Epigenetic mechanisms in the placenta related to infant neurodevelopment

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As the ‘third brain’ the placenta links the developing fetal brain and the maternal brain enabling study of epigenetic process in placental genes that affect infant neurodevelopment. We described the characteristics and findings of the 17 studies on epigenetic processes in placental genes and human infant neurobehavior. Studies showed consistent findings in the same cohort of term healthy infants across epigenetic processes (DNA methylation, genome wide, gene and miRNA expression) genomic region (single and multiple genes, imprinted genes and miRNAs) using candidate gene and genome wide approaches and across biobehavioral systems (neurobehavior, cry acoustics and neuroendocrine). Despite limitations, studies support future work on molecular processes in placental genes related to neurodevelopmental trajectories including implications for intervention.

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There are few settings in which gene-environment interactions are more profound, critical windows are of a more narrow duration, and the latency to onset of effect is shorter than the influence of an adverse intrauterine environment on neuroendocrine and neurobehavioral functioning in the newborn. This link between the intrauterine environment and early developmental outcomes represents an obvious manifestation of the Developmental Origins of Health and Disease (DOHaD) [1,2], which posits that the environment experienced by a developing fetus can shape the health trajectory of the offspring throughout life. Early development can have echoes across the lifetime. The clinical and epidemiologic literature around this paradigm has grown and has linked various developmental stressors, including psychosocial, chemical and physical factors, to various newborn, infant, childhood, and adult health outcomes. Impaired fetal growth increases the risk for developing a number of chronic diseases including coronary heart disease and other metabolic syndromes such as, hypertension, stroke, insulin resistance, Type 2 diabetes and dyslipidemia. The disease burden is further increased when there is a ‘mismatch’ between the prenatal and postnatal environments. Prenatal stress, especially through impact on the neuroendocrine system, is not only related to adult cardiovascular and metabolic disorders but also to neurobehavioral and behavioral disorders. Low birth weight is associated with mental illness including schizophrenia, depression and psychological distress. But birth size, in and of itself, is not the ‘cause’ of these disorders. Rather it is a proxy for the quality of the intrauterine environment. Factors in the prenatal environment affect developing systems that alter structure and function, which leads to the hypothesis that behavior, could also be affected providing a possible explanation for why low birth-weight is related to psychopathology. Developmental plasticity enables the fetus to change or reprogram structure and function by resetting physiological parameters in response to signals from the internal environment due to external events. The current research in this field is turning toward understanding the mechanisms and pathways responsible for this programming, considering that such an understanding could provide opportunities for early risk assessment and novel interventional approaches aimed at improving health.

A growing focus of work on the mechanistic basis of DOHaD has centered on the role of the placenta in mediating the effects of the intrauterine environment on offspring development and health. The placenta is the first complex mammalian organ to form and is required for successful development of all viviparous species. As master regulator of the intrauterine environment, the placenta plays a functional, mechanistic role in shaping fetal development, including growth and neurodevelopment, and sits at the interface between the maternal and fetal environments. The placenta is a unique endocrine and metabolic organ that mediates transmission of environmental signals, nutrition, and endocrine/immune and gas exchange between mother and fetus, and supports fetal brain development through adaptive responses to the maternal environment and protection from environmental insults [3]. It provides an ideal fetal record of the intrauterine environment and alterations to its function through epigenetic processes that could affect infant neurodevelopment [4]. Throughout gestation, the placenta regulates immune function and fetal exposure to endogenous and exogenous exposures, as well as acts as a neuroendocrine organ producing hormones, growth factors and neuropeptides. In fact, due to its role in neuroendocrine regulation and development, the placenta has been described as a ‘third brain’ that links the developing fetal brain and the mature maternal brain, and is thus a sensitive functional tissue to understand the prenatal environment’s effects on neurodevelopment [5].

The focus of much of the work on placental function and its impact on developmental programming has been on placental epigenetics. Epigenetics can be defined as heritable changes in gene expression/phenotype that do not involve alterations in DNA sequence [6]. This additional layer of regulation allows for the genome to be elaborated upon and functionally regulated without disruption of the underlying genetic code, and potentially on a time scale that will allow for response to a rapidly changing environment, such as that experienced over the course of pregnancy. The simplest and most widely studied epigenetic mechanism is DNA methylation, which may result in activation or silencing of key genes in critical pathways, which then remain stable through cell division [7]. Cytosines following by guanine (CpG) dinucleotides, which are under-represented in the genome, occur at greater than expected frequencies in gene promoter regions known as islands. The methylation occurs as the addition of a methyl-group to the 5'-carbon of the cytosine in CpG dinucleotides, and in the context of multiple methylated cytosines within a particular CpG island, it is often associated with total transcriptional silencing of the downstream gene. The presence of the methyl group alone in this context is not sufficient for transcriptional silencing, but instead recruits component proteins related to gene repression and creation/maintenance of a silenced chromatin conformation, complete with the appropriate post-translational modification of histone tails.

