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DNA methylation at GRIN2B partially mediates the association between prenatal bisphenol F exposure and cognitive functions in 7-year-old children in the SELMA study

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ABSTRACT

Background: Accumulating evidence suggests that prenatal chemical exposure triggers epigenetic modifications that could influence health outcomes later in life. In this study, we investigated whether DNA methylation (DNAm) levels at the glutamate ionotropic receptor NMDA type subunit 2B (*GRIN2B*) gene underlies the association between prenatal exposure to an endocrine disrupting chemical (EDC), bisphenol F (BPF), and lower cognitive functions in 7-year-old children.

Methods: Data from 799 children participating in the Swedish Environmental Longitudinal Mother and child Asthma and allergy (SELMA) pregnancy cohort was analyzed. Prenatal BPF exposure was assessed by measuring BPF levels in maternal urine. At age 7, DNAm of three CpG sites in a regulatory region of the *GRIN2B* gene was analyzed from buccal swabs using bisulfite-Pyrosequencing. Cognitive functions, including full-scale IQ and four subscales, were evaluated using the Wechsler Intelligence Scale for Children (WISC-IV). Associations between prenatal BPF exposure and *GRIN2B* DNAm, as well as between *GRIN2B* DNAm and cognitive functions, were determined using regression models adjusted for potential confounders. Generalized structural equation models (gSEM) were used to evaluate if *GRIN2B* DNAm mediates the association between prenatal BPF exposure and cognitive functions at 7 years of age.

Results: Prenatal BPF exposure was positively associated with *GRIN2B* DNAm levels at the third CpG site (CpG3), while CpG3 methylation was inversely associated with cognitive test scores. Mediation analyses showed that CpG3 methylation exerted 6–9% of the association between BPF exposure and full-scale IQ, as well as verbal comprehension and perceptual reasoning in boys, while not significant in girls.

Conclusions: This study is the first to identify locus-specific DNAm as a mediating factor underlying an epidemiological association between prenatal EDC exposure and cognitive functions in childhood. It also confirms previous findings, that *GRIN2B* DNAm is responsive to environmental exposures.

1. Introduction

The developing brain is vitally dependent on proper hormonal signaling of, e.g., thyroid and sex steroid hormones (Miranda and Sousa, 2018). Therefore, it is of concern that human populations are constantly exposed to chemicals that interfere with hormonal signaling, so called endocrine disrupting chemicals (EDCs), even from the time *in utero* (Bergman et al., 2013). Both animal studies and human epidemiological

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data repeatedly show that prenatal exposure to certain EDCs, like Bisphenol A (BPA), affects neurodevelopment (Engdahl and Rüegg, 2020; Wolstenholme et al., 2011). We have recently demonstrated that prenatal exposure to bisphenol F (BPF), used as a substitute for BPA in the production of polycarbonate plastics and epoxy resins (Chen et al., 2016), is associated with impaired cognition in children, primarily in boys, at 7 years of age (Bornehag et al., 2021; Tanner et al., 2020). The mechanism underlying this association is not known, but other studies have shown that changes in DNA methylation (DNAm) patterns can mediate persistent effects of early-life events on human health later in life (Checknita et al., 2018; Fasanelli et al., 2015; Tobi et al., 2018; Wiklund et al., 2019).

DNAm is a stable epigenetic modification that can, in interaction with other epigenetic modifications like histone modifications, change DNA accessibility and thereby regulate gene transcription over generation of cells (Bernstein et al., 2007; Miller and Grant, 2013). Epigenetic regulation plays a critical role in cell differentiation and tissue organization during development, not least of the brain (Smith and Meissner, 2013; Spiers et al., 2015). Animal studies have established that early-life exposure to environmental factors can induce epigenetic changes in the brain, which are linked to cognitive or behavioral changes later in life (Kundakovic and Jaric, 2017). Also, in humans, there is increasing evidence for associations between the prenatal environment (e.g., chemicals and metals, maternal smoking, alcohol consumption, and stress) and epigenetic changes in genes important for brain development and functions (Boschen et al., 2018; Jacobs et al., 2017; Tran and Miyake, 2017). Prenatal EDC exposure has been associated with altered mental and behavioral child development in a sex-dependent way (Braun et al., 2011; Evans et al., 2014; Harley et al., 2013; Kim et al., 2011; Kobrosly et al., 2014; Perera et al., 2016; Whyatt et al., 2012) as well as with altered DNAm patterns in a sex dependent way (Alavian-Ghavanini et al., 2018; Miura et al., 2019; Montrose et al., 2018).

In a previous study, we found that *in utero* exposure to BPA induces DNAm changes in a regulatory region of *GRIN2B*, the gene encoding the Glutamate ionotropic receptor [NMDA] subunit 2b, in the hippocampus of adult female rats, which correlated with changes in gene expression (Alavian-Ghavanini et al., 2018). Additionally, we found significant associations between *GRIN2B* methylation in buccal swabs samples from 7 year old children and prenatal BPA exposure in the SELMA study (Alavian-Ghavanini et al., 2018) and between *GRIN2B* methylation in saliva samples from adults and early-life stress in the PART study (Engdahl et al., 2021), suggesting that this region is susceptible to epigenetic changes in response to early life environmental impact also in humans. *GRIN2B* expression is important for brain development as mutations in *GRIN2B* have been associated with neurodevelopmental disorders such as intellectual disability, developmental dyslexia and autism spectrum disorders (ASD) (Hu et al., 2016; Mascheretti et al., 2015; Myers et al., 2019; Platzer and Lemke, 1993).

Here, we aimed at addressing whether *GRIN2B* methylation, analysed in buccal swab samples, is associated with cognitive function in the SELMA children at 7 years of age. Furthermore, we explored if *GRIN2B* methylation can mediate the statistical association between prenatal BPF exposure and cognitive outcomes.

2. Methods

2.1. Study population

We have analyzed data from the Swedish Environmental Longitudinal, Mother and child, Asthma and allergy (SELMA) study; a pregnancy cohort study designed to investigate early life exposure to environmental chemicals and health and development in children. SELMA recruited 2,582 pregnant women in the county of Värmland, Sweden at their first antenatal care visit between the years 2007 and 2010 with 39% response rate. Detailed recruitment selection criteria and sample collection procedures have been published previously (Bornehag et al., 2012). Participants provided written consent and the study was approved by the Regional Ethical Review Board (Uppsala, Sweden). For the current study, we used data from 799 children for which maternal BPF levels had been measured, child oral swab samples and cognitive functions collected at 7 years of age, and data on all covariates were available.

