FDR. Associations showing FDR corrected p-values <0.05 were considered as non-linear.

### 2.6.3. **Analysis of differentially methylated regions (DMRs)**—Biological functions are in general more robustly associated with genomic regions than single CpGs.
Therefore, we also identified differentially methylated regions (DMRs) associated with phenol concentrations with the *combin-p* Python module (Pedersen et al., 2012). The method combines p-values for CpGs detected in the EWAS in sliding windows accounting for their spatial correlations across the genome using Stouffer-Liptak-Kechris correction (Kechris et al., 2010). Obtained regional p-values were adjusted for multiple testing by Šidák correction (Šidák 1967). Significant DMRs (Šidák-corrected p-value <0.05) included at least two probes (p-value <0.001 to start a region) at a maximum distance of 500 bp. Information on genes encompassed by the DMRs identified as associated with phenol exposure were retrieved from the GeneCards Human Gene Database (Stelzer et al., 2016).

### 2.6.4. Associations between urinary phenol concentrations and methylation profiles

Complementary to the approaches described above, we conducted a GAMP analysis (Zhao et al., 2015). For each individual we characterized a methylation profile by approximating the density function and the cumulative distribution function of the methylation distribution using B-spline basis functions. The obtained B-spline coefficients were then used to represent each individual’s overall methylation distribution. The adjusted associations between B-spline coefficients and concentrations of each phenol were tested by computation of the variance component score test from the kernel machine framework. This method allowed for evaluating whether prenatal phenol exposure changed the overall profile or distribution of DNA methylation measurements for the subjects, instead of examining its effect on individual CpG methylation levels.

### 2.6.5. Identification of imprinted genes

To test the enrichment of phenol-associated DMRs with imprinted genes we looked for overlap between the DMRs containing at least five probes and the list of imprinted genes compiled from MetaImprint (https://openebench.bsc.es/tool/metaimprint, Wei et al., 2014), GeneImprint (http://www.geneimprint.com, Jirtle, 1999), and igc.otago (http://igc.otago.ac.nz, Morison et al., 2005) databases as well as the imprinted gene loci defined by Yuen et al., (2010) and Hamada et al., (2016). From the initial list of 304 imprinted genes, 297 were covered by the Illumina assay used in the current study. The difference between the proportions of the imprinted genes in the identified DMRs and those present among the 20,283 genes available for this study was evaluated using Fisher’s exact test.

### 2.6.6. Analysis of mediation via the placental cell composition

To estimate placental tissue heterogeneity, we followed the pipeline recommended by Decamps et al., 2020, as described in the Supplement. We identified six putative constituent cell types and estimated tissue heterogeneity using a reference-free based method from the *RefFreeE-WAS* R package (Houseman et al., 2016).

To explore whether placental cell heterogeneity could mediate the associations between phenol concentrations and DNA methylation levels, we estimated the percentage difference between regression estimates obtained for the models unadjusted and adjusted for the estimated placental tissue heterogeneity. Then, mediation analysis was performed using the *mediation* R package (Tingley et al., 2014), as described in the Supplement. Average
mediation effect p-values were reported as the measure of mediation and those <0.1 were treated as significant.

2.7. Research data and source code

All analyses were conducted using R v. 4.0.3 (R Core Team and R Foundation for Statistical Computing 2020), RStudio v. 1.3. 1093 (RStudio Team, 2020) and Python v. 3.7.4 (van Rossum and Drake, 2009). The data used in this study can only be provided upon reasonable request after approval by the EDEN steering committee. The source code is available upon request to the corresponding authors.

3. Results

3.1. Study population characteristics and urinary phenol concentrations

Median maternal age was 29.1 years and median gestational duration was 40.0 weeks (Appendix Table 1). Most of the mothers (62.9%) never smoked and had at least one child (56.4%). Frequency of detection of phenols ranged from 72% to 100% (Appendix Table 2). Methylparaben and triclosan were the most abundant biomarkers in maternal urine (median standardized concentrations were 101.6 and 43.1 μg/L, respectively). We observed moderate correlation between the standardized concentrations of dichlorophenols (Spearman’s coefficient rho = 0.63) and moderate to strong correlations (0.46 ≤ rho ≤ 0.82) between the parabens (Appendix Fig. 2).

3.2. EWAS examining individual CpGs

The p-value distributions of the CpGs included in the EWAS were close to the theoretical distribution as indicated by the BIF values (ranging from 0.94 to 1.05 depending on the phenol), and were notably smaller compared to the genomic inflation factor values (0.79–1.49, Appendix Fig. 3). In total, 596 CpGs were differentially methylated in association with urinary phenol concentrations, including 48 non-linearly associated probes (FDR-corrected p-value <0.05). Detected CpGs encompassed 406 known genes and 162 intergenic regions (Appendix Table 3). Among these, more than 90% of CpGs were positively associated with the exposure and over 91% of the associations were detected for triclosan (543 differentially methylated sites mapping to 365 known genes and 151 intergenic regions).

3.3. Regional DNA methylation analysis

The regional analysis identified 180 DMRs associated with pregnancy phenol urinary concentrations (Šidák-corrected p-value <0.05, Appendix Table 4), including 764 CpGs (107 were also identified as significantly associated with phenol biomarkers in the EWAS). For over 97% of identified DMRs (n = 175), DNA methylation levels increased. Most DMRs were related to triclosan (n = 162, Šidák-corrected p-value <0.05, Appendix Table 4). In the following sections, we focused on the 46 DMRs including five or more probes that encompassed 45 genes (Table 1, Appendix Table 5). Among those genes, seven (15.2%) were imprinted, which represents a significant enrichment compared to ~1.4% of imprinted genes covered in this study (p-value = 3.8e-06).
3.3.1. **Triclosan**—Thirty-six DMRs were positively associated and one was negatively associated [encompassing tenasin X (TNXB)] with prenatal concentrations of triclosan (Fig. 2). Identified DMRs encompassed 36 known genes, including six imprinted genes: APC regulator of WNT signaling pathway (APC), forkhead box G1 (FOXG1), stimulatory alpha subunit of the guanine nucleotide-binding protein (GNAS), GNAS antisense RNA 1 (GNASAS or GNAS-AS1), paternally expressed 10 (PEG10) and sarcoglycan epsilon (SGCE) (Fig. 3). DMRs located within GNAS and GNAS/GNASAS, TNXB and APC showed the highest total number of probes (3 DMRs, 79 probes; 1 DMR, 23 probes; 1 DMR, 14 probes, respectively). The remaining DMRs encompassed, among others, genes encoding proteins responsible for cell signaling (APOLD1, JAK3, NFAM1, PLEC1 or PLEC, SYNGAP1), transmembrane transport (AQP1, SLC16A11, SLC35C1) and cell adhesion (CD93, FERMT3, NUAK1, SPATA13), histone coding genes (HIST1H1A or H1–I, HIST1H2BK or H2BC12, HIST1H4I or H4C9), tumor suppressors (MAD1L1, PYCARD, RGM), genes involved in tumorigenesis (CLDN6, NUAK1, SPATA13), and several genes whose epigenetic profiles may serve as biomarkers of risk to develop diverse cancers (e.g., APC, CLDN6, JAK3, MAD1L1, NFAM1, PLEC1 or PLEC, PLEKHA6, PPP1R18, SPATA13). All identified genes, except from the RNA encoding GNASAS, are protein coding.

3.3.2. **Dichlorophenols**—2,4-dichlorophenol urinary concentration was associated with increase of methylation in DMRs encompassing rho guanine nucleotide exchange factor 37 (FLJ41603 or ARHGEF37), G protein subunit gamma 11 (GNG11), and interferon regulatory factor 7 (IRF7). All identified genes are protein coding. No DMRs were associated with 2,5-dichlorophenol.

3.3.3. **Benzophenone-3**—Benzophenone-3 was associated with increased methylation of two and decreased methylation of one DMR encompassing genes encoding death domain associated protein (DAXX), lactate dehydrogenase D (LDHD), and the imprinted vault RNA 2–1 (MIR886 or VTRNA2–1), respectively. DAXX and LDHD are protein coding.

3.3.4. **Parabens**—Both methylparaben and propylparaben were positively associated with DMRs encompassing the same gene encoding solute carrier family 17 member 9 (SLC17A9). Methylparaben, but not propylparaben, was positively associated with an additional DMR located within a gene encoding potassium voltage-gated channel interacting protein 2 (KCNIP2). Both genes are protein coding. No associations were observed for the other parabens.

3.4. **Effect of placental cell heterogeneity**

Additional adjustment of the EWAS for the six estimated placental cell proportions caused notable changes (mostly decrease) of regression coefficient values for 280 (278 for triclosan) CpGs showing ≥20% absolute difference between adjusted and unadjusted effect estimates (Appendix Table 3). For triclosan, adjustment resulted in a substantial decrease in the number of differentially methylated CpGs from 543 to 36 (FDR corrected p-value <0.05, Appendix Table 3). This result suggested a mediation effect of cell heterogeneity, which was confirmed by the formal analysis indicating mediation effect for 136 out of the 278 (48.9%)
CpGs (p-values <0.1, Appendix Table 3). Finally, adjustment for placental cell heterogeneity caused a drop of significant DMRs containing at least five CpGs and associated with triclosan (from 37 to 5, Appendix Table 4).