A major functional role of DNA methylation is in establishing the tissue specific genomic regulation necessary for cellular identity and function. The placenta demonstrates a highly unique pattern of DNA methylation across its genome, likely reflective of its broad functional roles throughout development [8]. This has spurred a focus of research on the intrauterine and maternal environment’s impact on the placental epigenome and examinations of the links between placental DNA methylation variation and offspring outcomes. Specifically, the focus of placental epigenetics and neurodevelopment research has been on the neuroendocrine system, in particular, programming of the hypothalamic-pituitary-adrenal (HPA) axis, which regulates the stress response, as well as a number of other systemic processes including emotions, energy expenditure, and the immune system [9]. The neuroendocrine system is highly active in placental tissue [10], and strongly implicated in the role of fetal programming in DOHaD. In this review, we will discuss studies of the placenta’s role in the field of behavioral development, growing from those focused on DNA methylation of single genes to those involving genome-wide DNA methylation assessments, and also touch on new avenues for research on behavioral epigenetics examining the impact of imprinted genes and microRNA in this process.

Overall description of the studies reviewed

Table 1 shows the 17 empirical, peer-reviewed publications that we have identified relating epigenetic processes in placental genes to neurodevelopmental outcome. These studies were from a cohort of several hundred term, healthy infants born to mothers with uncomplicated medical histories [11]. Table 1 shows the epigenetic process studied, genomic regions assayed, neurodevelopmental outcome measure(s) and the major finding of each study. Most ($n = 12$) were studies of DNA methylation of candidate genes, three of the DNA methylation studies included gene expression and two included genotype, one of which also included gene expression. Gene expression alone was examined in two studies of imprinted genes and there is one study of RNA expression and miRNAs. Two studies examined epigenome wide DNA methylation utilizing an epigenome wide association study (EWAS) framework. Out of the candidate gene studies, eight were reports of single genes, three included two genes and one study examined four genes. The most frequently studied gene was *HSD11B2* ($n = 5$ studies) followed by *NR3C1* in ($n = 4$

Table 1. Characteristics of studies of epigenetic effects on placental genes and infant neurodevelopment.

Epigenetic process	Genomic region assayed	Neurodevelopmental outcome measures(s)	Major finding	Ref.
DNA methylation	<i>HSD11B2</i>	NNNS sum scores	Increased methylation related to low birthweight, in turn related to poorer quality of movement	[11]
DNA methylation	<i>HTR2A</i>	NNNS sum scores	Lower methylation related to better quality of movement and attention	[43]
DNA methylation	<i>NR3C1</i>	NNNS sum scores	Methylation related to attention, regulation, lethargy, examiner soothing; maternal smoking related to lethargy	[21]
DNA methylation	<i>NR3C1</i>	Cry acoustics	Increased methylation related to a weaker and more high pitched cry (Figure 2)	[28]
DNA methylation	<i>NR3C1</i>	Infant–mother interaction and cortisol stress reactivity at 5 m	Increased DNA methylation related to increased cortisol reactivity and infant self-regulation	[31]
DNA methylation	<i>NR3C1</i> , <i>HSD11B2</i>	NNNS sum scores	Maternal depression related to increased methylation of <i>NR3C1</i> in turn to poorer regulation, more hypotonia and lethargy. Maternal anxiety related hypotonia through increased methylation <i>HSD11B2</i>	[18]
DNA methylation	<i>NR3C1</i> , <i>HSD11B2</i>	NNNS sum scores	Methylation of combinations of cortisol response genes related to different neurobehavioral phenotypes	[26]
DNA methylation	<i>NR3C1</i> , <i>HSD11B2</i> , <i>FKBP5</i> , <i>ADCYAP1R1</i>	NNNS sum scores	Methylation of cortisol response gene factors related to more stress abstinence and lower attention and arousal	[45]
EWAS	>485,000 CpG loci	NNNS profiles	High concentrations Hg and an at risk profile associated with ten loci in <i>EMID2</i> gene	[19]
EWAS	19,361 genes, 270,981 CpG loci	NNNS sum scores	Genome wide effects of <i>FHIT</i> and <i>ANKRD11</i> on attention, 50 brain development genes related to attention, lethargy, quality of movement and arousal (Figure 3)	[24]
DNA methylation, expression and genotype	<i>FKBP5</i>	NNNS sum scores	Increased methylation <i>FKBP5</i> related to higher arousal, genetic variation related to stress	[45]
DNA methylation and genotype	<i>NR3C1</i>	NNNS sum score	Increased methylation related to poorer quality of movement, lower attention and genotype	[44]
DNA methylation and gene expression	<i>LEP</i>	NNNS profiles	Increased methylation related to profiles characterized by lethargy and hypotonia only in males (Figure 1)	[20]
DNA methylation and gene expression	<i>HSD11B2</i> , <i>SCL6A4</i>	Cortisol levels	<i>HSD11B2</i> methylation and <i>SLC6A4</i> gene expression mediate relations between maternal depression during pregnancy and cortisol	[16]
Gene expression	108 imprinted genes	NNNS profiles	Gene expression of ten genes (Figure 4) related to two neurobehavioral patterns, movement and reflex problems and increased stress	[22]
Gene expression	22 imprinted genes	NNNS sum scores	Classes of expression related to quality of movement and amount of handling	[46]
miRNA expression	<i>miR-16</i> , <i>miR-21</i> , <i>miR-93</i> , <i>miR-135b</i> , <i>miR-146a</i> and <i>miR-182</i>	NNNS sum scores	High <i>miR-16</i> expression related to higher attention, high <i>miR-146a</i> and <i>miR-182</i> expressions related to increased quality of movement score	[47]