2.2. Biological samples

First morning void urine samples were obtained in week 3–27 of pregnancy (median week 10, and 96% of the samples were taken before week 13) at enrollment to the study. Urine samples were collected in supplied glass containers at home and, at a laboratory, transferred into polypropylene tubes without any other assisting equipment. Samples were stored at –20 °C before being processed at the laboratory at the division of occupational and environmental medicine, Lund University, Sweden. The samples were prepared and quantitatively analyzed for urinary BPF concentrations according to a method described in (Berge et al., 2017; Gyllenhammar et al., 2017) using liquid chromatography triple quadrupole linear ion trap mass spectrometry (QTRAP 5500; AB Sciex, USA; LC-MS/MS). The laboratory has qualified as HBM4EU laboratory for BPF analysis in urine.

DNA material from the mucosal lining of the mouth of the children was collected with buccal swabs by a nurse at the health examination at 7 years. The buccal swabs were then stored at −70 °C in a biobank until analysis.

2.3. Cognitive function measurements

Children's cognitive functions at age 7 were evaluated by trained psychologists using the Wechsler Intelligence Scale for Children (WISC-IV; (Wechsler, 2003)), 4th edition. The test measures intellectual functioning of children in four sub-domains (indices) that make up the full scale IQ score. The four indices are: verbal comprehension index (VCI) measuring verbal reasoning and concept formation; perceptual reasoning index (PRI) measuring visual perception and organization, and nonverbal concept formation; working memory index (WMI) measuring memory processes and mental control; and processing speed index (PSI) measuring the speed and accuracy of visual information processing (Prifitera et al., 2005).

2.4. DNA methylation

Methylation status of three CpG sites in a regulatory region of *GRIN2B* were assessed by bisulfite-Pyrosequencing (Alavian-Ghavanini et al., 2018). The sequenced region is located in a CpG island upstream of the *GRIN2B* gene on Chromosome 12, more specifically at chr12:14,134,920–14,134,943 (in the GRCh37/hg19 genome assembly). The first analyzed CpG in this sequence ("CpG1") is identical with the Illumina 450 K probe ID cg10091102, located at position chr12:14,134,922. CpG2 and CpG3 are located at position chr12:14,134,932 and chr12:14,134,934. In addition, a fourth CpG was analyzed at position chr12:14,134,936 but this position was excluded from statistical analyses due to a low rate of passing quality control check in the Pyrosequencing assay. The samples were analyzed in two separate batches. Genomic DNA was isolated from oral swabs using the BuccalAmp™ DNA extraction kit (Epicentre) according to manufacturers' protocol. DNA was bisulfite converted using the EZ-96 DNA Methylation-Gold MagPrep kit (Zymo Research Corporation). After that, the region of interest was amplified using the PyroMark PCR kit (Qiagen) together with 0.2 μM forward primer (5′- TGATTTAGGGGGGAGGAGAAATT − 3′) and biotinylated reverse primer (Batch 1 = 5′-AAACTACCTCCCCCAAAATCTTAACA − 3′, Batch 2 = 5′-AAAACTACCTCCCCCAAAATCTTAACAAA-3′) and with the addition of 1 μl $MgCl_2$ (25 mM) per 25 μl reaction, according to manufacturer's protocol. Pyrosequencing was performed on a PyroMark ID instrument (Batch

1 = Q24 format, Batch 2 = Q96 format; Qiagen) using PyroMark Gold reagents (Qiagen) and 0.4 μM sequencing primer (Batch 1 = 5′-GGAAGATATTGTTTTTGTTTTTAG – 3′, Batch 2 = 5′-TATTGTTTTTGTTTTTAGG-3′) according to the manufacturer's protocol. Methylation level at the four sequenced CpG sites were obtained using the PyroMark software (Qiagen).

Standard curves of commercial DNA with known methylation status were analyzed in both batches (batch 1 and batch 2: 0–100% methylation; Batch 2: 0–15% methylation) and showed linearity ($R^2 > 0.97$) between measured and predicted methylation. A technical control sample with very low methylation level at *GRIN2B* was used to determine the resolution for low methylation values. This sample was read both as low methylated (<5%) and unmethylated, indicating that the set limit between unmethylated and low positive may be close to the technical detection limit of this method.

2.5. Adjustments

To account for urine dilution, BPF levels were adjusted to creatinine urinary concentration, which had been analyzed according to an enzymatic method described by Mazzachi et al. (2000). The creatinine adjusted BPF values were $\log_{10}$ transformed prior to statistical analyses. Maternal IQ was assessed using the short version of Ravens matrices, a test of nonverbal intelligence (Van der Elst et al., 2013), and included as a potential confounder in the statistical models. Also, for CpG1 and CpG2, but not for CpG3, the cluster distribution of samples varied between analysis batch 1 and 2 (Table S1), indicating a batch effect in the data. Therefore, all statistical models were adjusted for analytical batch. Since cognition varied between boys and girls (Table 1), also sex was added as a covariate to the statistical models.

2.6. Statistical analysis

All analyses were performed using the statistical software STATA version 14, and R version 3.5.2.

2.6.1. Clustering analysis for DNAm data

DNAm levels of the three CpG sites were found to form highly skewed-to-right distributions because a large proportion of the DNAm levels was zero. To overcome this skewed distribution in the statistical models, DNAm levels were categorized into three clusters and defined as follows: the first cluster included unmethylated samples (i.e. DNAm level equivalent to 0%), the second cluster included low methylated samples (i.e. DNAm levels between 0.1 and 5%), and the third cluster included methylated samples (i.e. DNAm levels > 5%). The 5% cutoff was set based on the uncertainty to accurately determine low methylation in the range of <5% due to technical limitations of the method, determined by the low methylated control sample. The DNAm distribution for each CpG site is shown in Fig. S1. To obtain a better separation between the unmethylated samples from the methylated samples in the mediation analyses described below, we excluded the second cluster of low methylated samples from the main analyses. However, sensitivity analyses were also performed including all three clusters and are presented as supplementary information.

Table 1
Characteristics of the Study Population (N = 799).