3.5. Associations of phenol urinary concentrations with methylation of Alu and LINE-1 and with global DNA methylation

Average DNA methylation level was 16.2% (±1.1%) for Alu and 26.4% (±1.9%) for LINE-1. None of the phenol concentrations was significantly associated with the methylation levels of the repetitive elements (non-linear associations were not detected; the lowest p-value was 0.11 for methylparaben and LINE-1, Appendix Fig. 4) or with overall profile or distribution of DNA methylation in the GAMP analysis (the lowest p-value was 0.13 for the association between cumulative distribution of methylation and methylparaben concentration, Appendix Table 6).

4. Discussion

The epigenome-wide effects of pregnancy concentrations of selected synthetic phenols on DNA methylation in human placenta are explored here for the first time. We found that increased concentrations of several phenols were predominantly associated with an increase of DNA methylation. Most of the associations were observed for triclosan. A few associations were also observed for 2,4-dichlorophenol, benzophenone-3, methyl- and propylparaben, while other studied parabens (butyl- and ethylparaben), 2,5-dichlorophenol, and bisphenol A were not associated with differential DNA methylation. Out of the 46 identified DMRs containing at least five probes, seven (six for triclosan only) phenol-associated regions overlapped with imprinted loci controlling genes playing a key role in fetal and placental development. Finally, as revealed by the mediation analysis, the placental cell heterogeneity may act as a mediator between pregnancy exposure to triclosan and DNA methylation. Therefore, in our interpretation of the results, we focused on associations not adjusted for placental cell heterogeneity.

4.1. Triclosan

In our study population, triclosan was positively associated with 543 individual CpGs and 37 DMRs containing at least five CpGs. Identified DMRs encompassed 36 genes, including six imprinted loci. It has been shown that exposure to triclosan may have adverse effects on placenta in vivo and in vitro. Exposure of pregnant mice to triclosan (8 mg/kg/day) impaired placental development and nutrient transport by inducing hypothyroxinemia, resulting in placental weight and volume decrease (Cao et al., 2017). It was also associated with dose-dependent increase in fetal death accompanied by the enhanced placental thrombosis and alteration in reproductive and thyroid hormone levels when pregnant mice were treated with 10 and 100 mg/kg/day triclosan doses (Wang et al., 2015). Additionally, pregnant rats treated with this phenol (30–600 mg/kg/day) showed its accumulation in placenta followed by the upregulation of transcriptional expression levels of enzymes involved in hormone metabolism and sex steroid receptor genes, as well as reduction of gravid uterine weight and increased occurrence of abortion (Feng et al., 2016). Moreover, exposure to triclosan has also been shown to affect hormone secretion and cell viability of choriocarcinoma-
derived placental cells (Honkisz et al., 2012) and induce apoptosis in human placental syncytiotrophoblasts \textit{in vitro} (Zhang et al., 2015). It also inhibited estrogen sulfotransferase activity in placental cytosol derived from sheep cotyledons (James et al., 2010). Lastly, in a larger subsample of the EDEN cohort prenatal exposure to triclosan (assessed from urinary concentrations of triclosan) was associated with decreased placental weight (n = 473, Philippat et al., 2019).

4.1.1. \textbf{Imprinted genes—}The only study investigating the associations between prenatal triclosan and placental DNA methylation in humans relied on a candidate approach and considered the imprinted genes \textit{H19} and \textit{IGF2} (n = 179, LaRocca et al., 2014). The authors did not report significant associations for this phenol, neither when boys and girls were studied together nor in a sex-stratified analysis.

In our study, the most populated triclosan-associated DMRs showed increased methylation within the \textit{A/B} and \textit{GNASAS} (or \textit{GNAS-AS1}) promoters of the \textit{GNAS} complex (3 DMRs, 79 probes in total). Among its multiple products, \textit{GNAS} encodes the stimulatory alpha subunit of the G protein (Gsa), a ubiquitous signaling protein involved, among others, in hormonal regulation of adenylate cyclase enzyme and a variety of cellular responses (Syrovatkina et al., 2016) that control production of hormones by endocrine glands (Steer, 1975). Several previously described associations between differentially methylated \textit{GNAS} complex and health outcomes also point toward its role in endocrine function and metabolism regulation, among others. Somatic \textit{GNAS} mutations that cause constitutive Gsa activity have been detected in endocrine tumors (reviewed by Turan and Bastepe, 2015). Moreover, loss of methylation on maternal \textit{A/B} allele promoter, followed by a decrease of Gsa expression, has been observed in blood of patients with a form of pseudohyoparathyroidism, a syndrome characterized, among other symptoms, by resistance to renal parathyroid hormone (Mantovani, 2011) often accompanied by patients’ increased intrauterine growth (Bréhin et al., 2015). Apart from endocrine function and metabolism regulation, genome-wide studies have shown an increase of methylation within the placental \textit{GNAS} locus in association with being large for gestational age (n = 12, Shen et al., 2020). Moreover, differential methylation of \textit{GNAS} (depending on the gene region) in placenta and maternal blood has been observed in diandries, digynies and hydatidiform mole pregnancies (n = 36, Yuen et al., 2011). Lastly, transcriptome-wide studies in placenta showed association between downregulation of \textit{GNAS} expression and intrauterine growth restriction (n = 9, Dunk et al., 2012; n = 29, McMinn et al., 2006) and placental \textit{GNASAS} expression in a candidate approach study has been related to newborns neurobehavioral profile measured by the Network Neurobehavioral Scales (n = 615, Green et al., 2015). However, whether \textit{GNAS/GNASAS} placental DNA methylation correlates with expression needs further investigation.

Another imprinted gene positively associated with pregnancy triclosan concentration was the \textit{APC} gene located within the third biggest detected DMR (14 probes). \textit{APC} encodes tumor suppressor protein that acts as an antagonist of the Wnt signaling pathway which plays major roles in placentation by influencing embryonic development, stem cell maintenance, and differentiation and tissue homeostasis (reviewed by Sonderegger et al., 2010). \textit{APC} is also involved in other processes including cell migration and adhesion, transcriptional
activation, and apoptosis (O’Leary et al., 2016). Placentation displays many similarities with tumorigenesis, also at the epigenetic level, and silencing of tumor suppressor genes is an integral part of normal placentation development. In vitro studies have demonstrated that dysregulation of the epigenetic status of APC may contribute to trophoblast cancer cell progression (Novakovic et al., 2008; Wong et al., 2008). Lastly, increased methylation of the APC promoter has been also observed in cancer and pre-cancerous tissues of other origin (e.g., Li et al., 2017; Moreno-Bueno et al., 2002).

In our study, triclosan was associated with elevated methylation of the two overlapping SGCE/PEG10 genes whose regulation is critical for normal fetal development and placental formation (Chen et al., 2015; Ono et al., 2006). More specifically, PEG10 has a functional role in growth-promoting activities and placental formation during the first trimester of pregnancy. In the EDEN cohort, increased methylation of placental SGCE/PEG10 has been previously associated with smoking during pregnancy (n = 668, Rousseaux et al., 2020). Studies on cord blood showed an association between increased methylation of the candidate PEG10 gene with low birth weight (n = 90, Lim et al., 2012) and poor anthropometric scores at 1 year (n = 288) and at 3 years (n = 294) in males (Gonzalez-Nahm et al., 2018). Inversely, higher birth weight has been linked with an increased methylation of cord blood SGCE/PEG10 (n = 508, Liu et al., 2012) and SGCE (n = 496, Hoyo et al., 2014).

Considering the gene expression level, placental PEG10 upregulation has been detected in intrauterine growth restriction (n = 17, replicated in n = 38, Diplas et al., 2009) and spontaneous miscarriages or fetal deaths cases (n = 38, Dória et al., 2010), while its downregulation has been associated with preeclampsia (n = 44, Liang et al., 2014). However, whether PEG10 placental DNA methylation correlates with gene expression needs further investigation.

The last imprinted gene showing elevated DNA methylation associated with triclosan concentration was FOXG1 that has been shown to play a role in brain development (Martynoga et al., 2005; Xuan et al., 1995) and tumorigenesis (Bulstrode et al., 2017; Pradhan et al., 2013). FOXG1 has been previously detected in an epigenome-wide study in placenta as demethylated in association with maternal circadian disruption (n = 273, Clarkson-Townsend et al., 2019).

4.1.2. Non-imprinted genes—The second most populated (23 CpGs) triclosan-associated DMR we identified encompassed the TNXB gene and showed loss of methylation. TNXB encodes the tenascin-X glycoprotein that plays an important role in organizing and maintaining the structure of connective tissues by regulating the production and assembly of certain types of collagen. Increased maternal plasma levels of candidate TNXB gene transcripts were associated with a subtype of congenital heart defects (n = 70, Curti et al., 2016; n = 40, Morano et al., 2018).