EWAS: Epigenome-wide association study; NNNs: NICU network neurobehavioral scale.

studies). There were two studies of *FKBP5*, and one study each of *HTR2A*, *SCL6A4*, *FKBP5*, and *ADCYAP1R1*. A total of 16 of the 17 studies involved newborn infants, 14 of which used the NICU network neurobehavioral scale (NNNS) as the outcome measure [12]. The candidate genes studied focus on pathways involved in cortisol regulation and HPA axis function (*NR3C1*, *HSD11B2*, *FKBP5*, *ADCYAP1R1*) as well as on neuroendocrine signaling (*HTR2A* and *SLC6A4*), pathways chosen due to their active function in the placenta and their essential role in neurodevelopment, particularly in the domains studied using the NNNS. The NNNS is a well-established valid and reliable evaluation that provides measures of neurological function, behavior and stress and predicts behavior problems, school readiness and IQ through 4.5 years [13]. Summary scores on the NNNS (Table 2) were used in 11 studies. NNNS profiles were used in three studies (Figure 1). The studies typically used the exam once shortly after birth. It was also used multiple times to study the trajectory of neurobehavioral development between birth and 1 month. Outcome measures in the remaining three newborn studies included acoustic cry analysis and cortisol reactivity. Crying was recorded during the NNNS and processed through an automated, computerized cry analysis system [14]. This system uses an algorithm to extract acoustic parameters from standard digital audio files of the infant's cry (Figure 2) that have been extensively studied in high and low risk populations [15]. Cortisol stress

Table 2. NICU network neurobehavioral scale summary scores.	
Attention	Ability to localize and track objects, faces and voices
Handling	Handling strategies used during attention
Self-regulation	Organize behavior in response to stimulation
Arousal	Level of arousal during the examination
Excitability	High levels of motor, state and physiologic reactivity
Lethargy	Low levels of motor, state and physiologic reactivity
Hypertonicity	Hypertonic responses in arms, legs, trunk or tone
Hypotonicity	Hypotonic response in arms, legs, trunk or tone
Nonoptimal reflexes	Number of poor reflex scores
Asymmetric reflexes	Number of asymmetric reflex scores
Quality of movement	Smoothness, maturity, lack of startles, tremors
Stress/abstinence	Number of stress signs observed
Habituation	Assessment of state, observation of reaction to stimuli
Data taken from [29].

Figure 1. NICU network neurobehavioral scale profiles were calculated using a recursively partitioned mixture model algorithm using the NICU network neurobehavioral scale summary scores (x-axis). The scores are plotted as standard scores or standard deviation units (y-axis). The profiles are mutually exclusive subgroups of infants, ‘typologies’, with distinct patterns of performance across the summary scores.
Data taken from [22].

Figure 2. Digitized spectrogram plot. The plot is generated using a computerized acoustical analysis system. Time (~2 s) is on the x axis, Hz is on the y axis. The vertical red-yellow spikes are individual vocal utterances of the cry. The clarity of the cry signal ranges from ‘more’ (yellow) or phonation to ‘less’ (red) or dysphonation. Spaces in between the utterances are pauses or breathes.

Figure 3. Venn diagram of similarities from genes from the CpGs with highly significant correlations and NICU network neurobehavioral scale scores.

