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Examining the joint contribution of placental *NR3C1* and *HSD11B2* methylation for infant neurobehavior

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Summary Infant neurobehavior, a potential sentinel of future mental and behavioral morbidity characterized in part by reflex symmetry, excitability and habituation to stimuli, is influenced by aspects of the intrauterine environment partially through epigenetic alterations of genes involved in the stress response. DNA methylation of two related cortisol response genes, the glucocorticoid receptor (*NR3C1*), a nuclear receptor to which cortisol binds, and 11-beta hydroxysteroid dehydrogenase (*HSD11B2*), the enzyme responsible for conversion of cortisol into inactive cortisone, independently associate with infant neurobehavior. Although these factors are part of a common cortisol regulation pathway, the combined effect of DNA methylation of these factors on infant neurobehavior has not been characterized. Therefore, we conducted an examination of the joint contribution of *NR3C1* and *HSD11B2* DNA methylation on infant neurobehavior. Among 372 healthy term newborns, we tested the interaction between placental *NR3C1* and *HSD11B2* DNA methylation in association with neurobehavior as assessed with the validated NICU Network Neurobehavioral Scales. Controlling for confounders, interactions between DNA methylation of these genes were detected for distinct domains of neurobehavior (habituation, excitability, asymmetrical reflexes). Moreover, different patterns of DNA methylation across the cortisol regulation pathway associated with different neurobehavioral phenotypes. Those with low *NR3C1* methylation but high *HSD11B2* methylation had lower excitability scores; those with high *NR3C1* methylation but low *HSD11B2* methylation

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had more asymmetrical reflexes; those with high DNA methylation across the entire pathway had higher habituation scores. These results suggest that epigenetic alterations across the cortisol regulation pathway may contribute to different neurobehavioral phenotypes, likely though varying degrees of glucocorticoid exposure during gestation. While the postnatal environment may continue to affect neurobehavioral risk, this study provides novel insights into the molecular basis for fetal origins of mental conditions.

1. Introduction

In order to better understand disease etiology and identify novel avenues for prevention and intervention, examining the developmental origins of mental health conditions is of increasing interest (Schlotz and Phillips, 2009). Many mental and behavioral health conditions are now known to be attributable in part to an adverse intrauterine environment (Barker, 1998; Raikkonen et al., 2012; Sandman and Davis, 2012; Schlotz and Phillips, 2009). Neurobehavior assessed in infancy (characterized in part by reflex symmetry, excitability and habituation to stimuli) has been shown to be a sentinel of future mental, neurological and behavioral morbidity (Liu et al., 2010; Stephens et al., 2010; Tronick and Lester, 2013), while also sensitive to a range of deleterious prenatal exposures (Bagner et al., 2009; Coyle et al., 2005; de Moraes Barros et al., 2006, 2008a, 2008b; Law et al., 2003; Lester et al., 2002; Napiorkowski et al., 1996; Salisbury et al., 2005, 2007; Smith et al., 2008; Stroud et al., 2009; Tronick and Lester, 2013). While perturbations in the developing stress-response system (the hypothalamic-pituitary-adrenal axis (HPA)) have been implicated in the pathophysiology of mental illness, the molecular mechanisms linking HPA dysregulation to infant neurobehavior have not been fully elucidated (Lester et al., 2014; Monk et al., 2012). Therefore, we examined variability in infant neurobehavior according to placental epigenetic alterations of HPA-related genes in order to provide novel mechanistic insights into the developmental origins of mental disease.

Epigenetics involves mechanisms that control patterns of gene expression without modification of the underlying nucleotide sequence of DNA. Epigenetic mechanisms, such as DNA methylation, are particularly relevant for understanding the early origins of mental disease as they are sensitive to environmental exposures and can be altered during critical periods of development like gestation (Robins et al., 2011) and early childhood (Meaney and Szyf, 2005), although epigenetic alterations can be influenced by a range of environmental exposures occurring in adulthood as well (Madrigano et al., 2012). The fetal environment is regulated by the placenta, which plays an active immune-endocrine functional role in pregnancy, in addition to its role in nutrient, gas, and waste exchange (Marsit et al., 2012). The placenta is also involved in HPA development, including the development of the cortisol regulation pathway, through the activities of placentally expressed glucocorticoid receptor (NR3C1) and 11-β hydroxysteroid dehydrogenase type 2 (HSD11B2). NR3C1 is a nuclear receptor to which glucocorticoids like cortisol bind, and it facilitates cortisol's transcriptional activity, including regulation of *HSD11B2*. Placental HSD11B2 is responsible for converting cortisol into inactive cortisone, thereby protecting the developing fetus from overexposure to glucocorticoids during development. However, this protective mechanism has limits. If *NR3C1* is dysregulated potentially from significant prenatal stressors, the protective effect of placental HSD11B2 may be diminished, thereby allowing elevated levels of glucocorticoids into fetal circulation (Sarkar et al., 2001; Staud et al., 2006). Overexposure to glucocorticoids is associated with range of deleterious outcomes across the life course, including low birth weight, poor infant neurodevelopment, adult anxiety and cardiometabolic disorders (Cottrell and Seckl, 2009; Marsit et al., 2012; Wyrwoll et al., 2011).

There is emerging evidence to suggest that *NR3C1* and *HSD11B2* DNA methylation are each associated with infant neurobehavior. In previous work among 186 infants from the current sample, placental DNA methylation of *NR3C1* was marginally associated with infant neurobehavior in terms of quality of movement and attention regulation (Bromer et al., 2012); *HSD11B2* DNA methylation was associated with infant quality of movement and being born low birth weight (Marsit et al., 2012). Another study from this sample found greater *NR3C1* and *HSD11B2* placental methylation to be associated with worse neurobehavior among newborns whose mothers had either depression or anxiety during pregnancy (Conradt et al., 2013). These findings are congruent with work in other samples focused on stress-related gestational HPA programming that examined infant outcomes correlated with neurobehavior. One study of 82 infants found greater DNA methylation extent of cord blood *NR3C1* to predict dysregulated salivary cortisol response at 3 months (Oberlander et al., 2008), while another found that among 45 newborns studied, DNA methylation of placental *NR3C1* was associated with maternal smoking during pregnancy, and also with cortisol reactivity over the first month of life (Stroud et al., 2014). Another study of 25 infants whose mothers were exposed to high levels of stress (war trauma) during pregnancy found higher cord blood *NR3C1* DNA methylation to be associated with lower birth weight (Mulligan et al., 2012). Taken together, this emerging evidence suggests that gestational DNA methylation of *NR3C1* and *HSD11B2* is influenced by the intrauterine environment and their DNA methylation extent is associated with infant neurobehavior and related outcomes.

Although these factors are part of a common pathway, the neurobehavioral effects of *HSD11B2* and *NR3C1* DNA methylation have not been examined jointly. It is not known how neurobehavior may be affected if either or both of these gene promoters are simultaneously methylated.