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Placental CpG methylation of HPA-axis genes is associated with cognitive impairment at age 10 among children born extremely preterm

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1. Introduction

The neuroendocrine system serves as an interface between the brain and many of the peripheral endocrine systems and is integral for maintaining homeostasis throughout the body (Tsigos and Chrousos, 2002). Exposure to exogenous chemicals such as endocrine disrupting compounds (EDCs) in the environment may alter the neuroendocrine system resulting in detrimental health effects, including reproductive disorders and cancer (Toni, 2004; Uzumcu et al., 2012). One of the major components of the neuroendocrine system is the hypothalamic-pituitary-adrenal (HPA) axis. The HPA axis comprises interactions among the hypothalamus, the pituitary gland and the adrenal glands and plays a critical role in regulating physiological and biological responses to stressors (Kinlein et al., 2015). Importantly, it has been shown that components of the HPA system are sensitive to disruption by various environmental toxicants and stressors (Kitraki et al., 2015; Lee and Sawa, 2014).

Several genes are key to functioning of the HPA axis system (Lee and Sawa, 2014). Among these are Nuclear Receptor Subfamily Group 3C Member 1 (*NR3C1*), FK506 Binding Protein 5 (*FKPB5*), and Brain-Derived Neurotrophic Factor (*BDNF*). *NR3C1* encodes for the glucocorticoid receptor, which regulates mechanistic negative feedback actions that inhibit HPA axis activity (Keller-Wood and Dallman, 1984). *FKPB5* is involved in glucocorticoid signaling (Wochnik et al., 2005). *BDNF* promotes synaptic plasticity and regulation of Corticotropin-Releasing Hormone (CRH) in the hypothalamus, and is involved in regulating and maintaining homeostasis in the HPA axis during times of stress (Cowansage et al., 2010; Jeanneteau et al., 2012; Naert et al., 2015). Importantly, all of these genes are critical to the brain's defenses against stress-inducing exposures (Lee and Sawa, 2014).

In addition to their role in neuronal processes, an interesting feature is that many HPA axis-associated genes also control fetal readiness for birth, survival after birth and timing of birth (Wood and Keller-Wood, 2016). The mechanistic basis for this is that HPA axis-associated genes control multiple biological functions in the placenta such as cellular proliferation, nutrient transport, and trophoblast growth (Gao et al., 2012; Kawamura et al., 2009; Padmini et al., 2012) (Fig. 1). In the context of environmental exposures, the placenta regulates gene expression and hormone production in response to exposures and thus functions as an environmentally-responsive biosensor during fetal development (Gheorghe et al., 2010). There is evidence that disruption of normal placenta physiology is associated with later life health effects. Specifically, placental physiological measures such as weight and vascularity have been linked to later life health such as cardiovascular disease and hypertension (Barker et al., 1990). Furthermore, recent studies have shown that epigenetic marks (*i.e.* DNA methylation) in the

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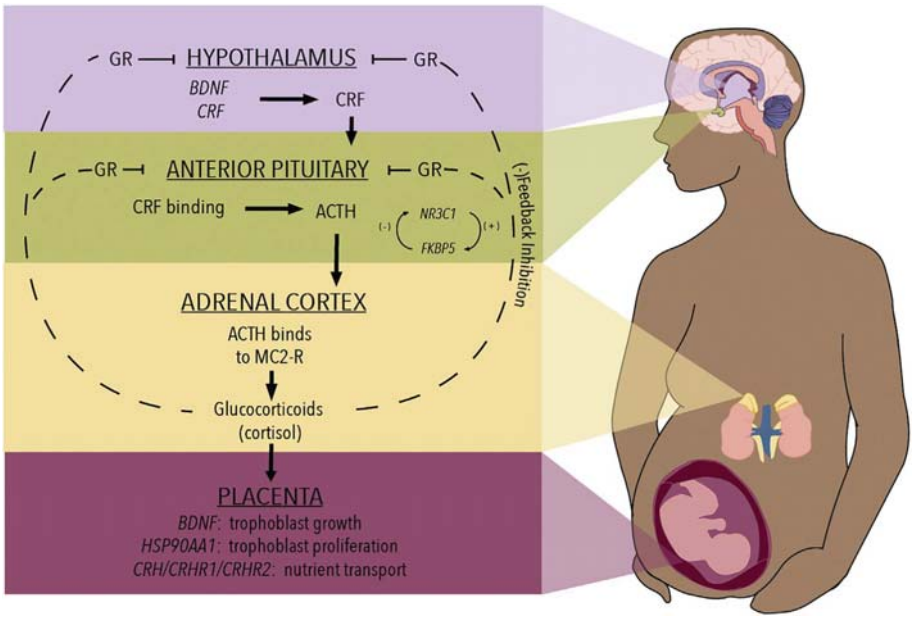

Fig. 1. The HPA axis involves interactions among the hypothalamus, anterior pituitary, and adrenal cortex. Genes that are critical to the HPA pathway include, Brain-derived neurotrophic factor (*BDNF*), FK506 binding protein 5 (*FKBP5*), Corticotropin-releasing hormone/factor (*CRF/CRH*), and Glucocorticoid receptor (*NR3C1*). Ultimately, the HPA axis cascade results in the production of cortisol, which has the potential to cross the placental barrier. Cortisol has been shown to regulate fetal readiness for birth and infant survival. HPA-axis associated genes are also involved in trophoblast growth and proliferation as well as nutrient transport.

placenta of HPA-axis genes are associated with neurobehavioral outcomes in infants (Appleton et al., 2015; Conradt et al., 2013; Monk et al., 2016; Paquette et al., 2014). The placenta is thus of great interest for study as it: (1) mediates fetal exposures to exogenous compounds, (2) regulates fetal nutrition, (3) controls the production of fetal and maternal cortisol, (4) produces additional hormones key for fetal development, and (5) is a key regulator of the fetal environment.

