DNA Hypermethylation of Brain-Derived Neurotrophic Factor is Associated with High Levels of Polybrominated Diphenyl Ethers in the Cord Serum: A Laizhou Wan Birth Cohort Study From China

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Abstract

Background

Recent studies have suggested that in utero exposure to environmental organic pollutants, such as polybrominated diphenyl ethers (PBDEs) and bisphenol A may induce epigenetic alterations, specifically by modifying the patterns of DNA methylation. We investigated the relationship between PBDE exposure and *BDNF* DNA methylation.

Methods

We measured the levels of eight PBDE congeners in umbilical cord serum samples using gas chromatography/mass spectrometry. Genomic DNA was extracted from whole cord blood samples. The methylation status of the *BDNF* gene was determined using methylation-specific PCR with primers specific for either methylated or unmethylated alleles. The unconditional logistic regression model was used to investigate the relationship between PBDE levels and *BDNF* DNA methylation.

Results

The total PBDE congener level (range) in cord serum samples was 23.17 (4.17–2459.46) ng/g lipid. After adjusting for potential confounders, the incidence of *BDNF* DNA methylation status may increase when cord serum BDE-28, BDE-47, BDE-99 and BDE-100 levels increased (OR = 3.32, 95% CI: 1.01, 10.85; OR = 7.89, 95% CI: 1.75, 35.66; OR = 7.34, 95% CI: 1.79, 30.09; OR = 3.96, 95% CI: 1.15, 13.68; respectively).

Conclusions

We found that the levels of BDE-28, -47, -99, and -100 were positively associated with *BDNF* DNA hypermethylation. Changes in cord blood *BDNF* DNA methylation may be a part of the biological pathway underlying in utero PBDE exposure and adverse foetal growth.

Background

Polybrominated diphenyl ethers (PBDEs) are organic compounds used as flame retardants in a range of products including electronic equipment, vehicles, furniture, computers, textiles, and infant products (Byun et al., 2015). As PBDEs are not chemically bound to these products, they leach from the products with time and use and finally bio-accumulate through the food chain (Shi et al., 2017; Ding et al., 2015). Previous studies have found that PBDEs are accumulated in human samples such as blood, placenta, breast milk, hair, and tissues (Wang et al., 2020; Fernández Cruz et al., 2020; Shi et al., 2017; Peng et al., 2020); further, they are also found in the environment, i.e., in sediments, soil, and dust (Da et al., 2020; Liao et al., 2020; Hoang et al., 2020).

Exposure to PBDEs has been linked to a wide range of adverse health effects; they can disrupt endocrine function, affect neurodevelopment, and be teratogenic (Ding et al., 2017; Lyche et al., 2015; Vuong et al., 2020; Ishfaq Ahmad Sheikh and Mohd Amin Beg 2020). Recent studies have shown that prenatal exposure to homologues of similar human load PBDEs is associated with irreversible impairment of brain function in offspring, suggesting that PBDEs can cause neurotoxicity and cause learning and memory impairment (Vuong et al., 2017; Ding et al., 2015). Therefore, investigating the effect of PBDE exposure on neurobehavioral development and the underlying mechanism has recently become a focus of research.

Brain-derived neurotrophic factor (*BDNF*) is an important member of the neurotrophin family. It has a central role in neural differentiation, survival of nerve cells, neurite outgrowth, and synaptic plasticity. It is involved in the process of long-term potentiation, which is one of the best known biochemical mechanisms that underlie learning and memory (Leal et al., 2014; Leal et al., 2017). Human *BDNF* gene is located on chromosome 11p13-14, and consists of 11 exons (*I-IX*, *Vh* and *VIIIh*) and 9 promoters (Pruunsild et al., 2007). *BDNF* promoter *IV* is one of the most widely investigated promoters in the context of DNA methylation changes associated with alterations in *BDNF* expression (Aarons et al., 2019). For example, McKinney et al (2015) found that *BDNFIV* methylation in the orbital frontal cortex in the old man was negatively associated with *BDNF* expression. Xie et al (2017) found that in the periphery blood, *BDNFIV* methylation was related to mild cognitive impairment. Studies also indicated that *BDNF* promoter *IV* appeared to be uniquely sensitive to neuronal stimuli in vitro (Tao et al., 1998; Martinowich et al., 2003) and in vivo (Fuchikami et al., 2008; Sui et al., 2010).