In our study we also observed increased methylation of the PYCARD gene (often referred to as ASC) in association with pregnancy triclosan exposure. PYCARD encodes apoptosis-associated speck-like protein with two protein-protein interaction domains (Stimson and Vertino, 2002). Protein domains are members of the death domain-fold superfamily that mediates assembly of large signaling complexes (inflammasomes) in the inflammatory

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and apoptotic signaling pathways (O’Leary et al., 2016). Placental PYCARD has been previously detected in an epigenome-wide study as mostly hypermethylated in association with prenatal smoking (n = 668, Rousseaux et al., 2020). It has been also shown that expression level of the ASC in fetal membrane, placental tissues, and maternal serum was higher in pregnancies with premature rupture of membranes (n = 60, Zhu et al., 2020). Finally, the ASC protein levels were increased in hyperglycemic pregnant women (n = 60, Corrêa-Silva et al., 2018).

Lastly, triclosan was associated with increased methylation of the AQP1 gene encoding aquaporin 1. Aquaporins play a crucial role in the preservation of maternal-fetal fluid balance critical during human (Zhang et al., 2012) and animal pregnancy (Luo et al., 2018; Mann et al., 2005; Zheng et al., 2014). Methylation changes of AQP1 have not been studied in either placenta or cord blood, but elevated AQP1 expression has been detected in the amnion in idiopathic polyhydramnios pregnancies (n = 8, Mann et al., 2006).

Taken together, our results for triclosan-induced placental DNA methylation changes of several imprinted and non-imprinted genes add evidence to its potential adverse role on placental health and development.

### 4.2. 2,4-dichlorophenol

2,4-dichlorophenol is a metabolite of 1,3-dichlorobenzene, a minor contaminant of 1,4-dichlorobenzene (Agency for Toxic Substances and Disease Registry, 2006). It is also an environmental transformation intermediate of triclosan and some herbicides (e.g., 2,4-dichlorophenoxyacetic acid and 2-(2,4-dichloro-phenoxy)propionic acid, Yang et al., 2010; Zona et al., 2002). Herein we identified increased DNA methylation in three DMRs in association with 2,4-dichlorophenol concentrations. One of the genes linked with the identified DMRs was IRF7, which plays an essential role in the response against viruses as a transcriptional regulator of type I interferon-dependent immune responses (Honda et al., 2005). IRF7 has been also shown to be altered in immune-mediated diseases [e.g., psoriasis (Wang et al., 2020), lupus (Coit et al., 2020), systemic sclerosis (Rezaei et al., 2017)] or cancer cells (e.g., Li et al., 2008; Li and Tainsky, 2011; Wiseman et al., 2020). An in vitro study showed that alkylphenols, a family of organic compounds obtained by the alkylation of phenols and displaying weak estrogen-like activity, may suppress IRF7 expression in blood-derived plasmacytoid dendritic cells (critical for immune regulation) and interfere with immunity against infection (Hung et al., 2013).

### 4.3. Benzophenone-3

In our study population, benzophenone-3 was positively associated with two DMRs (DAXX and LDHD) and negatively associated with one DMR (MIR886 or VTRNA2−I). An animal study investigating associations between prenatal benzophenone-3 exposure (injections, 50 mg/kg) and DNA methylation in pregnant mice showed inhibition of global DNA methylation and change of methylation status of estrogen receptor genes (Esr1, Esr2 and Gper1) and apoptosis-related genes (Bcl-2 and Bax) in the neocortical embryonic cells of the offspring (Wnuk et al., 2018). None of these genes was identified in our study, however we found a positive association with DNA methylation of DAXX, a different apoptosis-related
gene. DAXX placental methylation has been previously associated with prenatal exposure to air pollutant PM$_{10}$ in a genome-wide study carried in the EDEN cohort, however the direction of the association was opposite to the one observed herein (n = 668, Abraham et al., 2018). An *ex vivo* study also showed loss of DAXX methylation during differentiation of progenitor villous cytotrophoblasts and the same DAXX DMR was identified among the most consistently differentially methylated genes in placental samples from preeclampsia pregnancies (n = 6, Novakovic et al., 2017).

Another gene we identified as negatively associated with benzophenone-3 concentration was MIR886. This imprinted gene has been demonstrated to be demethylated in epigenome-wide studies in blood in association with benzophenone-3 concentrations: in a cross-sectional study in adolescents (n = 51, Almstrup et al., 2020) and in infants at risk to develop overweight and obesity during childhood and adolescence (n = 438, van Dijk et al., 2018). DNA methylation loss of blood MIR886 in adult offspring has been also linked to maternal malnutrition during pregnancy as shown in two epigenome-wide studies (n = 148, Finer et al., 2016; n = 123, Silver et al., 2015), suggesting an involvement of MIR886 in metabolic functions. Interestingly, prenatal benzophenone-3 concentrations have been previously associated with changes in birth outcomes, either increased birth weight (n = 346, Messerlian et al., 2018; n = 473 boys, Philippat et al., 2019) or decreased birth weight and length in girls (n = 847, Long et al., 2019). Finally, placental expression upregulation of MIR886 has been linked to neurobehavioral status of infants measured by the Network Neurobehavioral Scales in a candidate gene study (n = 615, Green et al., 2015).

### 4.4. Parabens

The above mentioned study of LaRocca et al. is the only one investigating the associations between pregnancy concentrations of parabens and placental DNA methylation and it focused on two imprinted genes (*H19* and *IGF2*) (n = 179, LaRocca et al., 2014). The authors reported methylation loss within the *IGF2* DMR2 in male placenta associated with increased maternal concentrations of butylparaben ($\beta = -0.52\%, 95\%\ CI: -0.89\%\; -0.15\%\$ and methylparaben ($\beta = -0.65\%, 95\%\ CI: -1.26\%\; -0.03\%\$). There were only seven common probes within the *IGF2* DMR2 between LaRocca et al. and our study and we did not identify any of them to be associated with butyl- or methylparaben. As for other loci within the *IGF2* gene, we found seven positively and two negatively associated CpGs for butylparaben, and eight positively and one negatively associated CpG for methylparaben (uncorrected p-values <0.05, the lowest FDR-corrected p-values were 1 and 0.52 for butyl- and methylparaben, respectively). The results discrepancy between the two studies may come from different methodologies used to assess DNA methylation (pyrosequencing vs. BeadChip technology), features of the studied population, adjustment for different confounders, or distinct exposure characteristics (e.g., different ranges of measured paraben concentrations in the two cohorts).

In our study, two out of four studied parabens (methyl- and propylparaben) were associated with increased placental DNA methylation for two individual CpGs and two DMRs linked with the *SLC17A9* gene. *SLC17A9* encodes protein involved in the transport of small molecules and participates in the vesicular uptake, storage, and secretion of adenoside
triphosphate and other nucleotides (O’Leary et al., 2016). Increased DNA methylation of cord blood SLC17A9 has been previously reported to be associated with prenatal exposure to perfluorooctane sulfonic acid (n = 190, Miura et al., 2018), a potential endocrine disruptor (Jensen and Leffers, 2008).

4.5. Effect of placental tissue heterogeneity

DNA methylation levels tend to differ among cell types within certain tissues, including placenta (Grigoriu et al., 2011). Therefore, some part of the DNA methylation variability observed across biological samples may be explained by the inter-individual heterogeneity of the studied tissue. This is why in epigenome-wide studies it is recommended to either adjust for cellular heterogeneity or remove the DNA methylation signal associated with cellular proportions in the pre-processing stage (Jaffe and Irizarry, 2014). However, more recently it has been postulated that treating the cell mix as a technical artifact by default may lead to discarding of the important information contained in the methylation signal determined by the cell composition (Holbrook et al., 2017; Konwar et al., 2019).

Animal and human studies indicated that prenatal exposure to triclosan may affect placental weight and size (Cao et al., 2017; Feng et al., 2016; Philippat et al., 2019) and induce placental thrombus and tissue necrosis (Wang et al., 2015). Therefore, we hypothesized that exposure to triclosan could potentially alter placental cell composition and affect inter-individual variation in DNA methylation associated with this phenol, and so we decided not to adjust our main EWAS for the placental cell heterogeneity. Indeed, adjustment for cell heterogeneity substantially changed most of the regression estimates of triclosan-associated CpGs identified in the main EWAS and reduced the number of differentially methylated CpGs and regions associated with this phenol. For the CpGs with the absolute difference in regression coefficient ≥ 20%, complementary mediation analysis confirmed that DNA methylation changes linked with triclosan exposure could be partially mediated by the placental heterogeneity. It should be noted that other features, such as the structure and/or function of placental cells, may also play a role in the effect of the pregnancy exposure to triclosan on the epigenetic modifications in placenta.

