Epigenetic Regulation of Placental *NR3C1*: Mechanism Underlying Prenatal Programming of Infant Stress Response by Maternal Smoking During Pregnancy?

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Background: Maternal smoking during pregnancy (MSDP) is associated with early and long-term neurobehavioral deficits; however mechanisms remain unknown. We tested the hypothesis that MSDP programs the hypothalamic pituitary adrenocortical (HPA) axis of the offspring via epigenetic mechanisms leading to adverse outcomes. In an intensive, prospective study, we investigated associations between MSDP and infant cortisol stress response and whether alterations in cortisol response were mediated by epigenetic modulation of the placental glucocorticoid receptor gene (*NR3C1)*. **Methods:** Participants were 100 healthy mother-infant pairs (53% MSDP-exposed; 42% female) from a low income, racially/ethnically diverse sample (55% minorities). MSDP was assessed by timeline followback interview verified by saliva and meconium cotinine. Infant cortisol responses to a neurobehavioral exam were assessed 7 times over the first postnatal month. Methylation of placental *NR3C1* promoter exon 1F was assessed using bisulfite pyrosequencing in a subsample (*n*=45). **Results:** MSDP-exposed infants showed significantly and persistently attenuated basal and reactive cortisol levels over the first postnatal month vs. unexposed infants. In addition, MSDP was associated with altered methylation of the placental *NR3C1* promoter; degree of methylation of the placental *NR3C1* was associated with infant basal and reactive cortisol over the first postnatal month and mediated effects of MSDP on infant basal cortisol. **Conclusions:** Results provide initial support for our hypothesis that MSDP programs offspring HPA (dys)regulation via epigenetic mechanisms. Epigenetic regulation of placental *NR3C1* may serve as a novel underlying mechanism. Results may have implications for delineating pathways to adverse outcomes from MSDP, and developing therapeutic targets to protect exposed offspring.

Keywords: Smoking, tobacco, pregnancy, infant, cortisol, stress, epigenetic, fetal, programming, methylation, glucocorticoid receptor (GR), placenta, *NR3C1*.