Recent studies have suggested that in utero exposure to environmental organic pollutants, such as PBDEs and bisphenol A (BPA), may induce epigenetic alterations, specifically by altering the patterns of *BDNF* DNA methylation (Kertes et al., 2017; Moser et al., 2015; Bennett and Lagopoulos, 2014; Wrigglesworth et al., 2019). In utero PBDEs exposure has been associated with adverse foetal growth. However, no studies investigate how PBDEs exposure through umbilical cord affects cord blood *BDNF* DNA methylation. In this study, we investigated *BDNF* DNA methylation and PBDEs levels in cord blood, and examined the effects of PBDE exposure during pregnancy on *BDNF* DNA methylation. Our findings may provide insights to understand the adverse effects of in utero PBDE exposure on foetal growth and development through novel epigenetic mechanisms.

Methods

Study population and biological sample collection

This study was a prospective birth cohort study (Laizhou Wan birth cohort, LWBC) which was started in 2010 to determine the effects of environmental exposures on the health of pregnant women and their children and was performed in the south coast area of Laizhou Wan (Bay) of the Bohai Sea, Shandong province, China. The detailed methods of this study are published elsewhere (Chen et al., 2015). In total, 164 pregnant women participated in the study from September 2010 to March 2011. Among these, 145 women agreed to take part in this study (response rate 88.4%). 23 cases were excluded for their

insufficient cord blood sample for PBDEs analysis and *BDNF* gene methylation detection, and 14 were excluded for missing values for major confounders. Finally, 108 women were regarded as the study population in the present study. No substantial differences were found in sociodemographic characteristics between the baseline population (n = 145) and the study population (n = 108). The study activities were approved by the Medical Ethics Committee of Shanghai Xinhua Hospital, Shanghai Jiao Tong University School of Medicine. Written informed consent was obtained from each participant.

Pregnant women were recruited at the time when they were preparing for labour and delivery in a county hospital (the only hospital with an obstetric ward) located in the southern coastal area of Laizhou Wan. All interviews were conducted by a specially trained nurse using structured questionnaires shortly after foetal delivery. Demographic and socioeconomic information (maternal age, height, pre-pregnancy weight, education level, household income, and home address) and maternal information on lifestyle habits (cigarette smoking and alcohol use during pregnancy, employment) were gathered at the enrolment interview. Information about exposure to PBDEs during pregnancy included the women's occupation, whether there were any brominated flame retardant (BFR) production factories near their home, and, if yes, what types of BFRs were produced in the factories. Information on paternal exposure to PBDEs and occupation were also collected during the interview. Prenatal and delivery medical records were abstracted including data about pregnancy and delivery complications, pregnancy weight gain, and infant birth outcome.

Umbilical cord blood was obtained immediately after delivery by medical professionals. Blood samples were collected from an umbilical vein immediately post-delivery using a syringe. Each sample was divided into two aliquots. One of the aliquots was collected into EDTA-containing collection tubes for genomic DNA extraction and the other was collected into two 10-mL red-topped tubes. The latter was allowed to clot and centrifuged at 1500 rpm for 20 min, following which the supernatant was decanted into pre-cleaned glass vials for residue analysis. All samples were coded, frozen, and stored at -80°C until further analysis.

PBDE measurement in umbilical cord serum

Detailed extraction, gravimetric lipid determination procedures, and instrumental analysis protocols of PBDE measurement are described elsewhere (Hovander et al., 2000; Ding et al., 2015). Briefly, after spiking with surrogate standards (¹³C-BDE-139), about 2 mL of each sample was extracted twice with a mixture of HPLC-grade hexane and methyl tertiary butyl ether (MTBE; 1:1 in volume). Lipid content was determined gravimetrically using the whole extract. The amount of serum lipid was used to express the concentration of BDEs on lipid weight basis. Each extract was cleaned by 2 mL concentrated H₂SO₄ and cleaned up by liquid–solid chromatography using a SiO₂/H₂SO₄ column (SiO₂/H₂SO₄ 2:1 by weight; 1 g). PBDEs were eluted from this column using hexane (8 mL) as a mobile phase. Finally, the 1–2 mL fraction was collected and concentrated to 40 µL. PBDEs were detected by GC/ECNI/MS on the Agilent 5975N mass spectrometer equipped with 6890 Gas Chromatograph. Eight PBDE congeners (BDE-28, -47, -85, -99, -100, -153, -154, and -183) were measured.