	All (n = 799)	Boys (n = 398)	Girls (n = 401)	P-value[a]
BPF[b] (ng/ml) [GM (GSD)]	0.16 (5.23)	0.16 (4.97)	0.15 (5.51)	0.566
Maternal IQ [mean ± SD]	114.9 ± 15.1	115.2 ± 15.6	114.5 ± 14.5	0.226
Child IQ				
Full scale IQ [mean ± SD]	99.8 ± 12.6	98.1 ± 12.8	101.5 ± 12.3	<0.001
Working memory [mean ± SD]	89.9 ± 13.7	88.7 ± 13.4	91.1 ± 13.8	0.013
Processing speed [mean ± SD]	98.6 ± 15.4	95.6 ± 15.3	101.5 ± 14.8	<0.001
Perceptual reasoning [mean ± SD]	106.3 ± 12.3	106.0 ± 12.6	106.6 ± 12.0	0.547
Verbal comprehension [mean ± SD]	100.7 ± 11.4	99.5 ± 11.7	101.8 ± 10.9	<0.001

[a] P-values from Mann-Whitney *t*-test, comparing boys and girls.
[b] Not creatinine adjusted BPF urinary concentrations

2.6.2. Descriptive analysis

Descriptive statistics were used to describe the study population in terms of prenatal BPF levels, cognitive test scores, and DNAm cluster proportions across three CpG sites, in the full sample and by sex. Differences by sex in continuous variables were evaluated using Mann-Whitney *t*-test as these variables had a significant Shapiro-Wilks normality test. The DNAm cluster proportions across three CpG sites were compared using the Chi-squared test. Spearman correlation coefficients between the three CpG sites showed significant but weak correlations ranging between 0.11 and 0.27.

2.6.3. Exploratory analyses

To establish the premise for mediation effects, we examined if DNAm at any CpG site (CpG1, CpG2, and CpG3) was associated with both cognitive functions and prenatal exposure to BPF. Statistically significant associations of the DNAm of a CpG site with both BPF and cognition would be considered a potential mediating factor of prenatal BPF exposure on cognitive functions, and were further analyzed for mediation effects. In these exploratory analyses, the DNAm clusters were regarded as categorical values representing the three clusters of unmethylated, low methylated and methylated samples, where the unmethylated cluster was used as the reference group.

For each CpG site we first investigated associations between DNAm and cognition, using separate generalized linear models (GLMs) on full scale IQ, and each of the four subscales, adjusting for child's sex, analytical batch (of pyrosequencing) and maternal IQ score. Second, we investigated associations between BPF exposure and DNAm, using multinomial logistic regression models on DNAm cluster adjusted for urinary creatinine, child's sex, and analytical batch. This model allowed to identify differences in the association between BPF exposure and DNAm clusters. Alpha values below 0.05 were considered significant.

2.6.4. Mediation analyses

We used the principles recommended by Baron and Kenny (Baron and Kenny, 1986), Judd and Kenny (Judd and Kenny, 1981), and James and Brett (James and Brett, 1984) - using three steps to establish mediation. The mediation effect of DNAm on the relation between BPF and cognitive functions was determined with the generalized structural equation model (gSEM) coding the CpG3 methylation in two clusters as a continuous variable. The two clusters used were the unmethylated group (0% methylation) and the methylated group (>5% methylation), excluding the middle cluster of low methylated samples in order to better distinguish between the unmethylated and methylated samples and to avoid the uncertainty of low methylated values due to the technical limitation of the assay. By using gSEM it is possible to construct a web of relationships between multiple variables, where all variables are included to build the model. The significance level of the indirect effect was hence estimated using robust corrections to standard errors that takes the batch effect, urinary creatinine, child's sex and maternal IQ into consideration. We then calculated mediation proportion as the indirect effect divided by the total effect. Those effects with an alpha below 0.05 were considered significant.

Based on the knowledge that associations between prenatal EDC exposure and neurodevelopmental outcomes often shows differences for boys and girls (Braun et al., 2011; Evans et al., 2014; Harley et al., 2013;

Kim et al., 2011; Kobrosly et al., 2014; Perera et al., 2016; Whyatt et al., 2012), we also conducted stratified analyses by sex.

As a sensitivity analysis, we ran the mediation analysis including the low methylated (0.1–5% methylation) group, i.e. this analysis used the full study population divided into three methylation clusters. Also in this analysis, the methylation clusters were treated as a continuous variable.

3. Results

3.1. Description of the study population

The results from the descriptive analysis are presented in Table 1. Prenatal urinary BPF concentrations had a geometric mean (GM) of 0.16 (ng/ml) and did not differ between sexes. On the other hand, girls exhibited significantly higher full IQ and higher scores in all subscales than boys, except for perceptual reasoning where boys and girls had similar scores. There was no difference in DNAm cluster distribution at 7 years of age between boys and girls (Table 2).

3.2. Exploratory analysis

Among the three *GRIN2B* CpG sites, prenatal BPF levels were significantly and positively associated with DNAm level of the third CpG site (CpG3) after adjusting for sex and analytical batch. A one-unit increase in BPF on the $\log_{10}$ scale was associated with 42% higher odds ($p = 0.047$) of having > 5% CpG3 methylation, compared to having this CpG site unmethylated. Cognitive test scores were significantly and inversely associated with DNAm at CpG3, but not CpG1 and CpG2, after adjusting for sex, analytical batch and maternal IQ scores. Having > 5% CpG3 methylation was associated with a 3.4 lower full-scale IQ score ($p = 0.033$), and a 3.6 lower score for the subscale verbal comprehension ($p = 0.014$), compared to having this CpG site unmethylated. BPF exposure was not associated with low (0.1–5%) CpG3 methylation, nor was low CpG3 methylation associated with cognitive test scores. Based on the significant associations between BPF exposure and CpG3 methylation (>5%), as well as between CpG3 methylation (>5%) and cognition, we continued with analyzing the role of only CpG3 methylation in the mediation analyses.

Table 2
Individuals allocated to each cluster for the three GRIN2B CpG sites analyzed from children's saliva at 7 years of age.