Data taken from [26].

reactivity was a difference score ($\mu\text{g/dl}$) measured from saliva collected before and after the NNNS conceptualizing the exam as a 'stressor' [16]. Cortisol stress reactivity was also measured at 5 months, collected during a mother infant interaction procedure that includes 'still face' episodes in which the mother is nonresponsive to her infant. Cortisol reactivity was a difference score ($\mu\text{g/dl}$) between baseline and the stressful 'still face' episodes [17].

The 17 studies can be divided into two basic designs, those in which epigenetic processes in the placenta were directly related to neurodevelopmental outcome and those in which epigenetic processes in the placenta mediated relations between prenatal factors and neurodevelopmental outcome. These factors include low birthweight [11], maternal depression during pregnancy [18], maternal obesity and exposure to toxicants [16,19]. Two studies reported sex differences in epigenetic effects on NNNS profiles [16,20] and one study suggested an epigenetic by genotype interaction on neurodevelopmental outcome [21].

NNNS findings across the 12 studies that used summary scores showed the strongest relations between epigenetic processes and quality of movement ($n = 7$) and attention ($n = 6$). The three studies that used the NNNS profiles showed epigenetic affects related to prenatal mercury exposure [19] to profiles characterized by movement and reflex problems in a genome wide study [22] and to an under-aroused profile in males only [20].

Discussion of the studies & their implications

This is a unique corpus of work relating epigenetic processes in the placenta to neurodevelopment that shows consistency of findings and proffers some noteworthy insights and suggestions for future work. Consistency is critical for the study of epigenetic processes in human behavior as it is in any scientific endeavor, and represents reliability and a form of replication. In the case of human behavior consistency of findings has unique consequences as we cannot conduct the kind of experimental research to examine brain tissue, cross fostering, intergenerational transmission and central infusion studies that can be performed in other species. Notwithstanding is a study of postmortem DNA methylation of *NR3C1* in the human hippocampus in relation to child abuse [23], keeping in mind that postmortem studies have their own limitations. Human behavioral epigenetic studies are, to date, associational, as are most human developmental studies. Experimental and quasi-experimental paradigms such as randomized clinical trials have been applied to human behavior but not, to our knowledge, in relation to epigenetic changes, although ethical issues would be of paramount importance.

Studies show consistent findings

These placental studies show a high degree of consistency of findings across expected domains. Systematic findings emerged in one cohort using same specimen (placenta) across epigenetic processes (DNA methylation, genome wide, gene expression and miRNA expression) including those more proximal (DNA methylation) and downstream (miRNA) from the promoter region and across the genomic region assayed (single and multiple genes, imprinted genes and miRNAs). Different analytic approaches (candidate gene and genome wide), realized complimentary

Figure 4. Heatmap of expression for ten genes used to assign membership to NICU network neurobehavioral scale clusters. Average scores of infant stress abstinence, quality of movement, asymmetrical reflexes and nonoptimal reflexes differed across the gene expression-based clusters. Infants in gene expression clusters 2 and 3 exhibited more signs of stress and abstinence, reduced quality of movement and increased signs of asymmetrical and nonoptimal reflexes.

Data taken from [24].

outcomes and interaction effects between genotype and epigenetic processes were detected. Findings were also consistent across the biobehavioral systems studied (NNNS neurobehavior, cry acoustics and neuroendocrine).

What are biobehavioral systems?

The term biobehavioral is used to acknowledge the biological basis of behavior, to understand behavior in terms of brain and physiological function. The neurodevelopmental measures used in these studies represent related biobehavioral processes. They measure stress reactivity at the neurobehavioral (NNNS), acoustical (cry) and neuroendocrine (cortisol) levels. As discussed later, thought provoking is that that these three measures have all been related to longer-term developmental outcome.

Interpretation of NNNS findings

Attention and quality of movement showed the greatest number of effects and were found in all of the regions assayed and epigenetic processes studied (Table 1). In one of the genome-wide studies, effects on attention and quality of movement (as well as lethargy and arousal) were common to 50 genes involved in biological processes relating to cellular adhesion and nervous system development [24] (Figure 3). Direct connections between specific neural mechanisms and infant neurobehavior are consistent with other findings relating NNNS scores to positive MRI findings in preterm infants [25].

In this group of placental studies, similar conclusions were drawn from studies using NNNS summary scores and NNNS profiles. This makes sense as it is understood that these two analytic methods are interdependent. The summary scores measure specific neurobehavioral domains. The profiles use these domains to identify subgroups of infants, 'typologies,' with distinct patterns of performance across the summary scores. The profiles disclose how

Table 3. Cry characteristics and associated mechanism.