To ensure the reproducibility of PBDE concentration measurement, one sample out of 20 was randomly selected and its concentration was measurement was repeated; the reproducibility of this analysis was good. At the same time, for every 12 samples, a blank control was processed to check for interference or contamination from solvents and glassware. The limit of detection (LOD) of the method was defined as the mean blank mass plus three standard deviations. The LOD ranged from 0.24 to 0.48 ng/g lipid in serum samples. The blank values were less than LOD for all PBDE congeners in the samples.

Detection of BDNF DNA methylation in the cord blood

Genomic DNA was extracted from whole blood samples (approximately 200 µL) using a Flexi Gene DNA Kit (Qiagen, USA). Purified DNA was quantified using an ND1000 spectrophotometer (Eppendorf, D30, Germany). The extracted genomic DNA (20 µL) was treated with sodium bisulphite modification using Fast DNA Bisulfite Kit (Qiagen, USA), according to the manufacturer's instructions. Elution was performed with 15 µL of Buffer EB. The methylation status of the *BDNFIV* gene was determined using methylation-specific PCR (MSP) with primers specific for either methylated or unmethylated alleles.

After bisulphite modification, a region-specific PCR with a biotinylated primer was performed for human *BDNFIV*. The PCR primer sequences were as follows: for the methylated reaction, 5'-GGTCGGCGGGGAGTAGTAT-3' (forward) and 5'-CCCATTCCCAACGCTTACCT-3' (reverse) and for the unmethylated reaction, 5'-GGTTGGTGGGGAGTAGTAT-3' (forward), 5'-CCCATTCCCAACACTTACCT-3' (reverse). The PCR reaction was carried out in a 20-µL solution containing 0.2 µL of bisulphite-modified genomic DNA, 1× PCR amplification buffer, 0.2 mM dNTPs, 1.5 mM MgCl₂, 0.2 µM primers, 1 U of platinum Taq DNA polymerase, and 14.1 µL pure water. The PCR conditions included an initial denaturation step for 2 min at 94°C and 35 cycles of 15 s at 95°C, 30 s at 60.7°C, and 15 s at 72°C. EpiTect Control DNA and Control DNA Set were used as positive controls for the methylated and unmethylated genes, respectively. Negative control samples without DNA were included in each set of PCR reaction. Pyro sequencing verification analyses were conducted at Sangon Biotech Co., Ltd. (Shanghai). PCR products were analysed on 2% agarose gel, stained with ethidium bromide, and visualised under UV light (**Supplementary Fig. 1**). Each MSP was repeated at least once to confirm the results.

In the measurements of methylation-specific polymerase chain reaction PCR gel electrophoresis, we determined that only the MSP band was amplified as methylation. If only the USP band was amplified, it was determined that no methylation occurred. Bands observed in both MSP and USP were considered partially methylated.

Statistical analysis

All data were analysed using the SPSS 20.0 software (SPSS Inc., Chicago, IL, USA). Descriptive statistics were calculated for demographic characteristics of mothers and infants. In our final analysis, the partial methylation and complete methylation in the samples *BDNFIV* were combined, and the *BDNFIV* methylation status was divided into methylated and unmethylated. We analysed *BDNFIV* methylation as

categorical variables. As four PBDE congeners (BDE-28, -47, -99, and -100) were detected in more than 85% of the cord serum, we analysed these four congeners to assess the relationship between cord blood PBDEs and cord blood *BDNFIV* methylation. An aggregate variable ($\sum_4 \text{PBDE}$) was generated using the molar sum of these four congeners. Cord blood PBDE levels were classified into two groups: detectable levels below the median detectable level, and detectable levels above the median. Two independent Chi-square tests were used to examine the correlation between *BDNFIV* methylation and PBDE levels.

We estimated adjusted odds ratios (ORs) and their 95% confidence intervals (CIs) of PBDEs associated with *BDNFIV* methylation using unconditional logistic regression model, and the effects of potential confounders were assessed using a forward stepwise regression process. Maternal age, education, pre-pregnancy body mass index (pre-pregnancy BMI), weight gain during pregnancy, parity, household monthly salary, type of delivery, infant sex, and gestational age were initially considered as confounders. Confounders were finally selected for regression models if they were related to PBDE levels in the literature and were associated ($P < 0.2$) with *BDNF* DNA methylation. Potential confounders included maternal age, education, and household monthly salary, pre-pregnancy BMI, parity, and infant sex.

Results

The study included 108 participants. Details regarding their demographic characteristics are presented in **Table 1**. The average maternal age was 28.51 years (SD = 5.12); two-thirds (66.67%) were primiparous, and half (52.80%) had graduated from high school or above. The majority of the women (93.52%) lived in households with a monthly income less than 5000 (¥). More than half (64.81%) of the women had a normal weight before pregnancy. Of the 108 births, 50 (46.3%) were boys and 58 were (53.7%) girls. The mean birth weight and gestational age were 3.40 kg (SD = 442.13) and 39.75 weeks, respectively.

