Brain, Behavior, and Immunity xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Gut microbiome composition is associated with temperament during early childhood

Lisa M. Christian [a,b,c,d,*], Jeffrey D. Galley [b,e], Erinn M. Hade [f], Sarah Schoppe-Sullivan [g], Claire Kamp Dush [g], Michael T. Bailey [b,e]

[a] Department of Psychiatry, The Ohio State University Wexner Medical Center, United States
[b] The Institute for Behavioral Medicine Research, The Ohio State University Wexner Medical Center, United States
[c] Department of Obstetrics and Gynecology, The Ohio State University Wexner Medical Center, United States
[d] Department of Psychology, The Ohio State University, United States
[e] Division of Biosciences, The Ohio State University, United States
[f] Center for Biostatistics, College of Medicine, The Ohio State University, United States
[g] Department of Human Sciences, The Ohio State University, United States

ARTICLE INFO

Article history:
Received 22 August 2014
Received in revised form 30 October 2014
Accepted 31 October 2014
Available online xxxx

Keywords:
Gut microbiome
Stress
Gut–brain axis
Temperament
Childhood
Children
Human
Mood
Early life

ABSTRACT

Background: Understanding the dynamics of the gut–brain axis has clinical implications for physical and mental health conditions, including obesity and anxiety. As such disorders have early life antecedents, it is of value to determine if associations between the gut microbiome and behavior are present in early life in humans. *Methods:* We used next generation pyrosequencing to examine associations between the community structure of the gut microbiome and maternal ratings of child temperament in 77 children at 18–27 months of age. It was hypothesized that children would differ in their gut microbial structure, as indicated by measures of alpha and beta diversity, based on their temperamental characteristics. *Results:* Among both boys and girls, greater Surgency/Extraversion was associated greater phylogenetic diversity. In addition, among boys only, subscales loading on this composite scale were associated with differences in phylogenetic diversity, the Shannon Diversity index (SDI), beta diversity, and differences in abundances of *Dialister, Rikenellaceae, Ruminococcaceae,* and *Parabacteroides*. In girls only, higher Effortful Control was associated with a lower SDI score and differences in both beta diversity and *Rikenellaceae* were observed in relation to Fear. Some differences in dietary patterns were observed in relation to temperament, but these did not account for the observed differences in the microbiome. *Conclusions:* Differences in gut microbiome composition, including alpha diversity, beta diversity, and abundances of specific bacterial species, were observed in association with temperament in toddlers. This study was cross-sectional and observational and, therefore, does not permit determination of the causal direction of effects. However, if bidirectional brain–gut relationships are present in humans in early life, this may represent an opportunity for intervention relevant to physical as well as mental health disorders.

1. Introduction

Our bodies are colonized by trillions of bacteria known as the microbiome which reside in many niches of the human body including the gut, skin, vagina, and oral cavity. There are remarkable differences in microbial communities across individuals (Huttenhower et al., 2012). The role of the gut microbiome in health is rapidly gaining attention; overall bacterial diversity as well as specific bacterial abundances in the gut have been implicated in not only obesity, but also allergy, asthma, and inflammatory bowel disease among other conditions (Kinross et al., 2011). In addition to affecting physical health, a central role of the gut microbiome in regulating mood and behavior is emerging. via communication along the gut–brain axis, bacterial communities may affect both the hypothalamic–pituitary–adrenal (HPA) axis and central nervous system via cytokine and neurotransmitter production among other mediators (for review see Collins and Bercik, 2009; Forsythe et al., 2010; Foster and McVey Neufeld, 2013). Relatedly, there is interest in the possibility of intervening on the gut microbiome to affect mental health disorders (Dinan and Cryan, 2012; Foster and McVey Neufeld, 2013).

* Corresponding author at: Institute for Behavioral Medicine Research, Room 112, 460 Medical Center Drive, The Ohio State University Wexner Medical Center, Columbus, OH 43210, United States.
E-mail address: Lisa.Christian@osumc.edu (L.M. Christian).

http://dx.doi.org/10.1016/j.bbi.2014.10.018
0889-1591/

Conversely, a causal direction from behavior to gut is also now clearly established. Stressor-induced activation of the autonomic nervous system affects gastric acid, bile, and mucus secretion as well as gut motility (Beckh and Arnold, 1991; Shigeshiro et al., 2012; Soderholm and Perdue, 2001), all factors that impact gut microbes (Boesjes and Brufau, 2014; Drasar et al., 1969; Santos et al., 1999; Saunders et al., 2002; Sommer et al., 2014; Sommer and Backhed, 2013; Tache and Perdue, 2004). Moreover, *in vivo* and *in vitro* studies demonstrate that microbial composition can be altered through a direct recognition of stress hormones, including norepinephrine and epinephrine (Freestone et al., 1999, 2002; Lyte, 2004; Lyte and Bailey, 1997; Lyte et al., 2003, 2011).