5. Strengths and limitations

In the present study we followed two complementary statistical approaches. The EWAS produces effect estimates for each CpG that are directly comparable to the estimates reported in previous studies, including those relying on a candidate approach, and can be used in meta-analyses. The approach relying on DMR identification accounts for genomic location of the differentially methylated CpGs and their potential interdependence. The well-established EDEN mother-child cohort enabled access to information on a broad range of potential confounders, however additional confounding by genetics or ancestry cannot be ruled out. Also the co-exposure to other environmental chemicals (e.g., persistent organic pollutants, heavy metals) may have affected the observed associations. However, because a previous study did not show strong correlations between phenol concentrations and other common environmental pollutants present in pregnant women’s samples (Tamayo-Uria et al., 2019), it is rather unlikely. In this study phenol biomarkers were assessed only for
boys which, although it is not a source of bias, hinders generalizability of our conclusions for female offspring. Moreover, we used a single spot urine sample to assess exposure to phenols or their precursors whose urinary concentrations have moderate to high intra-individual variability during pregnancy (Casas et al., 2018; Vernet et al., 2018), what can potentially lead to exposure misclassification, attenuation bias, and power reduction (Perrier et al., 2016). Discrepancies observed between our results and other studies investigating phenol-induced epigenetic modifications may come from different methodologies applied to assess DNA methylation (e.g., pyrosequencing vs. BeadChip technology), distinct features of the studied populations or adjustment for different confounders. It should be also noted that, in our study population, the average urinary concentrations of some phenols, especially triclosan, were relatively high [e.g., compared to the HEBC and POPS cohorts on which the previous study investigating phenol-induced changes in placental DNA methylation relied on (LaRocca et al., 2014)]. Finally, the observed changes in DNA methylation cannot be directly translated to gene expression (Lim et al., 2017), what makes it difficult to interpret how our results may link to pregnancy complications or health outcomes later in life.

6. Conclusions

This is the first epigenome-wide study describing associations between urinary concentrations of selected synthetic phenols during pregnancy and methylation of the placental DNA. Our results suggest that increased maternal concentrations of triclosan and, to lesser extent, of 2,4-dichlorophenol, benzophenone-3, methyl- and propylparaben were associated with increased methylation of 44 and decreased methylation of two placental DMRs containing at least five probes and encompassing 45 genes. Our results also suggest that part of the observed associations might be mediated by placental cell heterogeneity. Replication studies are needed to better characterize the associations between maternal concentrations of synthetic phenols and placental DNA methylation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

Abraham E, Rousseaux S, Agier L, Giorgis-Allemand L, Tost J, Galineau J, et al., 2018. Pregnancy exposure to atmospheric pollution and meteorological conditions and placental DNA methylation. Environ. Int 118, 334–347. 10.1016/j.envint.2018.05.007. [PubMed: 29935799]

Agency for Toxic Substances and Disease Registry, 2006. Toxicological profile for dichlorobenzenes. Available: https://www.atsdr.cdc.gov/toxprofiles/tp10.pdf. (Accessed 18 February 2021).

Almstrup K, Frederiksen H, Andersson A-M, Juul A, 2020. Levels of endocrine-disrupting chemicals are associated with changes in the peri-pubertal epigenome. Endocr. Connect 9, 845–857. 10.1530/EC-20-0286. [PubMed: 32759591]

Alvarado-Cruz I, Alegría-Torres JA, Montes-Castro N, Jiménez-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. 10.29024/aogh.909. [PubMed: 30873799]

Bai X, Zhang B, He Y, Hong D, Song S, Huang Y, et al., 2020. Triclosan and triclocarbon in maternal-fetal serum, urine, and amniotic fluid samples and their implication for prenatal exposure. Environ. Pollut 266, 115–117. 10.1016/j.envpol.2020.115117.

Benjami N, Hochberg Y, 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. R. Stat. Soc. Series B Stat. Methodol 57, 289–300. 10.1111/j.2517-6161.1995.tb02031.x.

Bréhin A-C, Colson C, Maupetit-Méhouas S, Grybek V, Richard N, Linglart A, et al., 2015. Loss of methylation at GNAS exon A/B is associated with increased intrauterine growth. J. Clin. Endocrinol. Metab 100, E623–E631. 10.1210/jc.2014-4047. [PubMed: 25603460]

Bulstrode H, Johnstone E, Marques-Torrejon MA, Ferguson KM, Bressan RB, Blin C, et al., 2017. Elevated FOXG1 and SOX2 in glioblastoma enforces neural stem cell identity through transcriptional control of cell cycle and epigenetic regulators. Genes Dev. 31, 757–773. 10.1101/gad.293027.116. [PubMed: 28465359]

Cao X, Hua X, Wang X, Chen L, 2017. Exposure of pregnant mice to triclosan impairs placental development and nutrient transport. Sci. Rep 7, 44803. 10.1038/srep44803. [PubMed: 28322267]

Casas M, Basagaña X, Sakhi AK, Haug LS, Philippat C, Granum B, et al., 2018. Variability of urinary concentrations of non-persistent chemicals in pregnant women and school-aged children. Environ. Int 121, 561–573. 10.1016/j.envint.2018.09.046. [PubMed: 30308014]

Chen H, Sun M, Liu J, Tong C, Meng T, 2015. Silencing of paternally expressed gene 10 inhibits trophoblast proliferation and invasion. PLoS One 10. 10.1371/journal.pone.0144845.

Chevrier C, Petit C, Philippat C, Mortamais M, Slama R, Rouget F, et al., 2012. Maternal urinary phthalates and phenols and male genital anomalies. Epidemiology 23, 353–356. 10.1097/EDE.0b013e318246073e. [PubMed: 22317818]
Clarkson-Townsend DA, Everson TM, Deyssenroth MA, Burt AA, Hermetz KE, Hao K, et al. 2019. Maternal circadian disruption is associated with variation in placental DNA methylation. PLoS One 14, e0215745. 10.1371/journal.pone.0215745. [PubMed: 31026301]

Coit P, Ortiz-Fernandez L, Lewis EE, McCune WJ, Maksimowicz-McKinnon K, Sawalha AH. 2020. A longitudinal and transancestral analysis of DNA methylation patterns and disease activity in lupus patients. JCI Insight 5, e143654. 10.1172/jci.insight.143654.

Corrêa-Silva S, Alencar AP, Moreli JB, Borbely AU, de S, Lima L, Scavone C, et al. 2018. Hyperglycemia induces inflammatory mediators in the human chorionic villous. Cytokine 111, 41–48. 10.1016/j.cytoc.2018.07.020. [PubMed: 29114628]

Curti A, Lapucci C, Berto S, Prandstraller D, Perolo A, Rizzo N, et al. 2016. Maternal plasma mRNA species in fetal heart defects: a potential for molecular screening. Frenat. Diagn 36, 738–743. 10.1002/pd.4853. [PubMed: 27257136]

Decamps C, Privé F, Bacher R, Jost D, Waguet A, Achar R, et al. 2020. Guidelines for cell-type heterogeneity quantification based on a comparative analysis of reference-free DNA methylation deconvolution software. BMC Bioinf. 21, 16. 10.1186/s12859-019-3307-2.

Diplas AI, Lambertini L, Lee M-J, Sperling R, Wetmur J, et al. 2009. Differential expression of imprinted genes in normal and IUGR human placentas. Epigenetics 4, 235–240. 10.4161/epi.9019. [PubMed: 19483473]

Dória S, Sousa M, Fernandes S, Ramalho C, Brandão O, Matias A, et al. 2010. Gene expression pattern of IGF2, PHLD2A, PEG10 and CDKN1C imprinted genes in spontaneous miscarriages or fetal deaths. Epigenetics 5, 444–450. 10.4161/epi.5.5.12118. [PubMed: 20484977]

Dunk CE, Roggensack AM, Cox B, Perkins JE, Åsenius F, Keating S, et al. 2012. A distinct microvascular endothelial gene expression profile in severe IUGR placentas. Placenta 33, 285–293. 10.1016/j.placenta.2011.12.020. [PubMed: 22264586]

Feng Y, Zhang P, Zhang Z, Shi J, Jiao Z, Shao B. 2016. Endocrine disrupting effects of triclosan on the placenta in pregnant rats. PLoS ONE 11, e0154758. 10.1371/journal.pone.0154758. [PubMed: 27149376]

Finer S, Iqbal MS, Lowe R, Ogunkolade BW, Pervin S, Mathews C, et al. 2016. Is famine exposure during developmental life in rural Bangladesh associated with a metabolic and epigenetic signature in young adulthood? A historical cohort study. BMJ Open 6, e011768. 10.1136/bmjopen-2016-011768.

Gonzalez-Nahm S, Mendez MA, Benjamin-Neelon SE, Murphy SK, Hogan VK, Rowley DL, et al. 2018. DNA methylation of imprinted genes at birth is associated with child weight status at birth, 1 year, and 3 years. Clin. Epigenet 10, 90. 10.1186/s13480-018-0521-0.