Table 2 shows the distribution (25th, 50th, 75th, and 95th percentile) of the eight individual lipid-adjusted PBDE congeners. The mean (range) level of all PBDE congeners in cord blood was 23.17 (4.17–2459.46) ng/g lipid. The detection rate of PBDE-28, -47, -99, and -100 was over 85% in all samples.

DNA methylation of *BDNF* was analysed. In total, 83.33% cord blood samples showed *BDNFIV* methylation. Two independent Chi-square tests were used to examine the correlation between *BDNF* DNA methylation and PBDE levels. The findings are presented in **Table 3**. Among the 108 samples, the levels of BDE-47, -99, and -100 were associated with *BDNFIV* methylation ($\chi^2 = 6.67$, $P = 0.01$; $\chi^2 = 6.67$, $P = 0.01$; $\chi^2 = 4.27$, $P = 0.039$; respectively).

Unconditional logistic regression models were used to analyse the associations between cord blood *BDNFIV* methylation and PBDE exposure. Methylation level was modelled as a function of PBDE levels, adjusting for maternal age, education, household monthly salary, pre-pregnancy BMI, parity, and infant sex (**Table 4**). After adjusting for potential confounders, the incidence of *BDNFIV* methylation status increased when the levels of PBDE congeners increased. The statistical parameters of this association

are as follows. BDE-28: OR = 3.32, 95% CI: 1.01, 10.85; BDE-47: OR = 7.89, 95% CI: 1.75, 35.66; BDE-99: OR = 7.34, 95% CI: 1.79, 30.09; and BDE-100: OR = 3.96, 95% CI: 1.15, 13.68.

Discussion

As commonly used flame retardants, PBDEs are ubiquitous in the environment and in human bodies. Studies have reported high levels of PBDEs in cord blood samples, indicating that in utero PBDEs exposure is very common (Zhao et al., 2018; Ding et al., 2015; Zhao et al., 2016). The levels of cord serum PBDEs in our study (23.17 ng/g lipid) were generally lower than those reported in North America (33.32 ng/g lipid in the USA and 93.66 ng/g lipid in Canada) (Chen et al., 2013; Foster et al., 2011). However, they were five orders of magnitude higher than those reported in serum samples collected during other studies performed on general populations in various areas of China, such as Hong Kong (4.45 ng/g lw; Ho et al., 2015) and Shanghai (2.16 ng/g lw; Xu et al., 2018). The possible reason for the high PBDEs levels in our study might be associated with high amounts of exposure in our study area (Li et al., 2017), which is located near several large-scale BFR production factories.

Previous studies have observed that infants and children who are prenatally exposed to high levels of PBDEs are more likely to suffer from various adverse health consequences, such as adverse infant birth outcomes (Chen et al., 2015; Chen et al., 2018). The exact mechanism underlying the adverse effects associated with prenatal exposure to PBDEs remains unclear; however, epigenetic mechanisms have been suggested as a possible pathway linking in utero PBDE exposure to adverse health effects.

In the present study, we evaluated the association between prenatal exposure to PBDEs and *BDNF* DNA methylation levels measured in cord blood samples. We found that BDE-28, -47, -99, and -100 were positively associated with *BDNF* DNA (exon IV) methylation. As DNA methylation is involved in the normal processes of development and genomic imprinting, any changes in the normal regulation of this system might have a high negative impact on the human body (Bernstein et al., 2007). DNA methylation changes have been found in psychiatric post-mortem brain samples (Keller et al., 2010) as well as in peripheral blood of the psychiatric patients (Kang et al., 2013; Ikegame et al., 2013). Meanwhile, a cohort study conducted at the Columbia Center for Children's Environmental Health (CCCEH) found that *BDNF* DNA methylation in the human blood may be used as a predictor of brain *BDNF* DNA methylation and gene expression; moreover, it can predict behavioural vulnerability induced by early-life environmental exposure (Kundakovic et al., 2015). Thus, our present study showed that increased levels of PBDEs were related to *BDNFIV* methylation in the cord blood. Our results contribute to the growing body of evidence that PBDEs may act as developmental neurotoxicants.