Characteristic	Definition	Biological mechanism
Cry latency	Time from known stimulus to onset of the first utterance (cry sound)	Arousal from limbic-hypothalamic system
Threshold	Number of applications of stimulation to elicit a cry	Arousal from limbic-hypothalamic system
Utterances	Number of cry sounds across cry	Neural control of respiratory system
Short utterances	Number of unvoiced sounds across cry	Unstable respiratory control
Phonation	Cry mode resulting from vocal fold vibration between 350 and 750 vibrations or cycles <i>per second</i> (Hz)	Neural control of muscular tension in vocal folds and air flow through the glottis
Hyperphonation	Cry mode caused by a sudden upward shift in f0 to >1000 Hz	Neural constriction of the vocal tract
Dysphonation	Cry mode caused by noisy or inharmonic vibration of the vocal folds	Unstable respiratory control
Cry mode changes	Number of times cry modes change during an utterance	Instability in neural control of the vocal tract
Fundamental frequency (f0)	Base frequency during vocal fold vibration, heard as the pitch of the cry	Vagal input to larynx and lower vocal tract
First formant (F1)	Frequencies centered at first resonance of f0, approximately 1100 Hz	Neural control of size and shape of upper vocal tract
Second formant (F2)	Frequencies centered at second resonance of f0, approximately 3300 Hz	Neural control of size and shape of upper vocal tract
Duration	Time (msec) from onset to offset of cry utterance	Neural control of respiratory system
Duration of inspiration	Time (msec) between cry utterances or interutterance interval. Breath holding is evaluated by the 2nd inspiratory period	Neural control of respiratory system
Amplitude	Intensity or amplitude of the cry (dB). Heard as loudness	Neural control of respiratory system and capacity
Variability in f0	Changes in f0	Instability in neural control of the larynx and lower vocal tract
Variability in F1, F2	Changes in formant	Instability in neural control of upper vocal tract
Variability in Amplitude	Changes in intensity or loudness within an utterance or averaged across utterances	Instability in neural control of the respiratory system
Data taken from [18].		

the functional domains measured by the summary scores interact to produce singular neurobehavioral signatures that have predictive validity [13].

Different patterns of DNA methylation across the cortisol regulation pathway could reflect fetal programming influences on the reciprocity of neurobehavior. Infants with low *NR3C1* methylation but high *HSD11B2* had different phenotypes based on the NNNS summary scores than infants with high *NR3C1* methylation but low *HSD11B2* [26]. In other work, we have also seen how impaired developmental programming can influence transcriptional activity and have negative effects on NNNS profiles in preterm infants [27]. Worth mentioning too is that the NNNS has immediate clinical significance and is increasingly being used in hospital settings for the evaluation and treatment of preterm infants, infants suffering withdrawal from prenatal opiate exposure (neonatal abstinence syndrome) and other at risk populations.

Cry acoustics

The investigation of cry acoustics [28] is novel and marks a new direction in the study of epigenetic processes in placental genes identified with newborn neurodevelopment. The cry signal, and its specific features (Table 3), results from the coordination among several brain regions that control respiration and vocal cord vibration by cranial nerves (IX–XII) modulating the autonomic nervous system also involved in the HPA stress reactivity response [15,29]. The high pitched cry and alterations in the force or energy of the cry are indicators of poor regulation of the HPA system and are related to medical conditions, perinatal risk factors and neural integrity [15,30]. The association between these cry acoustic measures and methylation of the *NR3C1* gene is consistent with models linking epigenetic regulation of glucocorticoid receptor gene expression and neurological integrity and/or neurobehavioral markers of the stress response in the neonatal period. Moreover, consider that crying in this study [28] was recorded during the administration of the NNNS. The NNNS was used to elicit cortisol stress reactivity in a different investigation

of DNA methylation of the *NR3C1* gene [21] and measures of infant crying during the NNNS are fundamental components of many of the NNNS summary scores including arousal, excitability, self-regulation and stress. Indeed the administration of the exam is determined by when the infant is crying, alert or sleeping.

The neuroendocrine system, DOHaD & fetal programming

The tight coupling between the neuroendocrine system and behavior is legion and the studies reviewed here disclose a clear focus on those biobehavioral connections including programming of the HPA axis and epigenetic effects on neurodevelopment. Hence, the emphasis on genes related to cortisol activity (*HSD11B2*, *NR3C1*, *HTR2A*, *LEP*) and the use of stress paradigms to elicit HPA reactivity [21,28,31].