Green BB, Kappil M, Lambertini L, Armstrong DA, Guerin DJ, Sharp AJ, et al. 2015. Expression of imprinted genes in placenta is associated with infant neurobehavioural development. Epigenetics 10, 834–841. 10.1080/15592294.2015.1073880. [PubMed: 26198301]

Grigoriu A, Ferreira JC, Choufani S, Baczyn D, Kingdom J, Weksberg R. 2011. Cell specific patterns of methylation in the human placenta. Epigenetics 6, 368–379. 10.4161/epi.6.3.14196. [PubMed: 21131778]

Hamada H, Okae H, Toh H, Chiba H, Hiura H, Shirane K, et al. 2016. Allele-specific methylome and transcriptome analysis reveals widespread imprinting in the human placenta. Am. J. Hum. Genet 99, 1045–1058. 10.1016/j.ajhg.2016.08.021. [PubMed: 27843122]

Hansen K. 2016. IlluminaHumanMethylation450kanno.iplied.11.hg19: Annotation for Illumina’s 450k Methylation Arrays.

Harrell FE Jr. 2021. Rms: Regression Modeling Strategies.

Haug LS, Sakhi AK, Cequier E, Casas M, Maitre L, Basagana X, et al. 2018. In utero and childhood chemical exposure in six European mother-child cohorts. Environ. Int 121, 751–763. 10.1016/j.envint.2018.09.056. [PubMed: 30326459]

Heijmans BT, Tobi EW, Lumey LH, Slagboom PE. 2009. The epigenome: archive of the prenatal environment. Epigenetics 4, 526–531. 10.4161/epi.4.8.10265. [PubMed: 19923908]

Heude B, Forhan A, Slama R, Douhaud L, Bedel S, Saurel-Cubizolles M-J, et al. 2016. Cohort Profile: the EDEN mother-child cohort on the prenatal and early postnatal determinants of child health and development. Int. J. Epidemiol 45, 353–363. 10.1093/ije/dyw151. [PubMed: 26283636]
Holbrook JD, Huang R-C, Barton SJ, Saffery R, Lillycrop KA, 2017. Is cellular heterogeneity merely a confounder to be removed from epigenome-wide association studies? Epigenomics 9, 1143–1150. 10.2217/epi-2017-0032. [PubMed: 28749184]

Honda K, Yanai H, Negishi H, Asagiri M, Sato M, Mizutani T, et al., 2005. IRF-7 is the master regulator of type-I interferon-dependent immune responses. Nature 434, 772–777. 10.1038/nature0346. [PubMed: 15800576]

Honkisz E, Zieba-Przybylska D, Wojtowicz AK, 2012. The effect of triclosan on hormone secretion and viability of human choriocarcinoma JEG-3 cells. Reprod. Toxicol 34, 385–392. 10.1016/j.reprotox.2012.05.094. [PubMed: 22677473]

Hornung RW, Reed LD, 1990. Estimation of average concentration in the presence of nondetectable values. Appl. Occup. Environ. Hyg 5, 46–51. 10.1080/1047322X.1990.10389587.

Houseman EA, Kile ML, Christiani DC, Ince TA, Kelsey KT, Marsit CJ, 2016. Reference-free deconvolution of DNA methylation data and mediation by cell composition effects. BMC Bioinf. 17, 259. 10.1186/s12859-016-1140-4.

Hoyo C, Dalvit AK, Iversen E, Benjamin-Neelon SE, Fuemmeler B, Schildkraut J, et al., 2014. Erythrocyte folate concentrations, CpG methylation at genomically imprinted domains, and birth weight in a multiethnic newborn cohort. Epigenetics 9, 1120–1130. 10.4161/epi.29332. [PubMed: 24874916]

Hung C-H, Yang S-N, Wang Y-F, Liao W-T, Kuo P-L, Tsai E-M, et al., 2013. Environmental alkylphenols modulate cytokine expression in plasmacytoid dendritic cells. PLoS One 8, e73534. 10.1371/journal.pone.0073534. [PubMed: 24039973]

Iterson M van Zwet EW, Heijmans BT, 2017. Controlling bias and inflation in epigenome- and transcriptome-wide association studies using the empirical null distribution. Genome Biol. 18, 19. 10.1186/s13059-016-1131-9. [PubMed: 28129774]

Jaffe AE, Irizarry RA, 2014. Accounting for cellular heterogeneity is critical in epigenome-wide association studies. Genome Biol. 15 10.1186/gb-2014-15-2-r31.

James MO, Li W, Summerlot DP, Rowland-Faux L, Wood CE, 2010. Triclosan is a potent inhibitor of estradiol and estrone sulfonation in sheep placenta. Environ. Int 36, 942–949. 10.1016/j.envint.2009.02.004. [PubMed: 19299018]

Jensen AA, Leffers H, 2008. Emerging endocrine disrupters: perfluoroalkylated substances. Int. J. Androl 31, 161–169. 10.1111/j.1365-2605.2008.00870.x. [PubMed: 18315176]

Jirtle RL, 1999. Genomic imprinting and cancer. Exp. Cell Res 248, 18–24. 10.1006/excr.1999.4453. [PubMed: 10094809]

Kechriss KJ, Biels B, Kornberg TB, 2010. Generalizing moving averages for tiling arrays using combined p-value statistics. Stat. Appl. Genet. Mol. Biol 9 10.2202/1544-6115.1434.

Kishi R, Grandjean P, 2020. Health impacts of developmental exposure to environmental chemicals. Springer, Singapore.

Konwar C, Del Gobbo G, Yuan V, Robinson WP, 2019. Considerations when processing and interpreting genomics data of the placenta. Placenta 84, 57–62. 10.1016/j.placenta.2019.01.006. [PubMed: 30642669]

LaRocca J, Binder AM, McElrath TF, Michels KB, 2014. The impact of first trimester phthalate and phenol exposure on IGF2/H19 genomic imprinting and birth outcomes. Environ. Res 133, 396–406. 10.1016/j.envres.2014.04.032. [PubMed: 24972507]

Li B-Q, Liu P-P, Zhang C-H, 2017. Correlation between the methylation of APC gene promoter and colon cancer. Oncol. Lett 14, 2315–2319. 10.3892/ol.2017.6455. [PubMed: 28781669]

Li Q, Tainsky MA, 2011. Epigenetic silencing of IRF7 and/or IRF5 in lung cancer cells leads to increased sensitivity to oncolytic viruses. PLoS One 6, e28683. 10.1371/journal.pone.0028683. [PubMed: 22194884]

Li Q, Tang L, Roberts PC, Kranik JM, Fridman AL, Kulaeva OL, et al., 2008. Interferon regulatory factors IRF5 and IRF7 inhibit growth and induce senescence in immortal Li-Fraumeni fibroblasts. Mol. Canc. Res 6, 770–784. 10.1158/1541-7786.MCR-07-0114.

Liang XY, Chen X, Jin YZ, Chen XO, Chen QZ, 2014. Expression and significance of the imprinted gene PEG10 in placenta of patients with preeclampsia. Genet. Mol. Res 13, 10607–10614. 10.4238/2014.December.18.2. [PubMed: 25526181]
Lim AL, Ng S, Leow SCP, Choo R, Ito M, Chan YH, et al., 2012. Epigenetic state and expression of imprinted genes in umbilical cord correlates with growth parameters in human pregnancy. J. Med. Genet 49, 689–697. 10.1136/jmedgenet-2012-100858. [PubMed: 23042810]

Lim Y-H, Bae S, Kim B-N, Shin CH, Lee YA, Kim JI, et al., 2017. Prenatal and postnatal bisphenol A exposure and social impairment in 4-year-old children. Environ. Health 16. 10.1186/s12940-017-0289-2.

Liu Y, Murphy SK, Murtha AP, Fuemmeler BF, Schildkraut J, Huang Z, et al., 2012. Depression in pregnancy, infant birth weight and DNA methylation of imprint regulatory elements. Epigenetics 7, 735–746. 10.4161/epi.20734. [PubMed: 22677950]

Long J, Xia W, Li J, Zhou Y, Zhao H, Wu C, et al., 2019. Maternal urinary benzo phenones and infant birth size: identifying critical windows of exposure. Chemosphere 219, 655–661. 10.1016/j.chemosphere.2018.11.190. [PubMed: 30557721]

Lumley T, 2004. Analysis of complex survey samples. J. Stat. Software 9. 10.18637/jss.v009.i08.

Luo H, Xie A, Hua Y, Wang J, Liu Y, Zhu X, 2018. Aquaporin 1 gene deletion affects the amniotic fluid volume and composition as well as the expression of other aquaporin water channels in placenta and fetal membranes. Clin. Chim. Acta 482, 161–165. 10.1016/j.cca.2018.04.001. [PubMed: 29626438]

Mann SE, Dvorak N, Gilbert H, Taylor RN, 2006. Steady-state levels of aquaporin 1 mRNA expression are increased in idiopathic polyhydramnios. Am. J. Obstet. Gynecol 194, 884–887. 10.1016/j.ajog.2005.07.004. [PubMed: 16522429]

Mann SE, Ricke EA, Torres EA, Taylor RN, 2005. A novel model of polyhydramnios: amniotic fluid volume is increased in aquaporin 1 knockout mice. Am. J. Obstet. Gynecol 192, 2041–2044. 10.1016/j.ajog.2005.02.046. [PubMed: 15970890]

Mantovani G, 2011. Clinical review: pseudohypoparathyroidism: diagnosis and treatment. J. Clin. Endocrinol. Metab 96, 3020–3030. 10.1210/jc.2011-1048. [PubMed: 21816789]

Martin TC, Yet I, Tsai P-C, Bell JT, 2015. coMET: visualisation of regional epigenome-wide association scan results and DNA co-methylation patterns. BMC Bioinf. 16 10.1186/s12859-015-0568-2.