This study is the first to experimentally confirm that PBDE exposure is linked to *BDNF* DNA hypermethylation in the cord blood. Similarly, associations between DNA methylation levels and prenatal exposure to other compounds, including BPA (Kundakovic et al., 2015), phthalate (Zhao et al., 2015), and air pollutants (Janssen et al., 2013), have been reported. However, the influence of PBDEs on cord blood *BDNF* DNA hypermethylation levels has not been described. Most previous studies on the relationship

between PBDE exposure and epigenetic changes have reported DNA methylation in different tissues such as placenta and cord blood (Dao et al., 2015; Kappil et al., 2016; Zhao et al., 2016). A positive association was observed between placental global DNA methylation and total PBDE levels in a study by Kappil et al. (2016). Placental DNA hypomethylation was associated with high levels of PBDE exposure in cord blood (Zhao et al., 2016). On the contrary, Dao et al. (2015) found that high maternal BDE-47 exposure was associated with decreased cord blood *TNFA* DNA methylation.

The biological mechanism for the interference of PBDEs with *BDNF* DNA hypermethylation is still unclear. Previous studies have shown that prenatal exposure to PBDEs was associated with increased odds of low thyroid hormones (Herbstman et al., 2008; Lin et al., 2011) and disrupted thyroid homeostasis by binding of thyroid hormones with their receptors and transporter proteins (i.e., transthyretin) (Yu et al., 2010; Ernest et al., 2012). Sui et al. (2010) suggested that perinatal hypothyroidism induces epigenetic modulations of DNA methylation and the cross talks involved in chromatin reorganisation or remodelling, thus influencing *BDNF* expression. We speculate that PBDEs disrupt thyroid homeostasis and reduce the fidelity of the epigenetic machinery, resulting in *BDNF* DNA hypermethylation of cytosine residues.

Our study is one of the first to show a relationship between PBDE exposure and DNA methylation in cord blood samples. However, there are several limitations to our study. Firstly, we did not measure all PBDE congeners, including BDE-209, which is the main constituent of the deca-BDE mixture. Therefore, our study cannot provide any information on the relationship between BDE-209 and *BDNF* DNA methylation. Secondly, epigenetic modifications are tissue specific. In our study, we acknowledged that cord blood contained a variable number of nucleated newborn red blood cells that are globally more hypomethylated than white blood cell types, which may confound our methylation measurements. It indicates that distinctive cell counting and / or classification of cells from whole blood is required to adjust the findings from the methylation patterns by regression model or access the methylation patterns in different types of blood cells (Dao et al., 2015). However, in our present study we cannot obtain the cell distribution of the immune cells in the cord blood samples we utilized. Given the fact that cord blood may contain stem cells that can populate the brain in later life (Singh et al., 2015) and also provide abundant source of immune cells which are important producers of cytokines (Stock et al., 2000), the cord blood may provide a reasonable surrogate for our target organ/tissues (brain/immune cells) (Dao et al., 2015). Thirdly, as partial methylation was treated as methylated samples in the final analysis, information bias could not be ruled out. However, after restricting the samples to complete methylation and no methylation samples, BDE-28 and BDE-99 are still related to the methylation status of *BDNFIV*, suggesting the robustness of the results reported in the present study to some extent. Fourthly, a lack of negative control using unmethylated promoter linked highly expressed genes in this study may confine our conclusions. More specified studies, for instance, conservative gene sequences as negative controls, pyrosequencing, or genome-wide analyses, are required to elucidate the potential mechanisms that how PBDEs induce *BDNFIV* methylation. Lastly, as people are simultaneously exposed to a variety of environmental pollutants, it is possible that unmeasured confounders have a role to play, which may affect both the exposure levels and *BDNF* DNA methylation status. In addition, our small sample size may limit our power to detect significant relationship in logistic regression models, which calls for further steps to

demonstrate the potential influence of prenatal PBDE exposure on the foetal epigenome, by performing replicated studies in a larger sample pool and exploring the relationship between prenatal PBDEs exposure and epigenetic changes.

Conclusions

In conclusion, our present study found that the levels of PBDEs in the cord serum were much higher compared with those measured in other studies in the general population in China. A number of PBDE congeners, including BDE-28, BDE-47, BDE-99, and BDE-100, were positively associated with *BDNF* DNA hypermethylation in the cord blood. Our findings may provide insights to understand the adverse effects of in utero PBDE exposure on foetal growth and development through novel epigenetic mechanisms.

Declarations

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Ethical Approval and Consent to participate

The study activities were approved by the Medical Ethics Committee of Shanghai Xinhua Hospital, Shanghai Jiao Tong University School of Medicine.