Determining the dynamics of the behavior–gut associations in early life is important because many physical and mental health conditions (e.g., obesity, anxiety) have early life antecedents (Caspi et al., 1996; Parsons et al., 1999) and the gut microbiome may be more malleable in early versus later life (Clarke et al., 2013). Considerable changes in the structure of the gut microbiota occur during the first year of life in response to changing diet (i.e., introduction of solid foods) and environmental exposures (Dominguez-Bello et al., 2010; Favier et al., 2003). However, by approximately 2 years of age, profiles of gut microbiota resemble profiles found in adults (Koenig et al., 2011; Palmer et al., 2007). Once established, these profiles are relatively stable; although the gut microbiome changes in response to illness, diet, and exposures such as antibiotics, overall profiles and the majority of dominant microbes tend to revert back to the pre-exposure state after a given disruption has passed (David et al., 2014; De La Cochetiere et al., 2005; Dethlefsen et al., 2008). Thus, assessment of the gut microbiome as early as 2 years of age may provide insight as to long-term functioning.

In order to link gut microbiome composition to behavior in early life, behavior must be captured in a valid and relevant manner. Reflecting affective-motivational and attentional style, temperament is a central construct in behavioral measurement in early childhood. Parental as well as direct observational ratings of temperament in early childhood predict personality, behavior, and risk for psychopathology in later childhood, adolescence, and adulthood (Rothbart and Posner, 2006). In addition, temperament has been linked to differences in functioning of the HPA axis (Dougherty et al., 2013; Mackrell et al., 2014) as well as autonomic nervous system (Brooker and Buss, 2010; Huffman et al., 1998; Stifter and Fox, 1990), providing a plausible basis by which individual differences in temperament may be mechanistically linked to the gut microbiome.

In this study, we examined the association between the community structure of the gut microbiome, using next generation pyrosequencing, and maternal ratings of child temperament in 77 children assessed at approximately 2 years of age. In this exploratory investigation, we hypothesized that children would differ in their gut microbial structure, as indicated by diversity, richness, and evenness of communities, based on their temperamental characteristics. Consistent with the literature reviewed, we postulate direct physiological pathways linking temperament and gut microbiome composition. However, the role of diet must also be considered, as diet appreciably affects gut microbiome composition (David et al., 2014; Wu et al., 2011). Thus, we examined dietary patterns in relation to temperament and the gut microbiome in this cohort.

2. Methods

2.1. Study design

This study included 79 mother–toddler pairs. Mothers of toddler–aged children were recruited from the general community of Columbus, Ohio. Children were excluded if their mother reported the child had a major health condition or developmental delay. Children were also excluded if they were already toilet trained, as this hindered collection of stool samples. Each mother completed an online questionnaire that included assessment of her child's temperament and feeding behaviors, as detailed below.

Stool samples were collected by the mother from the child within 7 days of questionnaire completion by the mother, as per the protocol detailed below. A final sample of 77 mother–toddler pairs were used after removing two samples due to low sequence count (<5108). This study was approved by the Ohio State University Biomedical Institutional Review Board. All women completed written informed consent for themselves and provided written consent on behalf of their children. Women received modest compensation for their participation. Data collection occurred from May 2011 to December 2012.

2.2. Demographic characteristics and child diet

Women provided their age, race (self and child's father), marital status, and child's sex. Women also reported the occurrence and duration of breastfeeding and the age at which formula (if applicable), cereals/grains, fruits/vegetables, and meats were introduced as part of the child's diet. The current frequency of each food type was also reported, from less than once per month to two or more times per day.

2.3. Child temperament

Temperament was assessed with the **Early Childhood Behavior Questionnaire (ECBQ)**, a widely used and well-validated instrument appropriate for children 18–36 months. This is a finely differentiated measure providing 18 dimensions of temperament that load onto three composite scales: Negative Affectivity, Surgency/Extraversion, and Effortful Control (Putnam et al., 2006). Subscales are detailed and defined in Table 1.

2.4. Stool sample collection and storage

Stool samples were used for analysis of the child gut microbiome in lieu of tissue collection due to the advantages of non-invasive collection and the common use of stool in human microbiome analysis (Qin et al., 2014; Raman et al., 2013; Stiverson et al., 2014; Xiao et al., 2014). Women were provided with sterile wooden applicators and 50-ml plastic conical collection tubes for collection. The stool was sterilely collected from the child's soiled diaper with the wooden applicator and placed in the collection tube. Samples were stored at 4 °C (i.e., refrigerated) for up to 24 h until collection by study personnel from the participant's home or delivery by the participant to the Ohio State University Wexner Medical Center (OSUWMC). In the latter case, women were instructed to transport samples in a cooler with ice. While at OSUWMC, samples were stored at −80 °C until pyrosequencing was conducted.

2.5. bTEFAP

Bacterial tag-encoded FLX-Amplicon Pyrosequencing (bTEFAP) was performed as previously described (Dowd et al., 2008a,b). The 16s RRNA universal primers 27f (AGA GTT TGA TCM TGG CTC AG) and 519r (GWATTACCGCGGCKGCTG) were used in a single-step 30 cycle PCR with the following thermoprofile: a single cycle of 94 °C for 3 min, then 28 cycles of: 30 s at 94 °C; 40 s at 53 °C, 1 min at 72 °C, with a single 5 min cycle at 72 °C for 5 min for elongation. Amplicons were pooled at equivalent concentrations and purified (Agencourt Bioscience Corporation, MA, USA). Sequencing

Please cite this article in press as: Christian, L.M., et al. Gut microbiome composition is associated with temperament during early childhood. Brain Behav. Immun. (2014), http://dx.doi.org/10.1016/j.bbi.2014.10.018