Martynoga B, Morrison H, Price DJ, Mason JO, 2005. Foxg1 is required for specification of ventral telencephalon and region-specific regulation of dorsal telencephalic precursor proliferation and apoptosis. Dev. Biol 283, 113–127. 10.1016/j.ydbio.2005.04.005. [PubMed: 15893304]

McMinn J, Wei M, Schupf N, Cusmai J, Johnson EB, Smith AC, et al., 2006. Unbalanced placental expression of imprinted genes in human intrauterine growth restriction. Placenta 27, 540–549. 10.1016/j.placenta.2005.07.004. [PubMed: 16125225]

Messerlian C, Mustielas V, Miguez-Alarcon L, Ford JB, Calafat AM, Souter I, et al., 2018. Preconception and prenatal urinary concentrations of phenols and birth weight of singleton infants born to mothers and fathers from the Environment and Reproductive Health (EARTH) study. Environ. Int 114, 60–68. 10.1016/j.envint.2018.02.017. [PubMed: 29477955]

Miura R, Araki A, Miyashita C, Kobayashi S, Kobayashi S, Wang S-L, Chen C-H, Miyake K, Ishizuka M, Iwasaki Y, Ito YM, Kubota T, Kishi R, 2018. An epigenome-wide study of cord blood DNA methylations in relation to prenatal perfluoralkyl substance exposure: The Hokkaido study. Environ. Int 115, 21–28. 10.1016/j.envint.2018.03.004. [PubMed: 29544137]

Morano D, Berto S, Lapucci C, Walczer Baldinazzo L, Prandstraller D, Farina A, 2018. Levels of circulating mRNA for the tenascin-X (TNXB) gene in maternal plasma at the second trimester in pregnancies with isolated congenital ventricular septal defects. Mol. Diagn. Ther 22, 235–240. 10.1007/s40291-018-0321-4. [PubMed: 29470764]

Moreno-Bueno G, Hardisson D, Sánchez C, Sarrió D, Cassia R, García-Rostán G, et al., 2002. Abnormalities of the APC/beta-catenin pathway in endometrial cancer. Oncogene 21, 7981–7990. 10.1038/sj.ong.1205924. [PubMed: 12439748]

Morison IM, Ramsay JP, Spencer HG, 2005. A census of mammalian imprinting. Trends Genet. 21, 457–465. 10.1016/j.tig.2005.06.008. [PubMed: 15990197]

Nahar MS, Liao C, Kannan K, Harris C, Dolinoy DC, 2015. In utero bisphenol A concentration, metabolism, and global DNA methylation across matched placenta, kidney, and liver in the human fetus. Chemosphere 124, 54–60. 10.1016/j.chemosphere.2014.10.071. [PubMed: 25434263]
Novakovic B, Evain-Brion D, Murthy P, Fournier T, Saffery R, 2017. Variable DAXX gene methylation is a common feature of placental trophoblast differentiation, preeclampsia, and response to hypoxia. Faseb. J 31, 2380–2392. 10.1096/fj.201601189RR. [PubMed: 28223336]

Novakovic B, Rakyan V, Ng HK, Manuelpillai U, Dewi C, Wong NC, et al., 2008. Specific tumour-associated methylation in normal human term placenta and first-trimester cytotrophoblasts. Mol. Hum. Reprod 14, 547–554. 10.1093/molehr/gan046. [PubMed: 18708652]

O’Leary NA, Wright MW, Brister JR, Ciufio S, Haddad D, McVeigh R, et al., 2016. Reference sequence (RefSeq) database at NCBI: current status, taxonomic expansion, and functional annotation. Nucleic Acids Res. 44, D733–D745. 10.1093/nar/gkv1189. [PubMed: 26553804]

Ono R, Nakamura K, Inoue K, Naruse M, Usami T, Wakisaka-Saito N, et al., 2006. Deletion of Peg10, an imprinted gene acquired from a retrotransposon, causes early embryonic lethality. Nat. Genet 38, 101–106. 10.1038/ng1699. [PubMed: 16341224]

Pedersen BS, Schwartz DA, Yang IV, Kechris KJ, 2012. Comb-p: software for combining, analyzing, grouping and correcting spatially correlated P-values. Bioinformatics 28, 2986–2988. 10.1093/bioinformatics/bts545. [PubMed: 22954632]

Perrier F, Giorgis-Allemand L, Slama R, Philippat C, 2016. Within-subject pooling of biological samples to reduce exposure misclassification in biomarker-based studies. Epidemiology 27, 378–388. 10.1097/EDE.0000000000000460. [PubMed: 27035688]

Philippat C, Botton J, Calafat AM, Ye X, Charles M-A, Slama R, 2014. Prenatal exposure to phenols and growth in boys. Epidemiology 25, 625–635. 10.1097/EDE.0000000000000132. [PubMed: 25061923]

Philippat C, Heude B, Botton J, Alfaidy N, Calafat AM, Slama R, et al., 2019. Prenatal exposure to select phthalates and phenols and associations with fetal and placental weight among male births in the EDEN cohort (France). Environ. Health Perspect 127, 17002. 10.1289/EHPP3523. [PubMed: 30624098]

Philippat C, Wolff MS, Calafat AM, Ye X, Bausell R, Meadows M, et al., 2013. Prenatal exposure to environmental phenols: concentrations in amniotic fluid and variability in urinary concentrations during pregnancy. Environ. Health Perspect 121, 1225–1231. 10.1289/ehp.1206335. [PubMed: 23942273]

Pradhan MP, Desai A, Palakal MJ, 2013. Systems biology approach to stage-wise characterization of epigenetic genes in lung adenocarcinoma. BMC Syst. Biol 7, 141. 10.1186/1752-0509-7-141. [PubMed: 24369052]

R Core Team, R Foundation for Statistical Computing, 2020. R: A Language and Environment for Statistical Computing.

Rezaei R, Mahmoudi M, Gharibdoost F, Kavosi H, Dashti N, Imeni V, et al., 2017. IRF7 gene expression profile and methylation of its promoter region in patients with systemic sclerosis. Int. J. Rheum. Dis 20, 1551–1561. 10.1111/1756-185X.13175. [PubMed: 28952189]

Rolland M, Lyon-Caen S, Sakhi AK, Pin I, Sabaredzovic A, Thomsen C, et al., 2020. Exposure to phenols during pregnancy and the first year of life in a new type of couple-child cohort relying on repeated urine biospecimens. Environ. Int 139, 105678. 10.1016/j.envint.2020.105678. [PubMed: 32248023]

Rousseaux S, Seyve E, Chuffart F, Bourouva-Flin E, Benmerad M, Charles M-A, et al., 2020. Immediate and durable effects of maternal tobacco consumption alter placental DNA methylation in enhancer and imprinted gene-containing regions. BMC Med. 18 10.1186/s12916-020-01736-1.

RStudio Team, 2020. RStudio: Integrated Development for R. RStudio, Inc.

Santamaria CG, Meyer N, Schumacher A, Zencussen ML, Teglia CM, Culzoni MJ, et al., 2020. Dermal exposure to the UV filter benzophenone-3 during early pregnancy affects fetal growth and sex ratio of the progeny in mice. Arch. Toxicol 94, 2847–2859. 10.1007/s00204-020-02776-5. [PubMed: 32430675]

Shen Z, Tang Y, Song Y, Shen W, Zou C, 2020. Differences of DNA methylation patterns in the placenta of large for gestational age infant. Medicine (Baltim.) 99, e22389. 10.1097/MD.0000000000022389.
Šidák Z, 1967. Rectangular confidence regions for the means of multivariate normal distributions. ChAMP: updated methylation analysis pipeline for Illumina BeadChips 62, 626–633. 10.1080/01621459.1967.10482935.

Silver MJ, Kessler NJ, Hennig BJ, Dominguez-Salas P, Laritsky E, Baker MS, et al. , 2015. Independent genomewide screens identify the tumor suppressor VTRNA2–1 as a human epiallele responsive to periconceptional environment. Genome Biol. 16.10.1186/s13059-015-0660-y.

