Commentary

Epigenomics

Are epigenetic changes in the intrauterine environment related to newborn neurobehavior?

"...there is little doubt that epigenetic changes in the intrauterine environment are related to newborn neurobehavior."

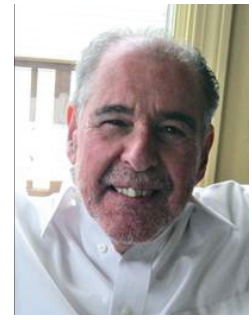
Keywords: human behavioral epigenetics • intrauterine environment • NNNS • newborn neurobehavior • placenta

Advances in the developmental origins of chronic diseases suggest that there are multiple environmental influences linked to normal variations in fetoplacental development [1]. The mechanism of this plasticity is thought to be through fetal programming involving epigenetic processes that alter gene expression and permanently set pathways linked to disease. A key question in the burgeoning field of human behavioral epigenetics is whether similar mechanisms can explain the development of behavioral 'diseases', in particular, mental health disorders [2]. However, the study of how variations in the intrauterine environment trigger epigenetic modifications that alter behavioral development is virtually uncharted territory.

A good place to start is the neurobehavior of the newborn infant. It is well known that the postnatal environment has a substantial influence on child development [3,4]. By focusing on *newborn* neurobehavioral phenotypes, we have an opportunity to hone in on the role of the prenatal period before postnatal environmental factors come into play. In addition, newborn neurobehavior may predict long-term developmental outcome, allowing the identification of individual infants most likely to suffer mental illness and the pursuit of interventions to prevent or ameliorate later deficits. Focusing on the epigenetics of newborn neurobehavior could provide the molecular context for individual differences in neurobehavior as well as an understanding of children's responses to the postnatal environment – that is, why some infants are more vulnerable or susceptible to poor developmental outcome than others. Newborn neurobehavior can be quantified with the NNNS (NICU Network Neurobehavioral Scale), a well-established evaluation that includes neurological and behavioral measures and indicators of stress [5] and has been shown to predict behavior problems, school readiness, and IQ through 4.5 years [6]. Here, we describe a series of studies relating epigenetic changes in placental genes and the NNNS in a sample of several hundred term, healthy infants who vary in physical growth, born to mothers with uncomplicated medical histories [7].

The placenta provides an ideal fetal record of the intrauterine environment and alterations to its function through epigenetic processes that could affect newborn neurobehavior [8]. The placenta modulates the fetal environment and 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 neurobehavior [9]. The neuroendocrine system, in particular, programming of the HPA axis is a good model for the exploration of epigenetic effects on placental genes and newborn neurobehavior. This is a well-studied system that includes an extensive literature on cortisol reactivity and the development of mental health disorders [10] and is known to be an active system within placental tissue [11].

In the placenta, *11β-HSD-2* is responsible for the inactivation of maternal cortisol. Increased DNA methylation of *11β-HSD-2*



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reduces expression, which increases cortisol levels that can be harmful to the fetus [12,13]. Findings from our first study [7] showed that increased placental DNA methylation of *11β-HSD-2* was related to a poorer quality of movement score on the NNNS, suggesting that increased exposure to cortisol *in utero* has a negative effect on the infant's motor control, smoothness of movements and motor maturity potentially through disruption of the HPA axis or other effects of excess cortisol.

Levels of fetal exposure to cortisol are also related to activity of the glucocorticoid receptor encoded by *NR3C1*, which facilitates cortisol's transcriptional activity including regulation of *11β-HSD-2.* DNA methylation of *NR3C1* is arguably the most studied epigenetic alteration related to human behavior but has not previously been studied in the placenta or in relation to newborn neurobehavior. Here we found that the extent of DNA methylation of the *NR3C1* promoter in the placenta was also related to the quality of movement as well as attention (ability to maintain alert states and track visual and auditory stimuli) scores on the NNNS [14]. There were also indications that infant genotype played a role in the effects of *NR3C1* methylation on attention suggesting that some infants may be more genetically susceptible to epigenetic alterations of the *NR3C1* gene that affect newborn attention than others.

Serotonin is of interest as it activates the HPA axis, can affect cortisol levels, and is involved in mood disorders. Examination of the DNA methylation status of the *HTR2A* promoter, regulating the serotonin transporter 2A, in the placenta showed higher methylation in males than in females [15]; perhaps because the rate of serotonin synthesis is higher in males than in females [16]. On the NNNS, increased DNA methylation was related to poorer quality of movement and better attention. However, better attention was found in males but not in females. Females' lower rates of serotonin synthesis and increased utilization of serotonin during stress [17] make them more prone to mood disorders, thus these sex differences in NNNS scores related to epigenetic mechanisms could be a consequence of differential responses to stress or epigenetic control of the serotonin system by sex.

Mood disorders were explored further by examining relations between DNA methylation of *NR3C1 and 11β-HSD-2* in mothers with depression or anxiety during pregnancy and newborn neurobehavior [18]. Infants of mothers with depression who also showed greater methylation of placental *NR3C1* had poorer self-regulation (able to modulate responsivity to stimulation), more hypotonia (low muscle tone), and more lethargy (low level of arousal) on the NNNS than infants of mothers without depression. Infants of mothers with anxiety who also showed greater methylation of placental *11β-HSD-2* were more hypotonic than infants of mothers without anxiety. Poor regulation, hypotonia and lethargy are 'depression-like' behaviors that we are seeing in newborn neurobehavior. Mood disorders in mothers can have long-term adverse effects on child mental health, especially when they continue post-partum [19]. Here we have an illustration of an important clinical condition that can trigger epigenetic changes that alter newborn neurobehavior and confer additional risk for the infant.