Much of the work in DOHaD has involved the neuroendocrine system and this is an important other area linking DOHaD with epigenetics [32] and health (broadly defined to include mental health) disparities in children [33]. This is also the system studied in the original work on maternal programming of rodent behavior [34] and has been extensively studied in relation to the development of mental health disorders [32,35]. For example, children exposed to physical maltreatment [36], infants of depressed mothers [18,37], adolescents whose mothers were exposed to intimate partner violence during pregnancy [38], and adults with a history of child abuse [23,39] have increased DNA methylation of the human homolog of the same rat exon 1₇ in the *Nr3c1* promoter region studied in the Meaney work [40]. In the study by Conradt *et al.* [31], DNA methylation of *NR3C1* in the placenta was related to cortisol reactivity and infant self-regulation at 5 months. To this, we add that findings of fetal programming of offspring brain and stress systems is a prominent proposed mechanism underlying links between prenatal adversity and offspring health and behavioral outcomes. In particular, dysregulation of the fetal HPA axis has been proposed as a final common pathway underlying links between prenatal adversity and long-term health and behavioral outcomes [41,42].

Direct & indirect effects

These studies of placental genes showed both direct and indirect epigenetic effects on infant neurobehavior that speak to the role of resetting of physiological parameters due to factors in the prenatal environment (fetal programming). Consider that in this corpus of placental epigenetic studies, relations between levels of DNA methylation and NNNS scores could vary as a function of fetal programming. Direct effect studies show decreases in DNA methylation (increased gene expression) related to better quality of movement on the NNNS in studies of *HTR2A* [43] and *NR3C1* [44], and attention in the case of *HTR2A* [43], increased DNA methylation of *FKBP5* was related higher arousal [45], increased *LEP* methylation related to more negative NNNS profiles [20]. In two studies of imprinted genes, decreased gene expression was associated with poor quality of movement [22,46], more reflex problems and increased stress [22] and more handling [46]. Increased miRNA expression was also related to better quality of movement [47].

Consider epigenetic effects as ‘footprints’

Although we recognize that there may be differences in epigenetic findings between, for example, rodent hippocampal tissue and human placental cells we also highlight the striking similarity of the findings in these human placental studies to the rodent work using brain cells, suggesting that they may be measuring similar processes. The approach from the field of epidemiology offers another perspective. That, even if the precise mechanism by which epigenetic phenomenon affects human behavior remains elusive, it is likely that findings such as these suggest an epigenetic ‘footprint’; that epigenetic processes were involved and it goes without saying that additional research is needed to elucidate the role of these ‘footprints.’ It would also be useful to determine if these footprints differ among specimens. Human work most often uses placenta, blood and buccal cells and it is unknown if these specimens provide similar or different information. Buccal cells may reflect the epigenetic status of the brain as these cells derive from the same primitive germ layer (the ectoderm). Thus, early programming effects during development may be coincident in these tissues.

It is vital that we study normal development?

Little is known about what are normal epigenetic marks, such as levels of methylation in candidate genes, in genome wide analysis, in different tissues and the longitudinal stability of epigenetic changes across the lifespan, including the identification of sensitive periods and inflection points in developmental trajectories. Consequently, it is notable that all of these studies were from a sample of clinically normal, healthy term infants. These findings then can be

taken as estimates of the variability of epigenetic effects in placenta in the population at large. Epigenetic studies are often conducted in at-risk or abnormal populations to help understand the role of epigenetics in pathological conditions, be it medical disease or psychopathology. Yet one wonders how we can understand the role epigenetics in aberrant development without knowing the normal developmental course of epigenetic processes. For example, we already know from this work that epigenetic effects in placenta were observed as both direct effects and indirect effects, the latter mediated by prenatal conditions that might be expected to result in epigenetic alterations affecting neurobehavior. Therefore, we now know that both direct and indirect effects can explain some of the variability in epigenetic findings in the normal population, and we know what are some of the indirect effects, epigenetic processes, genomic regions and biobehavioral systems involved. Another, we admit, somewhat simplistic, example is that if we had norms for DNA methylation, for example, including how those norms changed over the course of development, we could determine abnormal DNA methylation levels (as well as other epigenetic processes) that could be studied in relation to aberrant development.

Placental epigenetic effects can presage long-term neurodevelopmental outcome

The study of the newborn enables us to preview the role of placental mediated influences in determining the infant's neurodevelopmental 'presentation' before the powerful effects of the postnatal environment are implicated. The three biobehavioral measures used in these studies, NNNS, cry acoustics and cortisol stress reactivity have all been related to long-term developmental outcome [13,35,48]. Downstream post-transcriptional effects of miRNA expression in the placenta on neurodevelopment could also be a harbinger for long-term neurodevelopmental outcome as miRNA regulation of gene expression in adults has been related to major affective disorders and suicidal behavior [49].

Increased maternal glucocorticoids are thought to be a key mechanism underlying transmission of maternal adversity to the fetus. Maternal glucocorticoids are linked to risk for child and adult psychopathology, poor health outcomes and brain development [50,51]. For example, maternal prenatal cortisol is associated with more emotional problems and altered right amygdala volume in 7-year-old girls. Importantly, the tie-in between prenatal glucocorticoid exposure and childhood emotional problems was mediated by amygdala volume [50].