Because HPA axis-associated genes are known to regulate placental growth and function and these are known to influence overall fetal development, we hypothesized that placental DNA (CpG) methylation of targeted genes is predictive of later life cognitive function. To address this, we utilized placental samples from the Extremely Low Gestational Age Newborns (ELGAN) cohort to investigate the relationship between placental CpG methylation changes in HPA axis-associated genes and cognitive functioning at age 10. Our study is among the first to investigate the use of placental CpG methylation to predict later life cognitive function in mid-childhood. The data provide novel insights into potential mechanistic relationship of CpG methylation as a driver of fetal development and later life cognition in mid-childhood.

2. Methods

2.1. ELGANs study subject recruitment and sample collection

Details regarding the recruitment of ELGAN participants have been discussed elsewhere (O'Shea et al., 2009). Briefly, infants born before 28 weeks of gestation at one of the 14 ELGAN sites between 2002 and 2004 were eligible for enrollment in the study. Participating mothers provided informed consent following admission to the hospital, before birth, or immediately following birth. Study procedures were approved by the Institutional Review Board at each of the 14 participating ELGAN sites (O'Shea et al., 2009). After recruitment, a total of 1506 infants and 1249 mothers enrolled in the ELGAN study (ELGAN1). Of 1200 ELGAN survivors, 1102 (92%) underwent clinical evaluations at age 2 years. For the second clinical evaluation at age 10, 889 returned for follow up (ELGAN2). Although placental specimens were collected from the large majority of ELGAN participants, specimens of sufficient size for epigenetic analysis include 438 children. For the current study, a total of 228 mother-infant pairs were selected from the 889 ELGAN2 cohort based on the availability of placental samples with data on CpG methylation and cognitive function. Thus, the data presented here represents a subset of placenta from children who display deficits in cognitive function and controls.

Participating women gave permission for collection of a sample of their placenta for the ELGAN study. Upon delivery, placentas were placed into a sterile exam basin and taken to the sampling room, at which point, biopsies of the placentas were collected. To expose the chorion, the amnion was pulled back using sterile technique at the midpoint of the longest distance between the cord insertion and the edge of the placental disk. A tissue sample was collected by applying traction to the chorion and the underlying trophoblast tissue and cutting a sample out at the base of this tissue structure. The tissue sample was subsequently placed into a cryo-vial that was immediately submerged in liquid nitrogen. Placental samples were shipped to the University of North Carolina at Chapel Hill for processing and were stored at −80 °C prior to shipment (Onderdonk et al., 2008).

2.2. DNA extraction and assessment of DNA methylation

A small subsample of placental tissue (~0.2 g) was cut from the frozen biopsy sample and rinsed with sterile 1 X PBS to wash away any residual blood. Samples were then homogenized in Buffer RLT with β-mercaptoethanol (Qiagen, Valencia CA). An AllPrep DNA/RNA/miRNA Universal Kit (Qiagen, Valencia CA) was utilized to extract DNA and RNA sequences that were > 18 nucleotides in length, according to the manufacturer's instructions. To analyze placental CpG methylation, extracted DNA sequences were bisulfate-converted using the EZ DNA methylation kit (Zymo Research, Irvine, CA) and then subsequently hybridized on the Illumina HumanMethylation 450 BeadChip© array (n = 132) and the Illumina HumanMethylation850 Bead Chip array (n = 96) (Illumina, Inc., San Diego, CA), which assesses the DNA methylation levels of 486,428 and 853,307 individual probes at single nucleotide resolution, respectively. Data were integrated as it has been proposed that high variability methylation sites are well conserved between the two platforms (Logue et al., 2017). Methylation levels were calculated and expressed as β values (β = intensity of the methylated allele (M) / (intensity of the unmethylated allele (U) + intensity of the methylated allele (M) + 100). Data were normalized for both arrays using the *minfi* package in R (Aryee et al., 2014). Specifically, image files were used to produce background-corrected and quantile normalized β-values. Subsequently, β-values with a $p > 0.01$ were removed from analysis, leaving a total of 237 HPA-axis associated probes available for analysis. To demonstrate that the sites included in the analysis were suitable for integration, a regression analysis was

Table 1
Study subject characteristics.

Variable name	ELGAN subcohort subjects (n = 228) N (%) Mean (Range)	ELGAN2 subjects (n = 889) N (%) Mean (Range)
Infant sex		
Male	136 (60.2%)	471 (53.0%)
Female	92 (39.8%)	418 (47.0%)
Cognitive impairment		
No/low	158 (69.3%)	660 (74.3%)
Moderate/severe	70 (30.7%)	214 (24.1%)
Not reported		15 (1.6%)
Maternal age	29.8 (14.6–45.8)	29.2 (13.2–47.3)
Gestational age	25.7 (23.0–27.6)	25.9 (23.0–27.6)
Maternal education		
High school and above	184 (80.7%)	686 (77.2%)
Below high school	32 (14.1%)	123 (13.8%)
Not reported	12 (5.2%)	80 (9%)
Smoking		
Yes	23 (10.1%)	111 (12.5%)
No	197 (86.4%)	719 (80.9%)
Not reported	8 (3.5%)	59 (6.6%)
Race		
White	136 (59.7%)	515 (59.1%)
Non-white	92 (40.3%)	357 (40.2%)
Not reported		17 (1.9%)
Public insurance		
Yes	76 (33.2%)	314 (35.3%)
Multiple births		
Yes	97 (42.5%)	308 (34.6%)
Pregnancies with assisted reproductive technology		
Yes	37 (16%)	144 (13%)

performed on all HPA axis-associated gene probes (n = 237). It demonstrated strong concordance of the average beta values at the tested loci (R = 0.98, p < 0.001) for samples analyzed on the 450 k as compared to those analyzed to those on the 850 k (Supplemental Fig. 1).