The peptide hormone, leptin, is involved in energy homeostasis through actions in the hypothalamus and is related to neuroendocrine function [20] and brain development [21,22]. Our leptin findings showed that, in males only, increases in DNA methylation of the leptin promoter (LEP) were related to lethargy, hypotonia and low excitability (delayed reactivity) on the NNNS, consistent with less energy expenditure that could impact regulation of the HPA axis [23] and mimicking the behavioral phenotypes observed in leptin-deficient rodent models [24]. DNA methylation of LEP in the placenta has also been related to sex differences [23] and now appears to be carried forward to sex differences in newborn neurobehavior.

DNA methylation is the most studied epigenetic process in human behavioral epigenetics; however, it goes without saying that other epigenetic processes also need to be studied. In a genomic imprinting analysis of gene expression in 22 imprinted placental genes [25] worse scores on quality of movement and handling (infants who require excessive handling because they are difficult to manage) on the NNNS were related to two gene classes. One class included MEG3, HOXA11 and HOXD10, genes involved in nervous system, skeletal and muscular development. The other class included CDKAL1, ILK, MEST and PHLDA2, genes involved in cell cycle control and cell growth. Here is (not surprising) evidence that newborn neurobehavior is related to coordinated epigenetic alterations in a number of genes as well to a single altered gene.

Epigenetic control of the placental genome can also involve microRNAs (miRNAs). In an analysis of the expression of six candidate miRNAs (*miR-16, miR-21, miR-93, miR-135b, miR-146a and miR-182*) we found that high *miR-16* expression was related to poorer attention, and high expression of *miR-146a* and *miR-182* were related to better quality of movement. Here we see additional evidence for the role of epigenetic regulation on newborn neurobehavior by showing 'downstream' post transcriptional effects related to miRNA expression. These findings could also be a harbinger for long-term consequences as miRNA expres-

sion in adults has been related to psychopathology [26]. miRNAs have been implicated as fundamental regulators of gene expression in major affective disorders and suicidal behavior [26].

With respect to the theme of this editorial, there is little doubt that epigenetic changes in the intrauterine environment are related to newborn neurobehavior. These effects were seen in a number of epigenetic processes including DNA methylation in candidate genes, imprinted gene expression and miRNA expression. Epigenetic programming is a normal developmental process and the epigenetic effects described here were from a normal population. The developmental origins concept avers that normal variations in development give rise to the later onset of chronic diseases [1]. This may be true for mental health disorders as well. Our findings suggest that epigenetic effects, probably related to fetal programming, explain some of the variability in newborn neurobehavior that accounts for individual differences in the susceptibility to the development of mental health disorders. The ability to understand the molecular basis for why some infants develop mental disorders and others do not could have far reaching implications for personalized medicine. Especially in cases in which a specific risk factor has been identified, such as mood disorders, epigenetic biomarkers may lead to the early identification of those most predisposed to these disorders and key to developing early and personalized preventive interventions.

This is exciting work although still in the fetal stage. Many issues need to be addressed that are beyond the scope of this editorial but can be briefly mentioned. Epigenetic signatures are not the same in all tissues as has been shown when cord blood, placenta and saliva are compared [27]. Our work is limited to placenta; other tissues might not show comparable findings but may still have relevance when interpreted in the context of tissue specificity. These are associational studies, which limits mechanistic interpretation. Our findings are based on normal variations in the same sample from a population that would be considered 'low risk', a population in which fetal programming of chronic disease is thought to be fixed [1]. It is unlikely that fetal programming of behavioral pathways is as fixed as pathways leading to chronic disease. We are also aware that the postnatal environment plays a major role in child outcome that requires continued plasticity but we don't know the role of epigenetics in these developmental processes. The kind of work that we have described needs to be conducted in populations known to be at high risk for compromised developmental outcome where fetal programming of behavioral pathways may be more fixed and follow-up studies are needed to determine the meaning of newborn neurobehavior across a spectrum of at risk populations.

Although we saw epigenetic effects on neurobehavior from candidate genes, we know that it is simplistic to think that any effect is due to a single gene or that genes act independently, and our examinations of imprinted genes suggest coordinated alterations are critical. Overlap in the neurobehavioral outcomes related to these candidate genes makes sense and suggests that alterations to various genes are affecting a central key process, in this case the HPA axis. On the other hand, the differences in neurobehavior related to these alterations probably reflect gene specific effects on neurobehavioral dimensions involving other processes and pathways. For example, the effects of a specific factor, especially one that might be genetically 'loaded' such as mood disorders [18], are probably different than the cumulative effects of a number of stressors [28]. The cumulative stress model that includes both prenatal and postnatal stressors has been linked to the later onset of chronic disease and mental health disorders as a result of "allostatic load," the wear and tear on the HPA system due to the accumulating effects of environmental adversity [29]. Determining how these genes act in concert and independently, the dynamics of how each alteration affects the regulation of each other and this complex interaction on neurobehavior are all critical next steps.

Finally, the issue of sex differences needs to be explored. Sex differences in this sample have been observed several times in relation to behavior [23], as well as other factors [28]. It is well known that externalizing problems (e.g. conduct disorders) are more common in males and internalizing problems (e.g. mood disorders) are more common in females [30]. Pursuit of this line of inquiry could lead to understanding the molecular basis for the later development of sex-related mental health disorders. In conclusion, this is a new field and the work that needs to be done is extensive. The caveats are real but, if we may be so bold, so are the findings.

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