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Maternal prenatal stress is associated with the infant intestinal microbiota

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Summary Maternal prenatal stress has been often associated with infant physical development and health, as well as psychological functioning and behavior. However, the mechanisms underlying these relations remain elusive. The goal of the present study was to prospectively investigate the development of the intestinal microbiota as a potential pathway linking maternal prenatal stress and infant health. The development of the infant intestinal microbiota was followed over the first 110 days after birth in a healthy cohort of 56 vaginally born Dutch infants. Additionally, the relation between infant intestinal microbiota and gastrointestinal and allergic symptoms was examined. Results showed that maternal prenatal stress, i.e., either reported stress or elevated basal maternal salivary cortisol concentrations or both, was strongly and persistently associated with the infants' microbiota composition as determined by a phylogenetic microarray. Infants of mothers with high cumulative stress (i.e., high reported stress and high cortisol concentrations) during pregnancy had significantly higher relative abundances of Proteobacterial groups known to contain pathogens (related to *Escherichia*, *Serratia*, and *Enterobacter*), and lower relative abundances of lactic acid bacteria (i.e., *Lactobacillus*, *Lactoccus*, *Aerococcus*) and Bifidobacteria, altogether characteristics of a potentially increased level of inflammation. Furthermore, this aberrant colonization pattern was related to more maternally reported infant gastrointestinal symptoms and allergic reactions. In conclusion, clear links were found between maternal prenatal stress and the infant intestinal microbiota and health. Although causality cannot be concluded, the results suggest a possible mechanism by

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which maternal prenatal stress influences the offspring development. These results suggest a potential for bacterial interventions to enhance offspring health and development in pregnant women with stress.

1. Introduction

Although the underlying mechanisms remain unclear, an increasing number of studies link maternal prenatal stress to infant physical development and health, and psychological functioning and behavior. Stress during pregnancy predisposes to premature birth and low birth weight (Mulder et al., 2002; Beydoun and Saftlas, 2008), eczema (Sausenthaler et al., 2009), asthma (Cookson et al., 2009), and respiratory, general and skin illnesses (Beijers et al., 2010). Regarding psychological functioning and behavior, children of prenatally stressed mothers often show more impulsivity, anxiety problems, ADHD symptoms, and worse cognitive and psychomotor development (Beydoun and Saftlas, 2008). Recently, the development of the infant gut microbiota has been put forward as a possible factor underlying the links between maternal prenatal stress and infant development (Beijers et al., 2014). Rhesus monkey infants whose mothers had experienced stress during late pregnancy, in the form of repeated exposure to an acoustic startle, had lower levels of Bifidobacteria and Lactobacilli and more diarrheic symptoms than the infants of non-stressed mothers (Bailey et al., 2004). Also, in adult mice a social stressor (i.e., social disruption) provoked a decrease in the relative abundance of the genus *Bacteroides* together with an increase in the relative abundance of the genus *Clostridium* (Bailey et al., 2011). The goal of the present study is to investigate the relation between maternal prenatal stress (i.e., reported stress and cortisol concentrations) and the development of infant intestinal microbiota and health in the first 110 days of life in humans.

The intestinal microbiota are known to play an important role in the maturation of an infant's gastro-intestinal tract, immunity, metabolism, as well as the hypothalamic-pituitary-adrenal system (Sudo et al., 2004; Dimmitt et al., 2010; Bäckhed, 2011). An aberrant acquisition of intestinal bacteria or a reduced complexity of the microbiota may delay immune maturation or alter the development of the immune system and stress responses (Sudo et al., 2004; Adlerberth and Wold, 2009; Sekirov et al., 2010). Bacterial colonization of the infant gut is thought to begin in utero (Gosalbes et al., 2013), and to accelerate dramatically during and after delivery, and during the first months of life (Palmer et al., 2007; Fallani et al., 2010). Microbes from the mother and, to a lesser extent, of the environment are thought to be the first colonizers of the infant's gut (Tannock et al., 1990; Gosalbes et al., 2013). After the initial establishment of the intestinal microbiota during the first year of life, the microbiota begins to stabilize to a unique individual composition, continuing to develop gradually throughout childhood and adolescence. To what extent the early colonization dictates later development and finally the stable adult composition, is currently unknown. Due to the intimate interaction between the developing intestinal microbiota and the immune system, the early-life development of the intestinal microbiota may have long-lasting consequences (Bäckhed, 2011).

Distortions in the intestinal microbiota are associated with a wide range of diseases, including the risk of diarrheal illness, food allergy, inflammatory diseases (atopic diseases and inflammatory bowel disease), irritable bowel syndrome, obesity, and diabetes (Sekirov et al., 2010). Furthermore, as is the case with irritable bowel syndrome, gut-related diseases can develop or worsen during stressful periods (O'Mahony et al., 2009; De Palma et al., 2014). This may be due to the bidirectional communication between the central nervous system (CNS) and the gut (brain-gut axis; Dinan and Cryan, 2012), where both the autonomic nervous system (ANS) and the hypothalamic pituitary adrenal (HPA) axis play important roles (Rhee et al., 2009). When the HPA axis is activated in reaction to stress, cortisol is produced as an end product. In rats, experimentally increased cortisone levels in pregnant females resulted in lower levels of total bacteria and gram negatives in the intestine of the pups (Schiffrin et al., 1993). This suggests that cortisone may influence the maternal microbiota, and thereby the transmission of bacteria to offspring. In humans it is as yet unknown if maternal prenatal psychological stress and cortisol concentrations are related to the infant gut microbiota.

The goal of the present human study is to prospectively investigate the relation between maternal prenatal stress and the development of infant intestinal microbiota and health in the first 110 days of life. A limitation of the Rhesus monkey study of Bailey et al. (2004) is that the intestinal microbiota analyses were carried out with traditional culturing approaches and were not able to show the more complex microbiota signatures. The present study avoids this limitation by using a high-throughput phylogenetic microarray (Rajilic-Stojanovic et al., 2009).

2. Methods

2.1. Participants and procedure

This project is part of an ongoing longitudinal study in which 192 children are followed from the third trimester of pregnancy on. Infants were healthy, born at full term ($\geq$37 weeks) and had a 5-min APGAR score $\geq$7. Inclusion criteria were an uncomplicated, singleton pregnancy, clear understanding of the Dutch language, no drug use and no current physical health problems (see Beijers et al., 2010 or Tollenaar et al., 2011 for more details). All mothers gave written informed consent, and the study was approved by the Ethical Committee of the Faculty of Social Sciences, Radboud University Nijmegen (ECG/AvdK/07.563).

Fecal samples at 9 time points were available for investigating the development of the infant intestinal microbiota