2.3. Cognitive function assessment at ten years of age

General cognitive ability (or IQ) was assessed with the School-Age Differential Ability Scales–II (DAS-II) Verbal and Nonverbal Reasoning scales. Two subtests from the DAS-II and five subtests from the NEPSY-II were used to assess executive function. Working memory was evaluated with the DAS-II Recall of Digits Backwards, Recall Sequential Order test. The NEPSY-II Auditory Attention and Auditory Response Set, the NEPSY-II Animal Sorting test, and the NEPSY-II Inhibition and Inhibition Switching test were utilized to examine auditory attention and set switching, concept generation and mental flexibility, and simple inhibition and inhibition shifting, respectively (Bright et al., 2017). As a unitary measure of cognitive and executive function, we used Latent Profile Analysis (LPA), which empirically identifies subgroups of children who share similar profiles on a set of measures, to classify study participants into groups based on IQ and executive functioning (Heeren et al., 2017). For the current analyses participants were classified into two previously validated distinct profile groups (Heeren et al., 2017): normal or low normal cognitive function (n = 158) and moderate/severe cognitive impairment (n = 70).

2.4. Statistical analysis

Logistic regression analysis was performed in SAS (Cary, NC) to test whether methylation levels at 237 CpG sites associated with 14 HPA axis genes that predicted cognitive function at mid-childhood. These genes were selected based on their known involvement in the HPA axis and known relationship to environmental stressors (Lee and Sawa, 2014). Socioeconomic and clinical covariates were selected *a priori* based on known associations with both methylation and cognitive function including: race, public insurance, maternal education, sex of the infant, and gestational age. While methylation levels (β-values) are calculated as a proportion between 0 and 1, for the purposes of this analysis, β-value were adjusted to β-value * 100 in order to examine the change in the odds ratio (OR) for each percent increase in methylation. This transformation does not change the underlying distribution of the data or the sensitivity of the model. For the purposes of logistic regression, the dependent variable for this model was the binary outcome of either (i) normal or low normal cognitive function (n = 158) or (ii) moderate or severe cognitive impairment (n = 70), which were coded from four groups of no impairment, low impairment, moderate impairment, and severe impairment as derived using LPA. Sites of CpG methylation were considered to be significantly associated with moderate/severe cognitive impairment if the associated p-value was < 0.05. p-Values, beta estimates, parameter-likelihood ORs, and 95% confidence intervals (C.I.) for ORs are reported.

To gain insight on the potential functional outcomes that are associated with the identified HPA axis genes, network analysis was conducted using Ingenuity Pathway Analysis (IPA) (Ingenuity Systems®, Redwood City, CA, USA). Enrichment in canonical pathways associated with selected genes and behavioral outcomes were analyzed and reported. Significance was defined as a right-tailed Fisher's Exact test p-value < 0.0001.

3. Results

3.1. Study cohort

Demographic information for the ELGAN subcohort (n = 228) and the larger ELGAN2 cohort were collected at childbirth and are provided in Table 1. The data on children's cognitive impairment was collected at age 10 as described in the Methods section above. In the ELGAN subcohort for the current study (n = 228), there were 136 (60.2%) males and 92 (39.8%) females. The average maternal age was 29.8 years and the average gestational age was 25.7 weeks. In this subcohort 158 (69.3%) displayed normal or low normal cognitive function while 70 (30.7%) were classified as having moderate/severe cognitive impairment. Approximately 42% of the births in the subcohort were multigestation, and 16% of pregnancies involved assisted reproductive technology (ART). This subcohort displayed similar characteristics as the larger ELGAN2 cohort with respect to race, public insurance, maternal education, and gestational age. There is an enrichment of males in the 228 subcohort likely because of the known sexual dimorphism in neurocognitive outcomes of ELGANs (Kuban et al., 2016).

3.2. Placental CpG methylation predicts children's cognitive outcomes at age 10

A total of 14 genes comprising 237 probes were selected for analysis as they are known to play a role in the HPA axis, and have a known relationship to environmental stressors (Lee and Sawa, 2014). To test the hypothesis that placental CpG methylation of these genes is predictive of moderate/severe cognitive impairment multivariable logistic regression modeling was used across 228 placentas. These models assessed whether a 1% increase in placental methylation is associated with a change (either an increase or decrease) in the odds of moderate/severe cognitive impairment.

Of the 237 tested probes, 41 probes representing 10 HPA genes were identified to have CpG methylation that was significantly associated with moderate/severe cognitive impairment (Fig. 2). Increases in CpG methylation of a probe in the TSS200 region of *NR3C1* displayed the highest OR of 1.876 (CI: 1.067–3.298). The CpG probe (p = 0.0012) for which the association of increased CpG methylation and moderate/severe cognitive impairment was most significant was for Heat Shock