Sonderegger S, Pollheimer J, Knöfler M, 2010. Wnt signalling in implantation, decidualisation and placental differentiation – review. Placenta 31, 839–847. 10.1016/j.placenta.2010.07.011. [PubMed: 20716463]

Song S, He Y, Zhang T, Zhu H, Huang X, Bai X, et al. , 2020. Profiles of parabens and their metabolites in paired maternal-fetal serum, urine and amniotic fluid and their implications for placental transfer. Ecotoxicol. Environ. Saf 191, 10.1016/j.ecoenv.2020.110235. [PubMed: 31986458]

Steer ML, 1975. Adenyl cyclase. Ann. Surg 182, 603–609. 10.1097/00000658-197511000-00012. [PubMed: 172034]

Stelzer G, Rosen N, Plaschkes I, Zimmerman S, Twik M, Fishilevich S, et al. , 2016. The GeneCards Suite: from gene data mining to disease genome sequence analyses. Curr. Protoc. Bioinformatics 54, 10.1002/cpbi.5, 30.1–30.33. [PubMed: 27322403]

Stimson KM, Vertino PM, 2002. Methylation-mediated silencing of TMSI/ASC is accompanied by histone hypoacetylation and CpG island-localized changes in chromatin architecture. J. Biol. Chem 277, 4951–4958. 10.1074/jbc.M109809200. [PubMed: 11733524]

Svendsen AJ, Gervin K, Lyle R, Christiansen L, Kyvik K, Junker P, et al. , 2016. Differentially methylated DNA regions in monozygotic twin pairs discordant for rheumatoid arthritis: an epigenome-wide study. Front. Immunol 7 10.3389/fimmu.2016.00510.

Syrovatkina V, Alegre KO, Dey R, Huang X-Y, 2016. Regulation, signaling and physiological functions of G-proteins. J. Mol. Biol 428, 3850–3868. 10.1016/j.jmb.2016.08.002. [PubMed: 27515397]

Tamayo-Uria I, Maitre L, Thomsen C, Nieuwenhuijsen MJ, Chatzi L, Siroux V, et al. , 2019. The early-life exposome: description and patterns in six European countries. Environ. Int 123, 189–200. 10.1016/j.envint.2018.11.067. [PubMed: 30530161]

Taylor JK, 1987. Quality assurance of chemical measurements. Lewis Publishers, Chelsea, MI.

The International Panel on Chemical Pollution (IPCP), 2017. Overview Report I: Worldwide Initiatives to Identify Endocrine Disrupting Chemicals (EDCs) and Potential EDCs.

Tingley D, Yamamoto T, Hirose K, Keele L, Imai K, 2014. mediation: R package for causal mediation analysis. J. Stat. Software 59. 10.18637/jss.v059.i05.

Towers CV, Terry PD, Lewis D, Howard B, Chambers W, Armistead C, et al. , 2015. Transplacental passage of antimicrobial paraben preservatives. J. Expo. Sci. Environ. Epidemiol 25, 604–607. 10.1038/jes.2015.27. [PubMed: 25944699]

Turan S, Bastepe M, 2015. GNAS spectrum of disorders. Curr. Osteoporos. Rep 13, 146–158. 10.1007/s11914-015-0268-x. [PubMed: 25851935]

Valle-Sistac J, Molins-Delgado D, Díaz M, Ibáñez L, Barceló D, Silvia Díaz-Cruz M, 2016. Determination of parabens and benzophenone-type UV filters in human placenta. First description of the existence of benzyl paraben and benzophenone-4. Environ. Int 88, 243–249. 10.1016/j.envint.2015.12.034. [PubMed: 26773395]

Van der Most PJ, Kupfers LK, Snieder H, Nolte I, 2017. QCEWAS: automated quality control of results of epigenome-wide association studies. Bioinformatics 33, 1243–1245. 10.1093/bioinformatics/btw766. [PubMed: 28119308]

van Dijk SJ, Peters TJ, Buckley M, Zhou J, Jones PA, Gibson RA, et al. , 2018. DNA methylation in blood from neonatal screening cards and the association with BMI and insulin sensitivity in early childhood. Int. J. Obes 42, 28–35. 10.1038/ijo.2017.228.

van Rossum G, Drake F, 2009. Python 3 Reference Manual.

Vela-Soria F, Gallardo-Torres ME, Ballesteros O, Díaz C, Pérez J, Navalón A, et al. , 2017. Assessment of parabens and ultraviolet filters in human placenta tissue by ultrasound-assisted
 extraction and ultra-high performance liquid chromatography-tandem mass spectrometry. J. Chromatogr. A 1487, 153–161. 10.1016/j.chroma.2017.01.041. [PubMed: 28129936]

Venables WN, Ripley BD. 2002. Modern Applied Statistics With S, 4th. Springer, New York.

Vernet C, Philippat C, Calafat AM, Ye X, Lyon-Caen S, Siroux V, et al., 2018. Within-day, between-day, and between-week variability of urinary concentrations of phenol biomarkers in pregnant women. Environ. Health Perspect 126, 037005 10.1289/EHP1994. [PubMed: 29553460]

Vlahos A, Mansell T, Saffery R, Novakovic B. 2019. Human placental methylome in the interplay of adverse placental health, environmental exposure, and pregnancy outcome. PLoS Genet. 15, e1008236 10.1371/journal.pgen.1008236. [PubMed: 31369552]

Wang X, Chen X, Feng X, Chang F, Chen M, Xia Y, et al., 2015. Triclosan causes spontaneous abortion accompanied by decline of estrogen sulfotransferase activity in humans and mice. Sci. Rep 5 10.1038/srep18252.

Wang X, Liu X, Liu N, Chen H, 2020. Prediction of crucial epigenetically-associated, differentially expressed genes by integrated bioinformatics analysis and the identification of S100A9 as a novel biomarker in psoriasis. Int. J. Mol. Med 45, 93–102. 10.3892/ijmm.2019.4392. [PubMed: 31746348]

Wei Y, Su J, Liu H, Lv J, Wang F, Yan H, et al., 2014. MetaImprint: an information repository of mammalian imprinted genes. Development 141, 2516–2523. 10.1242/dev.105320. [PubMed: 24850854]

Wiseman DH, Baker SM, Dongre AV, Gurashi K, Storer JA, Somervaille TC, et al., 2020. Chronic myelomonocytic leukaemia stem cell transcriptomes anticipate disease morphology and outcome. EBioMedicine 58. 10.1016/j.ebiom.2020.102904.

Wnuk A, Rzemieniec J, Litwa E, Lasoń W, Kajta M, 2018. Prenatal exposure to benzophenone-3 (BP-3) induces apoptosis, disrupts estrogen receptor expression and alters the epigenetic status of mouse neurons. J. Steroid Biochem. Mol. Biol 182, 106–118. 10.1016/j.jsbmb.2018.04.016. [PubMed: 29704544]

Wong NC, Novakovic B, Weinrich B, Dewi C, Andronikos R, Sibson M, et al., 2008. Methylation of the adenomatous polyposis coli (APC) gene in human placenta and hypermethylation in chorioncarcinoma cells. Canc. Lett 268, 56–62. 10.1016/j.canlet.2008.03.033.

Xuan S, Baptista CA, Balas G, Tao W, Soares VC, Lai E, 1995. Winged helix transcription factor BF-1 is essential for the development of the cerebral hemispheres. Neuron 14, 1141–1152. 10.1016/0896-6273(95)90262-7. [PubMed: 7605629]

Yang AS. 2004. A simple method for estimating global DNA methylation using bisulfite PCR of repetitive DNA elements. Nucleic Acids Res. 32, 38e–338. 10.1093/nar/gnh032.

Yang J, Cao L, Guo R, Jia J, 2010. Permeable reactive barrier of surface hydrophobic granular activated carbon coupled with elemental iron for the removal of 2,4-dichlorophenol in water. J. Hazard Mater 184, 782–787. 10.1016/j.jhazmat.2010.08.109. [PubMed: 20864257]

Yuen RK, Jiang R, Peña Herrera MS, McFadden DE, Robinson WP. 2011. Genome-wide mapping of imprinted differentially methylated regions by DNA methylation profiling of human placentas from triploidies. Epigenet. Chromatin 4. 10.1186/1756-8935-4-10.

Zheng Z, Liu H, Beall M, Ma T, Hao R, Ross MG, 2014. Role of aquaporin 1 in fetal fluid homeostasis. J. Matern. Fetal Neonatal Med 27, 505–510. 10.3109/14767058.2013.820697. [PubMed: 23808411]
Zhu J, Ma C, Zhu L, Li J, Peng F, Huang L, et al., 2020. A role for the NLRC4 inflammasome in premature rupture of membrane. PLoS One 15, e0237847. 10.1371/journal.pone.0237847. [PubMed: 32833985]

Zona R, Solar S, Gehringer P, 2002. Degradation of 2,4-dichlorophenoxyacetic acid by ionizing radiation: influence of oxygen concentration. Water Res. 36, 1369–1374. 10.1016/s0043-1354(01)00323-2. [PubMed: 11902792]
Fig. 1.
Workflow of the study. Abbreviations: CpG = CG methylation site. DMR = differentially methylated region. EWAS = epigenome-wide association study. FDR = false discovery rate. GAMP = global analysis of methylation profiles.
Fig. 2.