	DNAm range in cluster[e]	All (n = 799) n (%)	Boys (n = 398) n (%)	Girls (n = 401) n (%)	P-value[a]
CPG 1					
Unmethylated [b]	0.00–0.00	544 (68%)	271 (68%)	273 (68%)	0.942
Low methylated [c]	2.51–4.99	91 (11%)	44 (11%)	47 (12%)	
Methylated [d]	5.01–29.10	164 (21%)	83 (21%)	81 (20%)	
CPG2					
Unmethylated [b]	0.00–0.00	553 (69%)	263 (66%)	290 (72%)	0.154
Low methylated [c]	2.24–5.00	106 (13%)	57 (14%)	49 (12%)	
Methylated [d]	5.01–26.50	140 (18%)	78 (20%)	62 (15%)	
CPG3					
Unmethylated [b]	0.00–0.00	673 (84%)	334 (84%)	339 (85%)	0.957
Low methylated [c]	2.33–5.00	64 (8%)	33 (8%)	31 (8%)	
Methylated [d]	5.01–28.2	62 (8%)	31 (8%)	31 (8%)	

[a] P-values from chi-square test, comparing CpG cluster distribution between boys and girls.
[b] Unmethylated cluster: 0% DNA methylation.
[c] Low methylated cluster: 0.1 – 5% DNA methylation.
[d] Methylated cluster: >5% DNA methylation.
[e] DNAm values of the samples included in the respective cluster.

3.3. Mediation analyses

To further evaluate the potential mediating role of *GRIN2B* CpG3 methylation in the relationship between prenatal exposure to BPF and cognition, mediation analyses were performed. When evaluating the individual associations in this model, both prenatal BPF exposure and CpG3 methylation were associated with lower cognitive test scores, but the association between BPF exposure and CpG3 methylation did not reach significance in the full study population (Table 3). When stratifying the data for sex, both the association between BPF exposure and CpG3 methylation, and the association between CpG3 methylation and cognitive outcomes were significant in boys (Table 3).

Mediation effects were then calculated in boys based on three significant relationships in the model (Fig. 1). In boys, CpG3 methylation was found to mediate 8% of the effect of prenatal BPF exposure on Full scale IQ, as well as 9% and 6% on the subscales verbal comprehension and perceptual reasoning, respectively (Table 3). However, not all associations were significant in girls, holding further mediation analysis.

3.4. Sensitivity analyses

To test the stability of the model, individuals allocated to the low methylation cluster (DNAm 0.1%-5%) were included in the analyses. In this 3-cluster model (Table S2), 6% of the association between prenatal exposure to BPF and full scale IQ was found to be mediated by DNAm of CpG3 in boys, which is similar to the result of the 2-cluster model (Table 3). The similarity of the results using the two models shows that the detected associations are stable despite some uncertainty of measuring methylation in the lowest range.

4. Discussion

The results of the current study show, for the first time, that DNAm levels of the *GRIN2B* gene mediate an association between prenatal EDC exposure (in this case BPF) and cognitive function in children at 7 years of age. More specifically, the results of our mediation analyses suggest that prenatal level of BPF is associated with a lower cognitive function through higher levels of DNAm at CpG3 of the *GRIN2B* gene in boys. These results lend support to our earlier findings of the adverse male-dominant effects of BPF on the IQ scores (Bornehag et al., 2021; Tanner et al., 2020) and shed light on molecular mechanisms underlying this association.

The observed sex-specific mediation effect of CpG3 methylation on cognitive outcomes highlights the complexity of epigenetic regulation, and that *GRIN2B* may have different roles in brain development depending on sex. Although DNA methylation of certain genes is known to differ between males and females, possibly underlying sex differences in susceptibility to certain neurological diseases and neurotoxicity of chemicals (Qureshi and Mehler, 2010; Ratnu et al., 2017; Torres-Rojas and Jones, 2018), more research is needed to decipher how *GRIN2B* CpG3 methylation can have different effects on neurodevelopmental outcomes in boys and in girls. However, our results that *GRIN2B* may play a specific role in mediating an adverse effect on cognitive outcomes in boys only, is supported by findings by Rooney et al. (2018) studying children that participated in the Casa Pia Clinical Trial of Dental Amalgams in Children (N = 330). They observed that lead (Pb) exposure at 8–12 years of age significantly impaired learning and memory test scores 2–7 years later in boys, but not in girls, carrying the minor allele of *GRIN2B* rs1806201.

GRIN2B encodes the GluN2B subunit of NMDA receptors. NMDA receptors are key mediators of excitatory synaptic transmission in the

Table 3
GRIN2B CpG3 mediation of the association between prenatal BPF exposure and cognitive outcomes at 7 years of age.

	BPF - CpG3		CpG3 - cognition		BPF - cognition		Indirect effect	Total effect	CpG3 mediation[a]
	β_1 (SE)	p-value	β_2 (SE)	p-value	β_3 (SE)	p-value			
Full sample (n = 735)									
CpG3 methylation [b]	0.055 (0.03)	0.113	NA	NA	NA	NA	NA	NA	NA
Full IQ	NA	NA	−1.57 (0.25)	**<0.001**	−1.70 (0.44)	**<0.001**	nd	nd	nd
Working memory	NA	NA	−0.53 (0.02)	**<0.001**	−1.54 (0.03)	**<0.001**	nd	nd	nd
Processing speed	NA	NA	−1.04 (0.20)	**<0.001**	−1.53 (1.22)	0.212	nd	nd	nd
Perceptual reasoning	NA	NA	−1.19 (0.46)	**0.010**	−1.19 (0.32)	**<0.001**	nd	nd	nd
Verbal comprehension	NA	NA	−1.71 (0.36)	**<0.001**	−1.11 (0.03)	**<0.001**	nd	nd	nd
Boys only (n = 365)									
CpG3 methylation [b]	0.069 (0.02)	**0.001**	NA	NA	NA	NA	NA	NA	NA
Full IQ	NA	NA	−2.69 (1.18)	**0.023**	−2.03 (0.82)	**0.014**	−0.19	−2.22	**8%**
Working memory	NA	NA	−1.79 (1.24)	0.148	−1.30 (0.64)	**0.043**	nd	nd	nd
Processing speed	NA	NA	−2.01 (2.19)	0.359	−0.99 (0.46)	**0.031**	nd	nd	nd
Perceptual reasoning	NA	NA	−1.86 (0.10)	**<0.001**	−1.93 (0.87)	**0.025**	−0.13	−2.06	**6%**
Verbal comprehension	NA	NA	−2.28 (0.50)	**<0.001**	−1.67 (0.46)	**<0.001**	−0.16	−1.83	**9%**
Girls only (n = 370)									
CpG3 methylation [b]	0.040 (0.08)	0.634	NA	NA	NA	NA	NA	NA	NA
Full IQ	NA	NA	−0.48 (1.20)	0.690	−1.42 (0.04)	**<0.001**	nd	nd	nd
Working memory	NA	NA	0.71 (0.76)	0.352	−1.74 (0.61)	**0.004**	nd	nd	nd
Processing speed	NA	NA	−0.18 (1.54)	0.909	−2.09 (1.91)	0.275	nd	nd	nd
Perceptual reasoning	NA	NA	−0.51 (0.62)	0.418	−0.54 (0.33)	0.104	nd	nd	nd
Verbal comprehension	NA	NA	−1.14 (0.90)	0.206	−0.62 (0.56)	0.273	nd	nd	nd