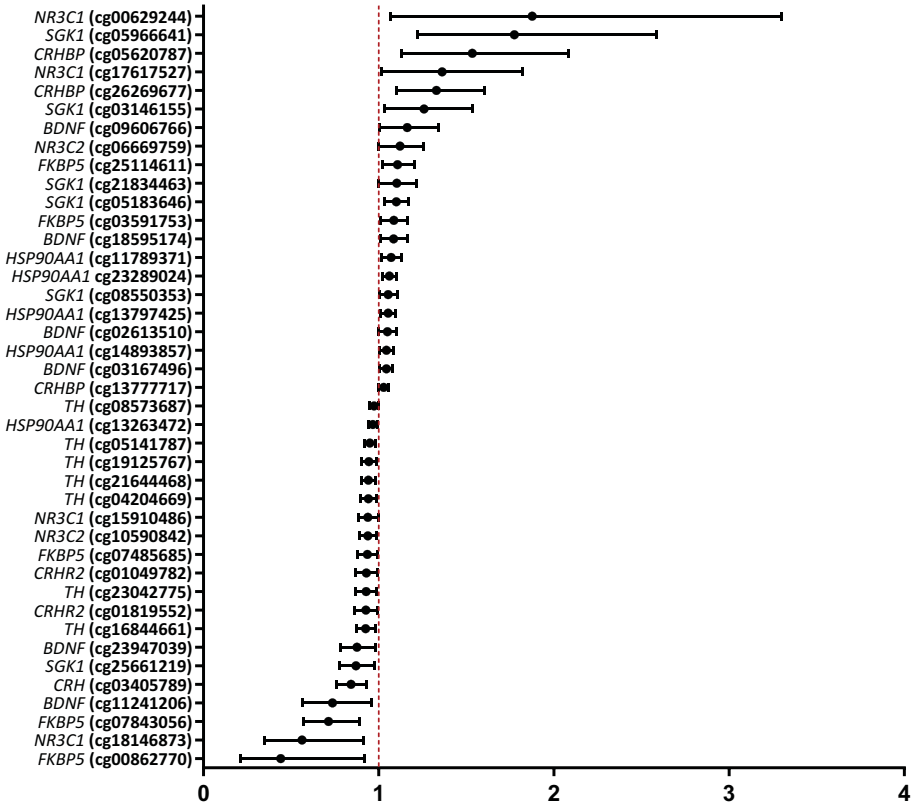

Fig. 2. Odds ratios and 95% confidence intervals for 41 CpG probes within 10 HPA axis-associated genes that displayed an association between increased placental CpG methylation and either moderate/severe cognitive impairment or low/low normal cognitive function at age 10 years.

Protein 90 Alpha Family Class A Member 1 (*HSP90AA1*) that displayed an OR of 1.061 (CI: 1.024–1.099). Increases in CpG methylation of two probes for Corticotropin Releasing Hormone Binding Protein (*CRHBP*) were consistently associated with moderate/severe cognitive impairment with OR ranging from 1.029–1.534.

Several probes displayed a negative association between CpG methylation and moderate/severe cognitive impairment, thus representing methylation that was associated with reduced risk of cognitive impairment. Among the probes where increases in CpG methylation were associated with reduced risk was *FKBP5* with an OR of 0.441 (CI: 0.212–0.917). The probe that displayed the most significant association ($p = 0.0009$) with a reduced risk was in the 3′ UTR region of Corticotropin Releasing Hormone (*CRH*) with an OR of 0.843 (CI: 0.762–0.933). Additionally, multiple probe sites on *CRHR2* (OR: 0.927–0.930) and multiple probe sites on *TH* (OR: 0.926–0.974) displayed consistent negative associations between CpG methylation and moderate/severe cognitive impairment. Genomic locations of the CpG sites for all tested probes ($n = 237$) and their CIs are provided in Supplemental Table 1. To summarize, increases in placental CpG methylation of HPA-axis related genes were associated with both higher and lower risk of cognitive impairment at age 10.

3.3. Pathway analysis results

To identify canonical pathways, upstream targets, and functional outcomes associated with the 10 significant genes identified network analysis was performed. For this analysis, networks were constructed based on connectivity through the literature, as enabled through IPA where over-represented pathways were determined using a right-tailed Fisher's Exact test.

The following genes that displayed altered methylation enrich for the glucocorticoid signaling pathway *FKBP5*, *NR3C1*, *NR3C2*, and *SGK1* ($p = 7.38 \cdot 10^{-6}$). Additionally, *NR3C1* and *NR3C2* enrich for the IL-4 signaling pathway ($p = 6.07 \cdot 10^{-4}$). In terms of environmental exposures, it has been postulated that environmentally-responsive transcription factors influence CpG methylation patterns (Martin and Fry, 2016). Thus, to explore enrichment for transcription factor binding sites within the genes that displayed altered CpG methylation transcriptional regulators of HPA genes were subsequently identified. Results revealed that the promoter region of the genes that displayed altered CpG methylation including *TH*, *SGK1*, *CRH*, *BDNF*, and *FKBP5* were enriched for binding sites of Methyl-CpG Binding Protein 2 (MECP2) ($p = 6.18 \cdot 10^{-10}$) (Fig. 3).

4. Discussion

Physiological signals within the placenta such as DNA methylation, gene expression and measures of placental physiology are associated with later life disease such as cognitive impairment, cardiovascular disease, and hypertension (Barker et al., 1990; Bromer et al., 2013; Paquette et al., 2015). Because placental physiology is dependent on transcriptional signals that may be controlled through epigenetic mechanisms, we set out to examine whether placental CpG methylation is predictive of children's cognitive outcomes at age 10 years. We analyzed 228 placentas from infants enrolled in the ELGAN cohort with a focus on 14 HPA axis-associated genes. These genes were selected for their known roles in stress response in the HPA axis as well as their roles in the placenta. Using multi-variable logistic regression modeling, the methylation levels for a set 237 probes representing 14 HPA axis genes were tested. Of these, 41 probes representing 10 HPA axis-associated genes were identified to be significantly associated with moderate/severe cognitive impairment at 10 years of age. The study is among the first to provide support for the placental epigenome as a potential biological “recording” of the *in utero* environment that is related to cognition at age 10.

