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Sex-specific associations between placental leptin promoter DNA methylation and infant neurobehavior

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Summary
Background: Leptin (*LEP*) is a hormone central for energy homeostasis and has been implicated in neurodevelopment. This adipokine is produced by the placenta and is epigenetically regulated by promoter DNA methylation. Recent evidence has suggested a role for *LEP* in behavioral development. In this study, we investigated associations between profiles of human newborn neurobehavior and placental *LEP* DNA methylation.
Methods: We determined *LEP* promoter methylation in 444 placental samples from healthy term infants and measured *LEP* gene expression in a random subset of these samples. Infant neurobehavior was assessed with the NICU Network Neurobehavioral Scales (NNNS) and we examined the relationship between *LEP* promoter methylation and profiles of infant neurobehavior derived from these scores generated using a hierarchical model-based clustering method.
Results: *LEP* methylation is negatively correlated with gene expression only in placentas from male infants ($r = -0.6$, $P = 0.006$). A 10% increase in *LEP* DNA methylation was associated with membership in a profile of infant neurobehavior marked by increased lethargy and hypotonicity (OR = 1.9; 95% CI: 1.07—3.4), and consistently with reduced risk of membership in a profile characterized by decreased lethargy and hypotonicity (OR = 0.54; 95% CI: 0.3—0.94) only in male infants ($n = 223$). No statistically significant associations were observed amongst female infants.

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Discussion: These results suggest that increased placental *LEP* DNA methylation, related to reduced expression, may play a role in human newborn neurodevelopment, particularly in reactivity to various stimuli, but that these effects may be sexually dimorphic.

1. Introduction

The developmental origins of health and disease (DOHaD) hypothesis (Gluckman et al., 2005) postulates that environmental influences during intrauterine and early life can affect adult metabolic disease risk. Importantly, this concept has been extended to non-metabolic chronic diseases including mental health outcomes such as childhood cognitive and behavioral problems, personality disorders and schizophrenia (Lester et al., 2012; Schlotz and Phillips, 2009). The DOHaD paradigm entails the existence of early life plasticity that programs the organism to adapt to the intrauterine environment (Gluckman et al., 2005). Increasing evidence has suggested that epigenetic marks could mediate such plasticity (Low et al., 2012). Epigenetic modifications are heritable changes in gene expression without DNA sequence alterations; the principal mechanisms of epigenetic regulation are DNA methylation, histone modifications and noncoding RNAs (Bird, 2002). DNA methylation involves the addition of a methyl group to a cytosine within CpG dinucleotides, which usually occur in CpG islands in gene promoters and is frequently associated with gene silencing (Deaton and Bird, 2011). DNA methylation is particularly interesting in the context of fetal programming because these marks are reset during development and their reestablishment occurs in a tissue-specific fashion (Godfrey et al., 2007). Moreover, studies have shown that DNA methylation, although stable during adult life, can be altered by environmental cues (Christensen and Marsit, 2011; Jirtle and Skinner, 2007; Novakovic and Saffery, 2013). Hence, is plausible that some of the adaptive mechanisms involved in fetal programming are mediated through altered DNA methylation during intrauterine life. A common and critical feature of epigenetic regulation, including DNA methylation, is its tissue specificity, thus defining the appropriate tissue for examination of the role of DNA methylation in mediating the intrauterine environment's role in long-term child health is critical.

The placenta is the key regulator of the intrauterine environment mediating maternal-fetal interactions, such as nutrient and gas exchange and endocrine regulation. Maternal physiological or pathological signals are translated into the placenta and can affect fetal programming (Jansson and Powell, 2007). For instance, maternal insults such as infection and malnutrition increase placental pathology susceptibility like intrauterine growth retardation (IUGR), that itself is associated with psychopathologies such as schizophrenia and autism (Hsiao and Patterson, 2012) and other fetal outcomes. Hence, the placenta can serve as an ideal fetal record of intrauterine life, as well as a functional tissue in which to study how alterations in epigenetic regulation of key genes and pathways in this tissue impact fetal development and future child health (Maccani and Marsit, 2009; Novakovic and Saffery, 2012).

Leptin is a peptide hormone initially shown to be involved in energy homeostasis through actions in the hypothalamus, but has more recently been related to neuroendocrine, immune, and reproductive functions in normal and gravid physiology (Alexe et al., 2006). Leptin has also been implicated in fetal growth and development, including brain development (Bouret, 2010; Udagawa et al., 2007). Evidence from rodent studies have shown that leptin has an array of neurodevelopmental activities, that at the cellular level impact neurogenesis, axon growth, dendrite proliferation, and synapse formation and that environmental cues during development can alter these activities through alterations of leptin levels (Bouret, 2010). Functionally, leptin has been involved in energy homeostasis, motivation, learning and memory, cognition and neuroprotection (Morrison, 2009). During pregnancy, this peptide is produced by the placenta and by maternal and fetal adipose tissue (Moschos et al., 2002). In human placental tissue, both leptin and its receptor have been identified, and this adipokine has autocrine and paracrine functions involved in proliferation and survival of trophoblast cells (Maymo et al., 2011). DNA methylation of the leptin promoter (*LEP*) has been shown to regulate placental leptin gene expression and has been linked to pregnancy pathology (Bouchard et al., 2010). More recently, we observed differences in placental *LEP* by infant sex (Lesseur et al., 2013). In summary, leptin is an important placental signal, epigenetically regulated by DNA methylation that exhibits differences between male and female placentas, has been linked to brain development and may be related to newborn neurobehavior. Hence, in this study we aimed to explore: first, possible associations between placental *LEP* methylation and profiles of newborn neurobehavior, which we can assess using the NICU Network Neurobehavioral Scales (NNNS). And, secondly to examine if infant sex can modified these associations.

2. Methods

2.1. Study population

Study participants are part of the ongoing Rhode Island Child Health Study (RICHS), which enrolls mother-infant dyads following delivery at Women and Infants Hospital of Rhode Island. Term infants born small for gestational age (SGA, $<$10th percentile), or large for gestational age (LGA, $>$90th percentile), based on birth weight and gestational age calculated from the Fenton growth chart (Fenton, 2003), are selected; infants appropriate for gestational age (AGA, $\geq$10th percentile and $\leq$90th percentile) matched on gender, gestational age ($\pm$3 days), and maternal age ($\pm$2 years) are also enrolled. Only singleton, viable infants are eligible for the study. Other exclusion criteria include maternal age $<$18 years, maternal life-threatening medical complication, and infant congenital or chromosomal abnormalities.