Beta coefficients with standard errors, and corresponding p-values from gSEMs for the associations between: β_1) Prenatal BPF exposure and CpG3 methylation; β_2) CpG3 methylation and cognitive outcomes; β_3) prenatal BPF exposure and cognitive outcomes. In the mediation analysis, nd (not done) denotes that the indirect/total effects and mediation proportion were not calculated because not all paths (i.e., β_1, β_2, β_3) were significant, $p < 0.05$. NA = not available (i.e., not possible to calculate). The full sample model was adjusted for urinary creatinine, maternal IQ, analytical batch and child's sex, and the stratified models were adjusted for urinary creatinine, maternal IQ and analytical batch.

[a] Mediation proportion x100, expressing to what extent CpG3 methylation mediates the relationship between BPF exposure on cognitive outcomes.

[b] Categorized as unmethylated (0% DNAm) and methylated (>5% DNAm).

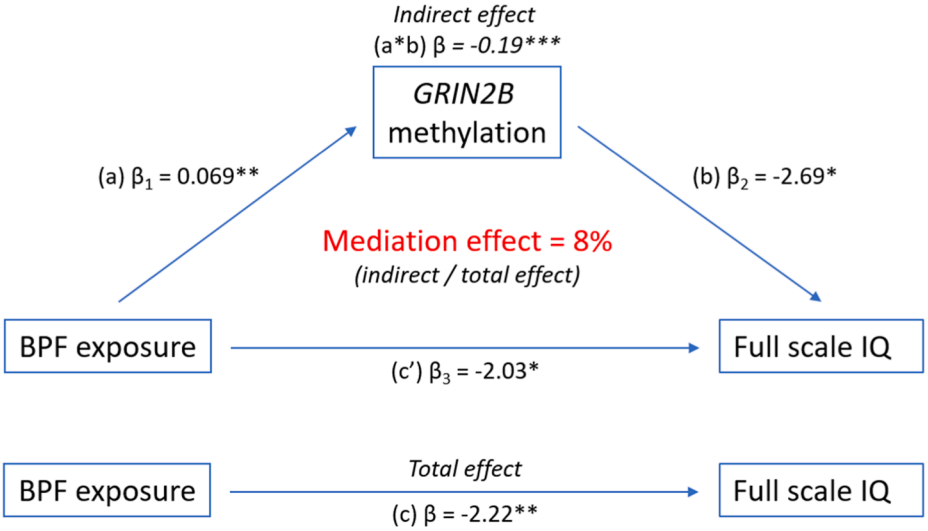

Fig. 1. Mediation analysis model investigating the mediating role of *GRIN2B* CpG3 methylation in the association between prenatal BPF exposure and cognitive function in boys at 7 years of age. Generalized structural equation models (gSEM) were used for assessing relationship between the independent variable BPF, the mediator DNAm at CpG3 and the dependent variable Full scale IQ adjusting for urinary creatinine, maternal IQ and analytical batch: a) the effect of prenatal BPF exposure on DNAm; b) the effect of DNAm on IQ; c') the direct effect of BPF exposure on IQ; c) the total effect of BPF exposure on IQ (i.e., (a*b) + c'). β = unstandardized beta values. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

brain and they consist of two GluN1 (coded by *GRIN1*) subunits and two regulatory subunits that can be for example GluN2B and/or GluN2A (coded by *GRIN2A*). Depending on subunit composition, the NMDA receptor has different channel properties such as channel kinetics and synaptic localization (Paoletti et al., 2013; Wyllie et al., 2013). During development, when synapse maturation and acquisition of learning abilities occurs, a switch in subunit composition takes place from primarily GluN2B to primarily GluN2A subunits (Dumas, 2005). This switch is evolutionary conserved and occurs in humans mainly during the first year of life (Law et al., 2003). In mice, males display higher GluN2B:GluN2A ratio in the frontal cortex during postnatal development compared to females (Sinclair et al., 2016), indicating that GluN2B-containing NMDA receptors may have a more pronounced role in males.

Our results are consistent with emerging evidence for the link between prenatal exposure to chemical or psychological factors and epigenetic patterns of the *GRIN2B* gene. For example, we have previously shown that prenatal BPA exposure could lead to hypermethylation in one CpG site of the same *GRIN2B* gene region as in the present study ("CpG1"; (Alavian-Ghavanini et al., 2018)). Furthermore, in another cohort, we have found that increased *GRIN2B* CpG3 methylation in saliva of adult individuals is associated with experience of childhood adversity (CA; defined as major conflicts, financial problems, and death within the family during childhood) (Engdahl et al., 2021), indicating that both bisphenols and CA affect molecular pathways that modulate methylation at this region. In the present study, we show that prenatal

BPF exposure is associated with lower IQ at 7 years of age. Interestingly, CA has also been associated with impaired cognitive performance (Pechtel and Pizzagalli, 2011), such as lower IQ in adulthood (Aas et al., 2012; Martins et al., 2019). One possible common molecular pathway may be that both BPF and CA can affect hormones that regulate transcriptional responses associated with cognitive outcomes. Thus, it is possible that *GRIN2B* DNAm, rather than being directly affected, serves as biomarker for chromatin accessibility resulting from transcriptional changes in response to these exposures (Davis and Pattenden, 2019).

We have not addressed the role of genetic variation in modulating *GRIN2B* DNAm in this study. There are no known SNPs within the analysed CpGs that could directly affect methylation, yet the analysed region (chr12:14,134,920–14,134,935) lies between the two SNPs rs1019385 (chr12:14,134,843) and rs3764028 (chr12:14,135,064). Genetic variations at both locations have been implicated to affect *GRIN2B* expression as well as disease susceptibility: The G allele of rs1019385 has been reported to inhibit nerve growth factor (NGF)-induced GRIN2B expression and to be more common in schizophrenic patients than in controls (Miyatake et al., 2002). The CC genotype of rs3764028 has been reported to decrease transcriptional activity of the *GRIN2B* promoter, and this genotype has been associated with sporadic Alzheimer's disease (Jiang and Jia, 2009). While these genetic associations emphasise the importance of this region for *GRIN2B* regulation, there are, to our knowledge, no reports that they, or other SNPs, influence methylation status of the CpGs analysed in our study. However, we cannot exclude that *GRIN2B* DNAm at this region is also influenced by genetic variation, which would make it an interesting target to study gene-environment interactions.