Our findings show that increased CpG methylation in the placenta

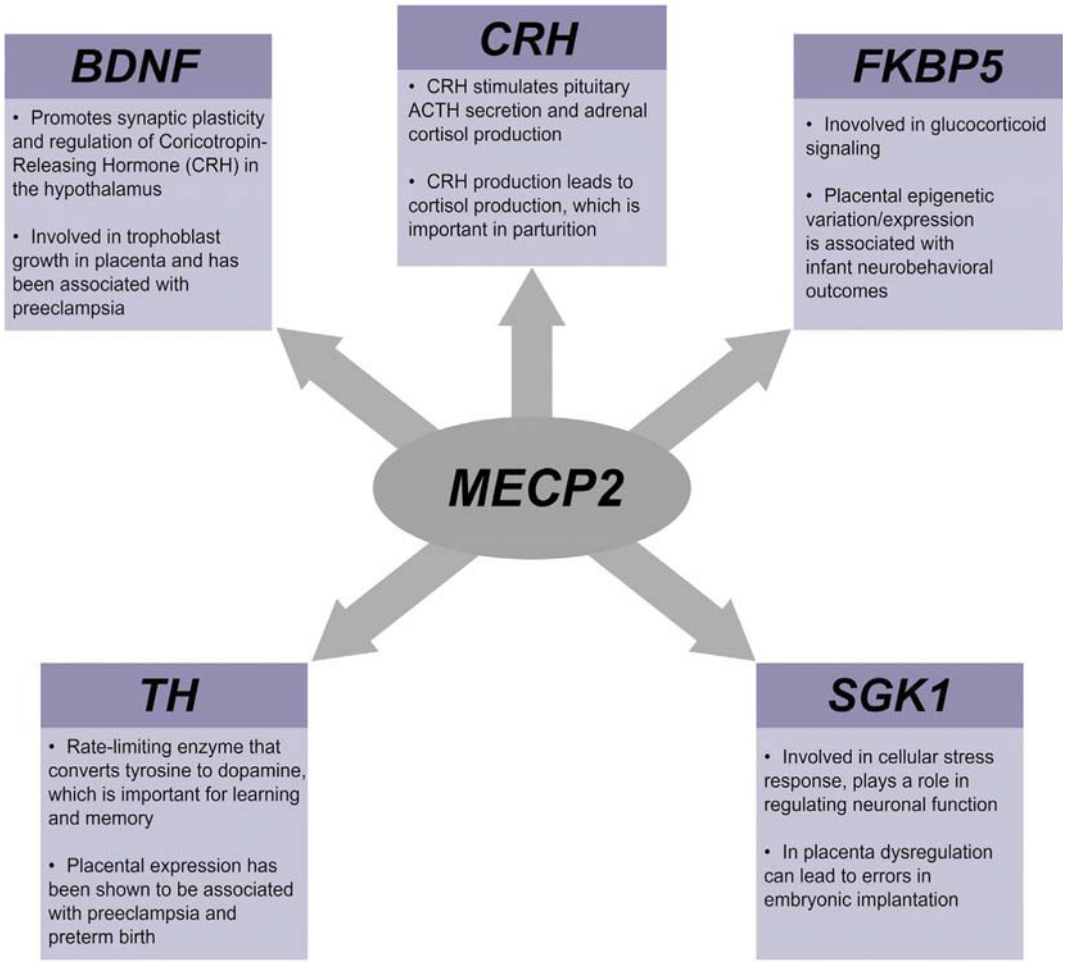

Fig. 3. Pathway analysis identifying Methyl-CpG Binding Protein 2 (MECP2) as an enriched transcriptional regulator of HPA axis genes. The function of these genes in the placenta and the HPA axis are detailed.

was associated with increased odds of developing moderate to severe cognitive function at age 10. This was observed for multiple probes for the gene *CRHBP*. In this regard it is notable that *CRHBP* is involved in cortisol signaling in the placenta and has been associated with early onset preeclampsia a disorder known to be associated with preterm birth (Hobel et al., 1999; Hogg et al., 2013). These data are important as they demonstrate that epigenetic marks of HPA axis genes in the placenta are associated with cognitive outcomes in mid-childhood, and these epigenetic marks may have functional impacts on HPA axis function and activity.

Increased CpG methylation of some CpG sites in the placenta was associated with decreased odds of developing moderate to severe cognitive impairment at age 10. This relationship was observed for CpG sites in *TH*, *CRH*, and *CRHR2*. In the placenta *TH* has been shown to be associated with preeclampsia and preterm birth (Manyonda et al., 1998). Likewise, CRH receptors, such as *CRHR2*, have been postulated to be involved in glucose transport across the placenta, which plays a major role in fetal development, nutrition, and cellular signaling (Gao et al., 2012). While in this study environmental toxicant exposure during pregnancy has not yet been assessed, it is interesting to note that dioxin exposures have been associated with induction of *TH* mRNA levels in rodent neuronal cells and may play a role in neurological dysfunction as *TH* is the major rate-limiting enzyme that converts tyrosine to dopamine throughout the body (Akahoshi et al., 2009; Daubner et al., 2011). These data, in conjunction with existing literature, demonstrate the potential ability of placental CpG methylation to predict cognitive function and development in mid-childhood.