β regression coefficient estimates according to genes encompassed by DMRs identified as associated with pregnancy triclosan concentration (number of CpGs within DMR ≥ 5, Šídák-corrected p-value <0.05, n = 202,379,904 CpGs). Circles represent CpGs within genes associated with triclosan exposure. β coefficient estimates correspond to a change in the DNA methylation level for doubling of the urinary phenol concentration. EWAS regression models on which the DMR analysis was based were adjusted for recruitment center, maternal active smoking in the three months preceding pregnancy and during pregnancy, maternal age, parity, maternal education level, season of conception, batch, plate, and chip. Abbreviations: CpG = CG methylation site. DMR = differentially methylated region. EWAS = epigenome-wide association study.
Fig. 3.
Regional Manhattan plots for the adjusted associations of pregnancy triclosan concentrations and placental DNA methylation within DMRs encompassing imprinted genes (number of CpGs within DMR ≥ 5, Šidák-corrected p-value <0.05, n = 202, 379,904 CpGs). Circles represent CpGs; orange boxes indicate exons; orange lines represent introns; green boxes indicate CpG islands. The heat map represents Spearman correlations between the CpGs included in each DMR. EWAS regression models on which the DMR analysis was based were adjusted for recruitment center, maternal active smoking in the three months preceding pregnancy and during pregnancy, maternal age, parity, maternal education level, season of conception, batch, plate, and chip. Abbreviations: CpG = CG methylation site. DMR = differentially methylated region. EWAS = epigenome-wide association study.
Table 1

DMRs (number of CpGs ≥5) associated with pregnancy urinary concentrations of phenols (46 DMRs, Šidák-corrected p-value <0.05, n = 202, 379,904 CpGs).

| Phenol               | Gene\(^a\) | DMR (chromosome:start-end) | No. of CpGs | SLK p-value | Šidák p-value | Direction of association |
|----------------------|------------|----------------------------|-------------|-------------|---------------|-------------------------|
| 2,4-dichlorophenol   | FLJ41603   | chr5:148960866-148961012   | 6           | 1.66E-08    | 4.31E-05      | +                       |
| 2,4-dichlorophenol   | GNG11      | chr7:93550986-93551133      | 5           | 1.92E-08    | 4.97E-05      | +                       |
| 2,4-dichlorophenol   | IRF7       | chr11:615,945-616,113       | 6           | 1.96E-08    | 4.44E-05      | +                       |
| Benzophenone-3       | DAXX       | chr6:33288296-33288373      | 6           | 7.98E-09    | 3.94E-05      | +                       |
| Benzophenone-3       | LDHD       | chr16:75150744-75150881     | 6           | 8.31E-10    | 2.31E-06      | +                       |
| Benzophenone-3       | MIR886     | chr5:135416331-135416530    | 7           | 8.45E-09    | 1.61E-05      | –                       |
| Methylparaben        | KCNIP2     | chr10:103599617-103599758   | 5           | 2.83E-08    | 7.62E-05      | +                       |
| Methylparaben        | SLC17A9    | chr20:61583910-61584109     | 6           | 3.04E-09    | 5.80E-06      | +                       |
| Propylparaben        | SLC17A9    | chr20:61583910-61584160     | 7           | 5.34E-12    | 8.12E-09      | +                       |
| Triclosan            | APC        | chr5:112073348-112073770    | 14          | 8.06E-12    | 7.26E-09      | +                       |
| Triclosan            | APOLD1     | chr12:12938161-12938426     | 5           | 2.31E-08    | 3.32E-05      | +                       |
| Triclosan            | AQP1       | chr7:30951272-30951499      | 7           | 8.80E-09    | 1.47E-05      | +                       |
| Triclosan            | C6orf25    | chr6:31692234-31692376      | 6           | 7.39E-07    | 1.98E-03      | +                       |
| Triclosan            | CD93       | chr20:23066944-23067160     | 8           | 2.76E-12    | 4.86E-09      | +                       |
| Triclosan            | CLDN6      | chr16:3068315-3068530       | 7           | 1.02E-07    | 1.80E-04      | +                       |
| Triclosan            | COL7A1     | chr3:48632484-48632724      | 6           | 2.31E-07    | 3.66E-04      | +                       |
| Triclosan            | DNAJC6     | chr1:65730339-65730495      | 6           | 7.21E-07    | 1.75E-03      | +                       |
| Triclosan            | FERMT3     | chr11:63974123-63974230     | 5           | 1.43E-07    | 5.07E-04      | +                       |
| Triclosan            | FOXG1      | chr14:29255904-29256035     | 8           | 2.71E-07    | 7.87E-04      | +                       |
| Triclosan            | GDF2       | chr10:48416839-48416978     | 6           | 1.49E-07    | 4.06E-04      | +                       |
| Triclosan            | GNAS       | chr20:57463325-57464130     | 30          | 2.49E-16    | 1.05E-13      | +                       |
| Triclosan            | GNAS       | chr20:57464970-57465176     | 7           | 2.34E-08    | 4.32E-05      | +                       |
| Triclosan            | GNASAS/GNAS| chr20:57425979-57427275     | 42          | 8.86E-15    | 2.60E-12      | +                       |
| Triclosan            | GP9        | chr3:128779498-128779602    | 6           | 4.48E-07    | 1.63E-03      | +                       |
| Triclosan            | HCRT       | chr17:40337411-40337624     | 5           | 2.17E-09    | 3.87E-06      | +                       |
| Triclosan            | HIST1H1A   | chr6:26018003-26018128      | 5           | 7.39E-07    | 2.24E-03      | +                       |
| Triclosan            | HIST1H2BK/HIST1H4I | chr6:27106988-27107394 | 6 | 9.50E-14 | 8.89E-11 | + |
| Phenol  | Gene | DMR (chromosome:start-end) | No. of CpGs | SLK p-value | Šidák p-value | Direction of association |
|--------|------|-----------------------------|-------------|-------------|--------------|--------------------------|
| Triclosan | JAK3 | chr19:17958505-17959083 | 12          | 5.54E-13    | 3.64E-10     | +                        |
| Triclosan | KIAA1949 | chr6:30652202-30652,830 | 12          | 6.08E-11    | 3.68E-08     | +                        |
| Triclosan | MADIL1 | chr7:2059921-2060,180      | 5           | 3.54E-10    | 5.19E-07     | +                        |
| Triclosan | MAFG  | chr17:79881468-79881544    | 5           | 1.16E-09    | 5.82E-06     | +                        |
| Triclosan | NFAM1 | chr22:4282836-42828517     | 6           | 3.90E-07    | 1.13E-03     | +                        |
| Triclosan | NUAK1 | chr12:106533840-106533904  | 5           | 3.35E-09    | 1.99E-05     | +                        |
| Triclosan | PLEC1 | chr8:145018928-145019117   | 6           | 1.50E-08    | 3.02E-05     | +                        |
| Triclosan | PLEKH6 | chr1:204328843-204329308   | 7           | 6.73E-13    | 5.50E-10     | +                        |
| Triclosan | PYCARD | chr16:31214307-31214495    | 7           | 1.93E-07    | 3.90E-04     | +                        |
| Triclosan | RGMA  | chr15:93616943-93617081    | 7           | 8.71E-07    | 2.39E-03     | +                        |
| Triclosan | RNH1  | chr11:504,784-504938       | 5           | 1.75E-09    | 4.30E-06     | +                        |
| Triclosan | SGCE/PEG10 | chr7:94285270-94285398    | 8           | 9.30E-07    | 2.76E-03     | +                        |
| Triclosan | SLC16A11 | chr17:6947200-6947,404    | 7           | 4.24E-07    | 7.89E-04     | +                        |
| Triclosan | SLC35C1 | chr11:45825356-45825580   | 8           | 5.55E-08    | 9.42E-05     | +                        |
| Triclosan | SPTA13 | chr13:24844846-24844939   | 6           | 1.99E-08    | 8.12E-05     | +                        |
| Triclosan | SYNGAP1 | chr6:33410480-33401543    | 5           | 1.29E-08    | 7.79E-05     | +                        |
| Triclosan | TNXB  | chr6:32063901-32064678    | 23          | 5.10E-13    | 2.49E-10     | −                        |
| Triclosan | ZBBX  | chr3:167098017-167098171  | 7           | 4.69E-06    | 1.15E-02     | +                        |
| Triclosan |       | chr1:46632581-46632872    | 5           | 6.25E-09    | 8.17E-06     | +                        |

EWAS regression models on which the DMR analysis was based were adjusted for recruitment center, maternal active smoking in the three months preceding pregnancy and during pregnancy, maternal age, parity, maternal education level, season of conception, batch, plate, and chip. Imprinted genes are in bold.

UCSC (https://genome.ucsc.edu).

Abbreviations: CpG = CG methylation site. DMR = differentially methylated region. EWAS = epigenome-wide association study. SLK = Stouffer-Liptak-Kechris correction. UCSC = University of California, Santa Cruz.