Consent for publication

Written informed consent was obtained from each participant.

Availability of supporting data

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

Competing interests

The authors declare that they have no actual or potential competing financial interests.

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Authors' contributions

Ying Tian and Yu Gao conceived and designed the study. Limei Chen, Yan Zhang, Yijun Zhou, and Rong Shi performed the experiments. Limei Chen wrote the paper. Ying Tian and Yu Gao reviewed and edited the manuscript. All authors read and approved the manuscript.

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Tables

Table 1. Demographic characteristics of the study population, Shandong Province, China, 2010–2011 (n = 108).

Variable	n	Mean $\pm$ SD or %
Maternal Characteristics		
Age (years)	108	28.51 $\pm$ 5.12
< 30	70	64.81
30 – 34	24	22.22
$\geq$ 35	14	12.96
Education (years)		
< 9	51	47.20
$\geq$ 9	57	52.80
Pre-pregnancy BMI (kg/m ²)	108	21.35 $\pm$ 2.96
< 18.5	13	12.03
18.5 – 23	70	64.81
$\geq$ 23	25	23.15
Parity		
0	72	66.67
$\geq$ 1	36	33.33
Household monthly income (¥)		
< 3000	74	68.52
3000 – 5000	27	25.00
> 5000	7	6.48
Obstetrical & newborn parameters		
Birth weight (g)	108	3401.52 $\pm$ 442.13
Gestational age (weeks)	108	39.75 $\pm$ 1.99
< 37	5	4.63
$\geq$ 37	103	95.37
Sex		
Boys	50	46.30
Girls	58	53.70

Table 2. Lipid-adjusted PBDE concentrations in the serum of umbilical cord blood (n= 108).

PBDE congener	Detection		Selected percentiles (ng/g of lipid)				$\sum_8$ PBDEs: an aggregate variable generated by eight congeners
	n (%)	Range	25 th	50 th	75 th	95 th	
BDE-28	92 (85.19)	☒LOD – 100.24	1.63	3.31	5.55	12.14	LOD: limit of detection
BDE-47	102 (94.44)	☒LOD – 582.27	2.32	3.65	5.48	55.77	
BDE-85	76 (70.37)	☒LOD – 404.80	1.54	2.37	3.26	13.76	
BDE-99	103 (95.37)	☒LOD – 812.82	4.97	6.76	8.52	77.27	
BDE-100	98 (90.74)	☒LOD – 130.02	2.21	3.14	4.25	13.09	NA: not available
BDE-153	80 (74.07)	☒LOD – 814.31	2.04	3.26	7.86	26.27	
BDE-154	53 (49.07)	☒LOD – 38.79	1.07	1.45	2.27	11.70	
BDE-183	48 (44.44)	☒LOD – 842.38	1.69	2.43	4.65	28.30	
$\sum_8$ PBDEs	NA	4.17 – 2459.46	16.29	23.17	34.74	206.50	Table 3. Chi-square

test coefficients of PBDE congeners and BDNF gene methylation.

*: $P < 0.05$.

Table 4. Adjusted associations between cord blood PBDE levels and BDNF methylation levels ^a.

*: $P < 0.05$.

a: Adjusted for maternal age, education, household monthly salary, pre-pregnancy BMI, parity and infant sex.

Supplementary Files

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PBDE		BDNF DNA methylation		χ^2	P
Congeners		0	1		
BDE-28	Median	12	42	2.40	0.12
	Median	6	48		
BDE-47	Median	14	40	6.67	0.01*
	Median	4	50		
BDE-99	Median	14	40	6.67	0.01*
	Median	4	50		
BDE-100	Median	13	41	4.27	0.039*
	Median	5	49		
Σ_4 PBDE	Median	12	42	2.40	0.12
	Median	6	48		

		BDNF		β	S.E.	Wald	OR(95% CI)
		DNA methylation					
PBDE congeners		0	1				
BDE-28	Median	12	42	1.20	0.61	3.92	3.32 (1.01, 10.85)*
	Median	6	48				
BDE-47	Median	14	40	2.07	0.77	7.21	7.89 (1.75, 35.66)*
	Median	4	50				
BDE-99	Median	14	40	1.99	0.72	7.66	7.34 (1.79, 30.09)*
	Median	4	50				
BDE-100	Median	13	41	1.38	0.63	4.72	3.96 (1.15, 13.68)*
	Median	5	49				
$\sum_4$ PBDE	Median	12	42	15.32	10.72	2.04	3.13 (0.92, 10.67)
	Median	6	48				