Interestingly, probes sites within three genes, namely *NR3C1*, *BDNF*, and *FKBP5*, displayed a unique pattern where depending on the CpG site location, increased placental methylation was associated with either reduced or increased odds for moderate/severe cognitive impairment at age 10. Differences in OR for these CpG sites could be due to the impact of the location of the methylation mark as a differential driver of gene expression (Rojas et al., 2015). For example, CpG methylation of probes within the promoter regions of genes tend to be associated with gene silencing, while methylation of probes within the gene body are associated with gene activation (Rojas et al., 2015). Related to placental function, *NR3C1* is highly expressed and is thought to play a role in regulating fetal exposure to cortisol (Conradt et al., 2013). *BDNF* has been shown to promote trophoblast growth, and cell survival during placental development and low expression levels of *BDNF* in the placenta have been associated with pregnancy complications such as preeclampsia and preterm birth (D'Souza et al., 2014; Kawamura et al., 2009). Of relevance to cognitive outcomes, hypo/hyper methylation and subsequent altered expression of *NR3C1* and *FKBP5* in the placenta have been associated with adverse neurobehavioral outcomes (Appleton et al., 2015; Bromer et al., 2013; Conradt et al., 2013; Paquette et al., 2014; Paquette et al., 2015). In further support of our findings, hypermethylation of *BDNF* in hippocampal tissues and peripheral blood have been associated with depression, bipolar disorder, autism, and schizophrenia (Kundakovic et al., 2015).

In the placenta, with relevance to environmental EDCs, prenatal exposures to Bisphenol A (BPA) has been associated with increases in global methylation, which may ultimately alter expression of HPA

genes, thus, altering both cognitive outcomes and placental development (Nahar et al., 2015). In relation to other tissues, it is interesting that expression levels of *NR3C1*, *FKPB5*, and *BDNF* are altered by exposure to BPA. For example, perinatal BPA exposures in rats induce anxiety-related disorders as a result of decreased expression in *NR3C1* as well as alter methylation and expression levels of *FKBP5* in hippocampal tissues (Chen et al., 2015; Kitraki et al., 2015). Additionally, studies in humans and rat blood and hippocampal tissues have confirmed that prenatal exposures to BPA alter expression as well as methylation levels, and may lead to adverse cognitive functioning (Kundakovic et al., 2015). Understanding the function that these identified genes serve in the HPA axis is important as their dysregulation has been implicated in the development of numerous cognitive outcomes and may provide evidence for potential therapeutic techniques to prevent these outcomes (de Kloet et al., 2006; Schatzberg et al., 2014).

When interpreting the results of this study, several factors should be considered. The present study focuses on CpG methylation in the placenta. Unfortunately, we do not have access to brain tissue in the ELGAN study. It is established that there are tissue-specific patterns of CpG methylation (Ghosh et al., 2010) as well as CpG sites that display conserved methylation patterns across tissues (Woodfine et al., 2011). Thus, it is not the intent of this study to convey that CpG methylation in the placenta would be similar to CpG methylation in the fetal brain. Rather, the CpG methylation in the placenta is viewed as a "biological recording" of placental signaling pathways that are critical for fetal growth and development. This work contributes to a growing body of literature showing altered placental CpG methylation HPA axis genes that are associated with altered infant behavior (Bromer et al., 2013; Conradt et al., 2013; Monk et al., 2016; Paquette et al., 2014; Paquette et al., 2015). The present study is among the first to demonstrate that methylation of HPA axis-related genes in the placenta is critically related to neurocognitive outcomes in children born preterm. It must also be noted that the ELGAN cohort consists only of preterm births. Further research must be carried out to determine the generalizability of these findings in a non-preterm cohorts. Finally, placental RNA or protein are not currently available to functionally validate the expression levels in response to increases in CpG methylation. Similarly, access to environmental exposure data was not currently available for study. Future studies should integrate mRNA and protein expression with CpG methylation and environmental exposure data to gain further insight on how CpG methylation in the placenta impacts gene expression levels.

In summary, the results of this study highlight a set of 10 HPA axis-associated genes that displayed an association between increased placental CpG methylation and either moderate/severe cognitive impairment or low/low normal cognitive function at age 10 years. Many of these genes regulate both placental function and HPA axis function. The identified genes are also known to play integral roles in memory, learning, and the development of psychological disorders and there is evidence that exposure to EDCs may influence their expression as well. Furthermore, given the plasticity of the epigenome during the prenatal period, these alterations could be influenced by exposure to environmental contaminants, including EDCs. Growing research supports that exposure to common EDCs, including estrogenic compound like BPA, may dysregulate several genes involved in regulation of the HPA axis (Kundakovic et al., 2015; Weiser and Handa, 2009). This work provides a basis for which to subsequently investigate the role of EDCs on the HPA axis. Future work should incorporate exposure data as it relates to epigenetic modifications of the HPA axis-associated genes, as these data could provide more information as to how EDCs mechanistically disrupt the HPA axis and potentially provide biomarkers for exposure and later-life cognitive impairments in mid-childhood.

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Disclosure of potential conflict of interests

The authors claim no competing financial interests.

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References

Akahoshi, E., Yoshimura, S., Uruno, S., Ishihara-Sugano, M., 2009. Effect of dioxins on regulation of tyrosine hydroxylase gene expression by aryl hydrocarbon receptor: a neurotoxicology study. Environ. Health 8, 24.

Appleton, A.A., Lester, B.M., Armstrong, D.A., Lesseur, C., Marsit, C.J., 2015. Examining the joint contribution of placental NR3C1 and HSD11B2 methylation for infant neurobehavior. Psychoneuroendocrinology 52, 32–42.

Aryee, M.J., Jaffe, A.E., Corrada-Bravo, H., Ladd-Acosta, C., Feinberg, A.P., Hansen, K.D., Irizarry, R.A., 2014. Minfi: a flexible and comprehensive Bioconductor package for the analysis of Infinium DNA methylation microarrays. Bioinformatics 30, 1363–1369.

Barker, D.J., Bull, A.R., Osmond, C., Simmonds, S.J., 1990. Fetal and placental size and risk of hypertension in adult life. BMJ 301, 259–262.