While it is clear that ideally, DNAm should be assessed in target tissue, this is, in the majority of human studies, not possible and thus surrogate tissue such as blood, saliva or buccal swab samples are commonly used. Our results are based on methylation in non-invasive buccal cell samples, which, in children, consist almost exclusively of epithelial cells (90.3% based on microscopy analysis). The remaining cell types include immune cells like neutrophils, B cells, natural killer cells, CD4 + T cells and monocytes to varying degree (Theda et al., 2018; van Dongen et al., 2018). How *GRIN2B* methylation differs between these cell types is, to our knowledge, not investigated, and therefore we do not know if cell composition could affect our results.

Findings in surrogate tissue give only limited information on functional changes in the target tissue. Yet, there is some correlation between DNAm in buccal samples and in brain tissue. For example, DNAm, assessed by an untarged approach in 27 individuals (5–61 years) with medically intractable epilepsy, showed correlation (r) of 0.88 within gene regulatory regions, and 20.4% of "variable CpGs", i.e. CpGs showing large inter-individuals DNAm variations, were nominally correlated between buccal cells and brain cells (Braun et al., 2019). Furthermore, our previous studies in rat brain showed that CpG3 methylation in a homologous region correlates with *Grin2b* expression (Alavian-Ghavanini et al., 2018). While we are unable to determine the relationship between *GRIN2B* methylation levels and expression in buccal swabs and in relevant brain regions of the SELMA children, it is possible that the levels measured in the buccal swab samples reflect stable changes induced in early embryonic development in several tissues, including the brain, which in turn would affect *GRIN2B* expression. Thus, the observed mediation effect of CpG3 methylation on the association between BPF exposure and cognition might in fact reflect functional processes in the brain rather than being a mere biomarker in buccal cells.

4.1. Strengths and limitations

The strength of the current study is the longitudinal design based on a large pregnancy cohort with well-characterized environmental exposures and thorough psychological tests at school age. However, one limitation of the study is that the BPF urinary levels were measured only once during pregnancy. The exposure therefore reflects one time point and one chemical, but the children have been exposed to various chemicals from gestational day 1 to 7 years of age. In addition, we cannot exclude that we have overseen some confounders, for example oral diseases, genetic variations or other environmental exposures. Another limitation is that *GRIN2B* methylation was measured in buccal swab samples, and we do not have the possibility to address if this refelcts functional changes in the brain.

A statistical limitation is that the majority of individuals displayed 0% methylation of analyzed *GRIN2B* region, which made the data skewed and therefore not suitable for a linear approach. We solved this issue by allocating the samples in clusters based on methylation ranges, which were used instead of each individually measured methylation percentages, see methods section. A further limitation was that methylation levels were measured in two separate analytical batches. However, there was no significant difference in CpG3 cluster distribution of the samples between the two batches.

5. Conclusion

In this study, we showed that DNA methylation in a regulatory region of *GRIN2B* can mediate up to 9% of the association between prenatal BPF exposure and decrease in cognitive function in boys. The effects of *GRIN2B* methylation on cognitive functions were found only in boys, which warrants more research on the roles of hormones and other molecular targets in the relationship between BPF exposure, *GRIN2B* gene function, and cognition. Further research involving other neurodevelopmental genes is needed to investigate which other molecular targets contribute to the effect of prenatal exposure to BPF on cognitive functions.

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CRediT authorship contribution statement

Elin Engdahl: Investigation, Writing - original draft, Funding acquisition. **Katherine Svensson:** Methodology, Formal analysis, Writing - original draft. **Ping-I Daniel Lin:** Methodology, Formal analysis. **Ali Alavian-Ghavanini:** Investigation. **Christian Lindh:** Methodology. **Joëlle Rüegg:** Conceptualization, Writing - original draft, Funding acquisition. **Carl-Gustaf Bornehag:** Conceptualization, Resources, Writing - original draft, Funding acquisition.

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.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2021.106617.

References

Aas, M., Steen, N.E., Agartz, I., Aminoff, S.R., Lorentzen, S., Sundet, K., et al., 2012. Is cognitive impairment following early life stress in severe mental disorders based on specific or general cognitive functioning? Psych. Res. 198, 495–500.

Alavian-Ghavanini, A., Lin, P.I., Lind, P.M., Risen Rimfors, S., Halin Lejonklou, M., Dunder, L., et al., 2018. Prenatal bisphenol a exposure is linked to epigenetic changes in glutamate receptor subunit gene grin2b in female rats and humans. Sci. Rep. 8, 11315.

Baron, R.M., Kenny, D.A., 1986. The moderator-mediator variable distinction in social psychological research: Conceptual, strategic, and statistical considerations. J. Pers. Soc. Psychol. 51, 1173–1182.

Berge, T.L., Lygre, G.B., Jonsson, B.A., Lindh, C.H., Bjorkman, L., 2017. Bisphenol a concentration in human saliva related to dental polymer-based fillings. Clin. Oral Investig.

Bergman, A., Heindel, J.J., Kasten, T., Kidd, K.A., Jobling, S., Neira, M., et al., 2013. The impact of endocrine disruption: A consensus statement on the state of the science. Environ. Health Perspect. 121, A104–A106.

Bernstein, B.E., Meissner, A., Lander, E.S., 2007. The mammalian epigenome. Cell 128, 669–681.

Bornehag, C.-G., Engdahl, E., Unenge Hallerbäck, M., Wikström, S., Lindh, C., Rüegg, J., et al., 2021. Prenatal exposure to bisphenols and cognitive function in children at 7 years of age in the swedish selma study. Environ. Int. 106433.

Bornehag, C.G., Moniruzzaman, S., Larsson, M., Lindstrom, C.B., Hasselgren, M., Bodin, A., et al., 2012. The selma study: A birth cohort study in sweden following more than 2000 mother-child pairs. Paediatr. Perinat. Epidemiol. 26, 456–467.