Epigenetic changes in placental genes that regulate neuroendocrine activity were related to NNNS summary scores and profiles in the studies reviewed herein. Quality of movement and attention, the most frequent NNNS findings in these placental studies are pre-eminent in the standardized developmental outcome tests predicted by the NNNS through 4½ years of age including IQ, school readiness and behavior problems [13]. Similarly, the acoustics of crying includes the prosodic elements of speech and is thought to be involved in the later development of speech and language [52].

Trajectories could be used to study long-term development

In the Stroud study [21], DNA methylation of *NR3C1* was related to the trajectory NNNS summary scores between birth and 1 month. Epigenetic processes can be perturbed by the postnatal caregiving environment raising the possibility of an insidious cycle in which initial neurodevelopmental vulnerability due to epigenetic effects in placental genes affecting the HPA axis is further exacerbated by a negative postnatal environment rendering infants more susceptible to entropy in neurodevelopmental trajectories. Investigation of epigenetic processes in placental genes and neurodevelopmental trajectories in the prediction of long-term developmental outcome seems warranted. An optimistic alternative would permit neural plasticity to allow for recovery and describe upward neurodevelopmental inflection points that could identify windows of opportunities for preventive interventions.

Could epigenetics play a role in intervention?

Identifying those infants most at risk for poor neurodevelopmental outcomes is crucial to allow for targeted surveillance or preventative interventions to be instigated from birth. One intriguing possibility is to consider the use of epigenetic characteristics of the placenta at birth as a 'molecular barometer' of the in utero experience to predict future infant neurodevelopmental outcomes [53]. Specifically, epigenetic alterations in placental genes that perturb the HPA axis could predispose infants to neurobehavioral profiles interacting with postnatal environmental factors that forecast later developmental outcome. In addition, by broadly profiling the epigenetic landscape of the placenta and identifying the functional pathways and networks whose variability is linked to neurobehavioral variability, we can denote new directions for intervention, be they nutritional, pharmaceutical, or behavioral, driven by these novel discoveries.

Rethinking the meaning of neurobehavior

An alternative interpretation in keeping with a developmental systems biology and fetal programming perspective is to consider neurobehavior in terms of adaptation to the postnatal environment. Most of the studies reviewed here examined epigenetic processes in candidate genes. However, we know that it would be naive to think that genes operate in isolation. Indeed, there are also studies that examine epigenetic processes in several candidate genes, imprinted genes and in genome wide studies. In other work using buccal cells in preterm infants, we found negative correlations between DNA methylation of *NR3C1* and *HSD11B2* [27]. These are cortisol regulating genes that act in reciprocity through a negative feedback loop, to wit, the negative correlation between these two genes. In the same study, infants with a higher risk NNNS neurobehavioral profile had more DNA methylation of *NR3C1* and less methylation of *HSD11B2* indicative of the regulatory process of these genes on neurobehavior.

It is biologically plausible, that the same kind of reciprocity among genes that regulate cortisol levels *in utero* also holds for neurobehavior regulated by cortisol levels. A hallmark of the trajectory of child development is reciprocity among developing neurobehavioral systems. Developmental regression of one system enables another system to advance. A classic example is when infants start to walk; it is normal for them to become more irritable and distracted at the same time (which can be somewhat confusing, if not worrisome to parents). Disorganization of one or more neurodevelopmental systems enables the infant to reorganize internal resources fueling the onset of other systems, in this case, walking. Development is not linear. Rather, it is characterized by ‘fits and starts’ particularly around sensitive periods, of which the neonatal period is undeniably one. NNNS scores measure intertwined developmental processes that work in concert to regulate the infant’s overall neurobehavioral repertoire reminiscent of the reciprocal relationship between *NR3C1* and *HSD11B2* in the regulation of cortisol levels. Moreover, this is a normal developmental process ostensibly due to developmental programming as programming occurs postnatally as well as prenatally. This process is illustrated by the Paquette [43] study in which less DNA methylation of *HTR2A* was related to higher quality of movement and lower attention on the NNNS. This does not negate findings in which these same behaviors can be maladaptive, but reminds us of the importance of considering direct versus indirect epigenetic effects. Indirect epigenetic effects in placental genes are associated with adverse prenatal conditions such as low birthweight [11], maternal depression [18], tobacco [21] and mercury [19].