Bright, H.R., Babata, K., Allred, E.N., Erdei, C., Kuban, K.C.K., Joseph, R.M., O'Shea, T.M., Leviton, A., Dammann, O., Investigators, E.S., 2017. Neurocognitive outcomes at 10 years of age in extremely preterm newborns with late-onset bacteremia. J. Pediatr. http://dx.doi.org/10.1016/j.jpeds.2017.04.045.

Bromer, C., Marsit, C.J., Armstrong, D.A., Padbury, J.F., Lester, B., 2013. Genetic and epigenetic variation of the glucocorticoid receptor (NR3C1) in placenta and infant neurobehavior. Dev. Psychobiol. 55, 673–683.

Chen, F., Zhou, L., Bai, Y., Zhou, R., Chen, L., 2015. Hypothalamic-pituitary-adrenal axis hyperactivity accounts for anxiety- and depression-like behaviors in rats perinatally exposed to bisphenol A. J. Biomed. Res. 29, 250–258.

Conradt, E., Lester, B.M., Appleton, A.A., Armstrong, D.A., Marsit, C.J., 2013. The roles of DNA methylation of NR3C1 and 11beta-HSD2 and exposure to maternal mood disorder in utero on newborn neurobehavior. Epigenetics 8, 1321–1329.

Cowansage, K.K., LeDoux, J.E., Monfils, M.H., 2010. Brain-derived neurotrophic factor: a dynamic gatekeeper of neural plasticity. Curr. Mol. Pharmacol. 3, 12–29.

Daubner, S.C., Le, T., Wang, S., 2011. Tyrosine hydroxylase and regulation of dopamine synthesis. Arch. Biochem. Biophys. 508, 1–12.

D'Souza, V., Patil, V., Pisal, H., Randhir, K., Joshi, A., Mehendale, S., Wagh, G., Gupte, S., Joshi, S., 2014. Levels of brain derived neurotrophic factors across gestation in women with preeclampsia. Int. J. Dev. Neurosci. 37, 36–40.

Gao, L., Lv, C., Xu, C., Li, Y., Cui, X., Gu, H., Ni, X., 2012. Differential regulation of glucose transporters mediated by CRH receptor type 1 and type 2 in human placental trophoblasts. Endocrinology 153, 1464–1471.

Gheorghe, C.P., Goyal, R., Mittal, A., Longo, L.D., 2010. Gene expression in the placenta: maternal stress and epigenetic responses. Int. J. Dev. Biol. 54, 507–523.

Ghosh, S., Yates, A.J., Fruhwald, M.C., Miecznikowski, J.C., Plass, C., Smiraglia, D., 2010. Tissue specific DNA methylation of CpG islands in normal human adult somatic tissues distinguishes neural from non-neural tissues. Epigenetics 5, 527–538.

Heeren, T., Joseph, R.M., Allred, E.N., O'Shea, T.M., Leviton, A., Kuban, K.C.K., 2017. Cognitive functioning at age 10 years among children born extremely preterm: a latent profile approach. Pediatr. Res. 82, 614–619.

Hobel, C.J., Arora, C.P., Korst, L.M., 1999. Corticotrophin-releasing hormone and CRH-binding protein. Differences between patients at risk for preterm birth and hypertension. Ann. N. Y. Acad. Sci. 897, 54–65.

Hogg, K., Blair, J.D., McFadden, D.E., von Dadelszen, P., Robinson, W.P., 2013. Early onset pre-eclampsia is associated with altered DNA methylation of cortisol-signalling and steroidogenic genes in the placenta. PLoS One 8, e62969.

Jeanneteau, F.D., Lambert, W.M., Ismaili, N., Bath, K.G., Lee, F.S., Garabedian, M.J., Chao, M.V., 2012. BDNF and glucocorticoids regulate corticotrophin-releasing hormone (CRH) homeostasis in the hypothalamus. Proc. Natl. Acad. Sci. U. S. A. 109, 1305–1310.

Kawamura, K., Kawamura, N., Sato, W., Fukuda, J., Kumagai, J., Tanaka, T., 2009. Brain-derived neurotrophic factor promotes implantation and subsequent placental development by stimulating trophoblast cell growth and survival. Endocrinology 150, 3774–3782.