Boschen, K.E., Keller, S.M., Roth, T.L., Klintsova, A.Y., 2018. Epigenetic mechanisms in alcohol- and adversity-induced developmental origins of neurobehavioral functioning. Neurotoxicol. Teratol. 66, 63–79.

Braun, J.M., Kalkbrenner, A.E., Calafat, A.M., Yolton, K., Ye, X., Dietrich, K.N., et al., 2011. Impact of early-life bisphenol a exposure on behavior and executive function in children. Pediatrics 128, 873–882.

Braun, P.R., Han, S., Hing, B., Nagahama, Y., Gaul, L.N., Heinzman, J.T., et al., 2019. Genome-wide DNA methylation comparison between live human brain and peripheral tissues within individuals. Transl. Psychiatry 9, 47.

Checknita, D., Ekstrom, T.J., Comasco, E., Nilsson, K.W., Tiihonen, J., Hodgins, S., 2018. Associations of monoamine oxidase a gene first exon methylation with sexual abuse and current depression in women. J Neural Transm (Vienna) 125, 1053–1064.

Chen, D., Kannan, K., Tan, H., Zheng, Z., Feng, Y.-L., Wu, Y., et al., 2016. Bisphenol analogues other than bpa: Environmental occurrence, human exposure, and toxicity—a review. Environ. Sci. Technol. 50, 5438–5453.

Davis, I.J., Pattenden, S.G., 2019. Chapter 1-3 - chromatin accessibility as a strategy to detect changes associated with development, disease, and exposure and susceptibility to chemical toxins. In: McCullough, S.D., Dolinoy, D.C., (Eds.), Toxicoepigenetics. Academic Press, pp. 85–103.

Dumas, T.C., 2005. Developmental regulation of cognitive abilities: Modified composition of a molecular switch turns on associative learning. Prog. Neurobiol. 76, 189–211.

Engdahl, E., Rüegg, J., 2020. Prenatal exposure to endocrine disrupting chemicals and their effect on health later in life. In: Teperino, R. (Ed.), Beyond our genes. Springer, Cham. https://doi.org/10.1007/978-3-030-35213-4_4.

Engdahl, E., Alavian-Ghavanini, A., Forsell, Y., Lavebratt, C., Rüegg, J., 2021. Childhood adversity increases methylation in the grin2b gene. J. Psychiatr. Res. 132, 38–43.

Evans, S.F., Kobrosly, R.W., Barrett, E.S., Thurston, S.W., Calafat, A.M., Weiss, B., et al., 2014. Prenatal bisphenol a exposure and maternally reported behavior in boys and girls. Neurotoxicology 45C, 91–99.

Fasanelli, F., Baglietto, L., Ponzi, E., Guida, F., Campanella, G., Johansson, M., et al., 2015. Hypomethylation of smoking-related genes is associated with future lung cancer in four prospective cohorts. Nat. Commun. 6, 10192.

Gyllenhammar, I., Glynn, A., Jonsson, B.A., Lindh, C.H., Darnerud, P.O., Svensson, K., et al., 2017. Diverging temporal trends of human exposure to bisphenols and plastizisers, such as phthalates, caused by substitution of legacy edcs? Environ. Res. 153, 48–54.

Harley, K.G., Gunier, R.B., Kogut, K., Johnson, C., Bradman, A., Calafat, A.M., et al., 2013. Prenatal and early childhood bisphenol a concentrations and behavior in school-aged children. Environ. Res. 126, 43–50.

Hu, C., Chen, W., Myers, S.J., Yuan, H., Traynelis, S.F., 2016. Human grin2b variants in neurodevelopmental disorders. J. Pharmacol. Sci. 132, 115–121.

Jacobs, M.N., Marczylo, E.L., Guerrero-Bosagna, C., Ruegg, J., 2017. Marked for life: Epigenetic effects of endocrine disrupting chemicals. Annu Rev Env Resour 42, 105–160.

James, L.R., Brett, J.M., 1984. Mediators, moderators, and tests for mediation 69, 307–321.

Jiang, H., Jia, J., 2009. Association between nr2b subunit gene (grin2b) promoter polymorphisms and sporadic alzheimer's disease in the north chinese population. Neurosci. Lett. 450, 356–360.

Judd, C.M., Kenny, D.A., 1981. Process analysis: Estimating mediation in treatment evaluations. Eval. Rev. 5, 602–619.

Kim, Y., Ha, E.H., Kim, E.J., Park, H., Ha, M., Kim, J.H., et al., 2011. Prenatal exposure to phthalates and infant development at 6 months: Prospective mothers and children's environmental health (moceh) study. Environ. Health Perspect. 119, 1495–1500.

Kobrosly, R.W., Evans, S., Miodovnik, A., Barrett, E.S., Thurston, S.W., Calafat, A.M., et al., 2014. Prenatal phthalate exposures and neurobehavioral development scores in boys and girls at 6–10 years of age. Environ. Health Perspect. 122, 521–528.

Kundakovic, M., Jaric, I., 2017. The epigenetic link between prenatal adverse environments and neurodevelopmental disorders. Genes (Basel) 8, 104.

Law, A.J., Weickert, C.S., Webster, M.J., Herman, M.M., Kleinman, J.E., Harrison, P.J., 2003. Changes in nmda receptor subunit mrnas and cyclophilin mrna during development of the human hippocampus. Ann. N. Y. Acad. Sci. 1003, 426–430.

Martins, D.S., Hasse-Sousa, M., Petry-Perin, C., Arrial-Cordeiro, R.T., Rabelo-da-Ponte, F. D., Lima, F.M., et al., 2019. Perceived childhood adversities: Impact of childhood trauma to estimated intellectual functioning of individuals with bipolar disorder. Psychiatry Res. 274, 345–351.

Mascheretti, S., Facoetti, A., Giorda, R., Beri, S., Riva, V., Trezzi, V., et al., 2015. Grin2b mediates susceptibility to intelligence quotient and cognitive impairments in developmental dyslexia. Psychiatr. Genet. 25, 9–20.

Mazzachi, B.C., Peake, M.J., Ehrhardt, V., 2000. Reference range and method comparison studies for enzymatic and jaffe creatinine assays in plasma and serum and early morning urine. Clin. Lab. 46, 53–55.

Miller, J.L., Grant, P.A., 2013. The role of DNA methylation and histone modifications in transcriptional regulation in humans. Subcell. Biochem. 61, 289–317.