The significance or ‘meaning’ of neurobehavioral findings needs to be fully contextualized, understood *in situ*, within the surrounding intrauterine environment in which neurobehavior is shaped. The ultimate outcome of the infant will be determined, in part, by the interaction between the infant’s neurobehavior at birth and the characteristics of the postnatal environment as infant behavior affects parenting which in turn affects the infant [17]. This dynamic, reciprocal process was examined in the Conrads article in which increased DNA methylation of placental *NR3C1* was related to increased cortisol reactivity [31]. In the cry literature, the ‘goodness of fit’ between the mothers’ level of accuracy in perceiving her infants newborn cry and the acoustic characteristics of her infants cry was related to language and cognitive scores at 18 months [54]. The biological drivers of differences in cry acoustics impact the developing infant-parent communication system. This finding also supports the earlier assertion that some of the roots of speech and language are embedded in the infants cry. Understanding newborn neurobehavior becomes a window into the infant’s ability to adapt to the postnatal environment including vulnerability or susceptibility to environmental adversity.

Limitations

The work reviewed herein has limitations. This is a restricted literature with only 17 studies of a relatively few candidate genes, most of which were single gene studies, and DNA methylation was the major epigenetic process examined. With few exceptions these were studies of newborn infants using the same (NNNS) outcome variable in a relatively homogeneous sample of normal, full term, healthy sample with a primary focus on the neuroendocrine system. On the other hand, these limitations can also be regarded as strengths. As discussed above, these studies describe a set of consistent and reliable findings across epigenetic process, genomic regions and biobehavioral processes measured. Not to negate the importance of a broader cohort of studies, these limitations permit us to appreciate some of the epigenetic mechanisms in the placenta related to infant neurodevelopment in a more in depth manner and to realize their biological plausibility. These studies pinpoint the conclusion that development has echoes in epigenetic effects in placental genes that determine the infant’s newborn neurobehavioral profile; echoes that are accessible for study as they reverberate in the caverns of the postnatal environment.

Future perspective

The studies reviewed in this article suggest that the intrauterine environment can modulate neurodevelopment through alteration of the function of the placenta by epigenetic mechanisms. These epigenetic alterations lead to changes in gene expression, which alter the function of the placenta as regulator of the fetal environment and thus contribute to neurodevelopmental trajectories of the infant in the same way that these alterations could set the metabolic profile of the infant. The growing body of literature which has focused on candidate genes involved in stress response or which produce neuropeptides has demonstrated that epigenetic processes in the placenta play a fundamental role in setting neuroendocrine stress reactivity systems programmed to set the neurodevelopmental table that interacts synergistically with the postnatal caregiving environment. To be sure, these neurodevelopmental trajectories can be reprogrammed, in other words, altered by further epigenetic changes due to postnatal environmental conditions. But, again, as with physiological profiles, the 'set points' of these trajectories and their initial programming is influenced by prenatal effects on the placental epigenome. This is a unique hypothesis and shift in the paradigm of the developmental basis of neurodevelopmental outcome suggesting novel mechanistic insights into the way in which the prenatal period can influence long-term neurodevelopmental outcome.

In addition to these well-justified candidate pathways involved in neuroendocrine regulation, a number of additional functionally relevant pathways have been identified through genome-wide scans of DNA methylation in the placenta. These studies have identified other unique placental functions which can impact various aspects of neurobehavioral development, including processes related to the transport of various signaling molecules and hormones and those related to various morphological or cellular developmental programs [24]. Such findings again highlight the unique functional role of the placenta during development and may point to novel molecular pathways that are suitable to new approaches and interventional strategies that can be used to reduce risk even during the earliest points of development. Out of course, further characterization of these pathways, including factors, which can impact their variation and their downstream function, are necessary before these novel findings can be translated into clinically relevant approaches in children.

The study of epigenetic processes and human behavior is in its embryonic stage. Nonetheless, this is an unprecedented opportunity to identify what used to be, latent molecular processes in placental genes that will herald neurodevelopmental trajectories of normal and abnormal child behavior and development and enable the initiation of preventive intervention programs.

Executive summary

- The role of the placenta in understanding the prenatal environment's effects on neurodevelopment.
- Fetal programming and the neuroendocrine system.

Overall description of the studies reviewed

- Characteristics of studies on epigenetic processes in placental genes and human infant neurobehavior.
- Findings of the studies on epigenetic processes in placental genes and human infant neurobehavior.

Discussion & implications of these studies

- Consistency of findings.
- Biobehavioral systems.
- Meaning of epigenetic effects.
- Importance of studying normal development.
- Implications for understanding long-term developmental outcome.
- Intervention.
- Rethinking the meaning of neurobehavior.

Future perspective

- Epigenetic effects on placental genes and infant neurobehavior.
- Developmental trajectories and child development.
- Limitations.
- Placenta and echoes of development.

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