Keller-Wood, M.E., Dallman, M.F., 1984. Corticosteroid inhibition of ACTH secretion. Endocr. Rev. 5, 1–24.
Kinlein, S.A., Wilson, C.D., Karatsoreos, I.N., 2015. Dysregulated hypothalamic-pituitary-adrenal axis function contributes to altered endocrine and neurobehavioral responses to acute stress. Front. Psychiatry 6, 31.
Kitraki, E., Nalvarte, I., Alavian-Ghavanini, A., Ruegg, J., 2015. Developmental exposure to bisphenol A alters expression and DNA methylation of Fkbp5, an important regulator of the stress response. Mol. Cell. Endocrinol. 417, 191–199.
de Kloet, C.S., Vermetten, E., Geuze, E., Kavelaars, A., Heijnen, C.J., Westenberg, H.G., 2006. Assessment of HPA-axis function in posttraumatic stress disorder: pharmacological and non-pharmacological challenge tests, a review. J. Psychiatr. Res. 40, 550–567.
Kuban, K.C., Joseph, R.M., O'Shea, T.M., Allred, E.N., Heeren, T., Douglass, L., Stafstrom, C.E., Jara, H., Frazier, J.A., Hirtz, D., Leviton, A., Extremely Low Gestational Age Newborn Study, I., 2016. Girls and boys born before 28 weeks gestation: risks of cognitive, behavioral, and neurologic outcomes at age 10 years. J. Pediatr. 173 (69–75 e61).
Kundakovic, M., Gudsnuk, K., Herbstman, J.B., Tang, D., Perera, F.P., Champagne, F.A., 2015. DNA methylation of BDNF as a biomarker of early-life adversity. Proc. Natl. Acad. Sci. U. S. A. 112, 6807–6813.
Lee, R.S., Sawa, A., 2014. Environmental stressors and epigenetic control of the hypothalamic-pituitary-adrenal axis. Neuroendocrinology 100, 278–287.
Logue, M.W., Smith, A.K., Wolf, E.J., Maniates, H., Stone, A., Schichman, S.A., McGlinchey, R.E., Milberg, W., Miller, M.W., 2017. The correlation of methylation levels measured using Illumina 450K and EPIC BeadChips in blood samples. Epigenomics 9, 1363–1371.
Manyonda, I.T., Slater, D.M., Fenske, C., Hole, D., Choy, M.Y., Wilson, C., 1998. A role for noradrenaline in pre-eclampsia: towards a unifying hypothesis for the pathophysiology. Br. J. Obstet. Gynaecol. 105, 641–648.
Martin, E.M., Fry, R.C., 2016. A cross-study analysis of prenatal exposures to environmental contaminants and the epigenome: support for stress-responsive transcription factor occupancy as a mediator of gene-specific CpG methylation patterning. Environ. Epigenet. 2.
Monk, C., Feng, T., Lee, S., Krupska, I., Champagne, F.A., Tycko, B., 2016. Distress during pregnancy: epigenetic regulation of placenta glucocorticoid-related genes and fetal neurobehavior. Am. J. Psychiatry 173, 705–713.
Naert, G., Zussy, C., Tran Van Ba, C., Chevallier, N., Tang, Y.P., Maurice, T., Givalois, L., 2015. Involvement of endogenous brain-derived neurotrophic factor in hypothalamic-pituitary-adrenal Axis activity. J. Neuroendocrinol. 27, 850–860.
Nahar, M.S., Liao, C., Kannan, K., Harris, C., Dolinoy, D.C., 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.
Onderdonk, A.B., Delaney, M.L., DuBois, A.M., Allred, E.N., Leviton, A., Extremely Low Gestational Age Newborns Study, I, 2008. Detection of bacteria in placental tissues obtained from extremely low gestational age neonates. Am. J. Obstet. Gynecol. 198 (110), e111–117.
O'Shea, T.M., Allred, E.N., Dammann, O., Hirtz, D., Kuban, K.C., Paneth, N., Leviton, A., 2009. The ELGAN study of the brain and related disorders in extremely low gestational age newborns. Early Hum. Dev. 85, 719–725.
Padmini, E., Venkatraman, U., Srinivasan, L., 2012. Mechanism of JNK signal regulation by placental HSP70 and HSP90 in endothelial cell during preeclampsia. Toxicol. Mech. Methods 22, 367–374.
Paquette, A.G., Lester, B.M., Koestler, D.C., Lesseur, C., Armstrong, D.A., Marsit, C.J., 2014. Placental FKBP5 genetic and epigenetic variation is associated with infant neurobehavioral outcomes in the RICHS cohort. PLoS One 9, e104913.
Paquette, A.G., Lester, B.M., Lesseur, C., Armstrong, D.A., Guerin, D.J., Appleton, A.A., Marsit, C.J., 2015. Placental epigenetic patterning of glucocorticoid response genes is associated with infant neurodevelopment. Epigenomics 7, 767–779.
Rojas, D., Rager, J.E., Smeester, L., Bailey, K.A., Drobna, Z., Rubio-Andrade, M., Styblo, M., Garcia-Vargas, G., Fry, R.C., 2015. Prenatal arsenic exposure and the epigenome: identifying sites of 5-methylcytosine alterations that predict functional changes in gene expression in newborn cord blood and subsequent birth outcomes. Toxicol. Sci. 143, 97–106.
Schatzberg, A.F., Keller, J., Tennakoon, L., Lembke, A., Williams, G., Kraemer, F.B., Sarginson, J.E., Lazzeroni, L.C., Murphy, G.M., 2014. HPA axis genetic variation, cortisol and psychosis in major depression. Mol. Psychiatry 19, 220–227.
Toni, R., 2004. The neuroendocrine system: organization and homeostatic role. J. Endocrinol. Investig. 27, 35–47.
Tsigos, C., Chrousos, G.P., 2002. Hypothalamic-pituitary-adrenal axis, neuroendocrine factors and stress. J. Psychosom. Res. 53, 865–871.
Uzumcu, M., Zama, A.M., Oruc, E., 2012. Epigenetic mechanisms in the actions of endocrine-disrupting chemicals: gonadal effects and role in female reproduction. Reprod. Domest. Anim. 47 (Suppl. 4), 338–347.
Weiser, M.J., Handa, R.J., 2009. Estrogen impairs glucocorticoid dependent negative feedback on the hypothalamic-pituitary-adrenal axis via estrogen receptor alpha within the hypothalamus. Neuroscience 159, 883–895.
Wochnik, G.M., Ruegg, J., Abel, G.A., Schmidt, U., Holsboer, F., Rein, T., 2005. FK506-binding proteins 51 and 52 differentially regulate dynein interaction and nuclear translocation of the glucocorticoid receptor in mammalian cells. J. Biol. Chem. 280, 4609–4616.
Wood, C.E., Keller-Wood, M., 2016. The critical importance of the fetal hypothalamus-pituitary-adrenal axis. F1000Res. 5.
Woodfine, K., Huddleston, J.E., Murrell, A., 2011. Quantitative analysis of DNA methylation at all human imprinted regions reveals preservation of epigenetic stability in adult somatic tissue. Epigenetics Chromatin 4 (1).