Miranda, A., Sousa, N., 2018. Maternal hormonal milieu influence on fetal brain development. Brain Behav 8, e00920.

Miura, R., Araki, A., Minatoya, M., Miyake, K., Chen, M.-L., Kobayashi, S., et al., 2019. An epigenome-wide analysis of cord blood DNA methylation reveals sex-specific effect of exposure to bisphenol a. Sci. Rep. 9, 12369.

Miyatake, R., Furukawa, A., Suwaki, H., 2002. Identification of a novel variant of the human nr2b gene promoter region and its possible association with schizophrenia. Mol. Psychiatry 7, 1101–1106.

Montrose, L., Padmanabhan, V., Goodrich, J.M., Domino, S.E., Treadwell, M.C., Meeker, J.D., et al., 2018. Maternal levels of endocrine disrupting chemicals in the first trimester of pregnancy are associated with infant cord blood DNA methylation. Epigenetics 13, 301–309.

Myers, S.J., Yuan, H., Kang, J.Q., Tan, F.C.K., Traynelis, S.F., Low, C.M., 2019. Distinct roles of grin2a and grin2b variants in neurological conditions. F1000Res 8.

Paoletti, P., Bellone, C., Zhou, Q., 2013. Nmda receptor subunit diversity: Impact on receptor properties, synaptic plasticity and disease. Nat. Rev. Neurosci. 14, 383–400.

Pechtel, P., Pizzagalli, D.A., 2011. Effects of early life stress on cognitive and affective function: An integrated review of human literature. Psychopharmacology 214, 55–70.

Perera, F., Nolte, E.L.R., Wang, Y., Margolis, A.E., Calafat, A.M., Wang, S., et al., 2016. Bisphenol a exposure and symptoms of anxiety and depression among inner city children at 10–12 years of age. Environ. Res. 151, 195–202.

Platzer, K., Lemke, J.R., 1993. Grin2b-related neurodevelopmental disorder. In: Adam, M.P., Ardinger, H.H., Pagon, R.A., Wallace, S.E., Bean, L.J.H., Stephens, K., et al., (Eds.). Genereviews((r)), Seattle (WA).

Prifitera, A., Saklofske, D.H., Weiss, L.G., 2005. Wisc-iv clinical use and interpretation: Scientist-practitioner perspectives. Academic Press.

Qureshi, I.A., Mehler, M.F., 2010. Genetic and epigenetic underpinnings of sex differences in the brain and in neurological and psychiatric disease susceptibility. Prog. Brain Res. 186, 77–95.

Ratnu, V.S., Emami, M.R., Bredy, T.W., 2017. Genetic and epigenetic factors underlying sex differences in the regulation of gene expression in the brain. J. Neurosci. Res. 95, 301–310.

Rooney, J.P.K., Woods, N.F., Martin, M.D., Woods, J.S., 2018. Genetic polymorphisms of grin2a and grin2b modify the neurobehavioral effects of low-level lead exposure in children. Environ. Res. 165, 1–10.

Sinclair, D., Cesare, J., McMullen, M., Carlson, G.C., Hahn, C.-G., Borgmann-Winter, K.E., 2016. Effects of sex and dtnbp1 (dysbindin) null gene mutation on the developmental glun2b-glun2a switch in the mouse cortex and hippocampus. J. Neurodev. Disord. 8, 14.

Smith, Z.D., Meissner, A., 2013. DNA methylation: Roles in mammalian development. Nat. Rev. Genet. 14, 204–220.

Spiers, H., Hannon, E., Schalkwyk, L.C., Smith, R., Wong, C.C., O'Donovan, M.C., et al., 2015. Methylomic trajectories across human fetal brain development. Genome Res. 25, 338–352.

Tanner, E.M., Hallerbäck, M.U., Wikström, S., Lindh, C., Kiviranta, H., Gennings, C., et al., 2020. Early prenatal exposure to suspected endocrine disruptor mixtures is associated with lower iq at age seven. Environ. Int. 134, 105185.

Theda, C., Hwang, S.H., Czajko, A., Loke, Y.J., Leong, P., Craig, J.M., 2018. Quantitation of the cellular content of saliva and buccal swab samples. Sci. Rep. 8, 6944.

Tobi, E.W., Slieker, R.C., Luijk, R., Dekkers, K.F., Stein, A.D., Xu, K.M., et al., 2018. DNA methylation as a mediator of the association between prenatal adversity and risk factors for metabolic disease in adulthood. Sci. Adv. 4 (eaao4364).

Torres-Rojas, C., Jones, B.C., 2018. Sex differences in neurotoxicogenetics. Front Genet 9, 196.

Tran, N.Q.V., Miyake, K., 2017. Neurodevelopmental disorders and environmental toxicants: Epigenetics as an underlying mechanism. Int. J. Genomics 2017, 7526592.

Van der Elst, W., Ouwehand, C., van Rijn, P., Lee, N., Van Boxtel, M., Jolles, J., 2013. The shortened raven standard progressive matrices: Item response theory-based psychometric analyses and normative data. Assessment 20, 48–59.

van Dongen, J., Ehli, E.A., Jansen, R., van Beijsterveldt, C.E.M., Willemsen, G., Hottenga, J.J., et al. 2018. Genome-wide analysis of DNA methylation in buccal cells: A study of monozygotic twins and mqtls. Epigenetics Chromatin 11, 54–54.

Wechsler, D., 2003. Wechsler intelligence scale for children, fourth ed. The Psychological Corporation, San Antonio, TX.

Whyatt, R.M., Liu, X., Rauh, V.A., Calafat, A.M., Just, A.C., Hoepner, L., et al., 2012. Maternal prenatal urinary phthalate metabolite concentrations and child mental, psychomotor, and behavioral development at 3 years of age. Environ. Health Perspect. 120, 290–295.

Wiklund, P., Karhunen, V., Richmond, R.C., Parmar, P., Rodriguez, A., De Silva, M., et al., 2019. DNA methylation links prenatal smoking exposure to later life health outcomes in offspring. Clin. Epigen. 11, 97.

Wolstenholme, J.T., Rissman, E.F., Connelly, J.J., 2011. The role of bisphenol a in shaping the brain, epigenome and behavior. Horm. Behav. 59, 296–305.

Wyllie, D.J., Livesey, M.R., Hardingham, G.E., 2013. Influence of glun2 subunit identity on nmda receptor function. Neuropharmacology 74, 4–17.