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Associations Between Gut Microbes and Social Behavior in Healthy 2-Year-Old Children

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Abstract

Objective——Emerging research has connected abundances of specific bacteria to differences in psychosocial behaviors in animals and adult humans. However, research assessing mind-microbiome associations in children is sparse with extant work primarily focused on populations with autism; making it unclear whether links are also present in typically-developing children. The current study fills this gap by examining associations between prosocial-self-regulating temperaments (Effortful Control; EC) and the gut microbiome in typically developing children.

Methods——Maternal ratings of temperament were assessed in 77 toddlers, 18–27 months of age (46.7% female, M_{age}= 23.14 months). Next generation pyrosequencing of the V1-V3 region of the 16S rRNA gene was used to classify children's gut microbial composition from fecal samples. EC included the sub-categories; Cuddliness, Attentional Focusing, Attentional Shifting, Inhibitory Control, and Low Intensity Pleasure.

Results——After adjusting for covariates, EC was positively associated with relative abundances of *Akkermansia* $\Delta R^2 = 0.117$, $b = .022$, $SE = .007$, $p = 0.002$., with Cuddliness (i.e., joy and ease of being held) driving the relation. Further, Attentional Focusing was negatively associated with *Alistipes* $\Delta R^2 = 0.062$, $b = -0.011$, $SE = .005$, $p = 0.028$. Permutational analysis of variance revealed no significant differences in community structure between high and low EC groups on the phylum level ($R^2 = 0.00372$, $p = 0.745$) or the genus level ($R^2 = 0.01559$, $p = 0.276$).

Conclusions——Findings suggest that certain microbes may be linked to prosocial behaviors used to regulate emotion in typically-developing children. Further research is needed to test whether these observations replicate in larger samples.

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Conflicts of Interest

We have no conflict of to disclose.

Keywords

human microbiome; effortful control; prosocial behavior; microbial composition; preschool children

Trillions of diverse microorganisms inhabit the human gut to form personalized microbial ecosystems. The gut microbiome is composed of bacteria, archaea, and eukaryotes and includes both pathogenic and symbiotic microbes that influence health outcomes (1). Interestingly, emerging evidence has revealed that bidirectional communication is taking place between the mind and the gut microbiome (2) as shown in numerous animal studies (3). Similarly, research on adults has echoed these connections and linked gut microbial composition to psychological traits and processes like the stress response, mood disorders (3,4), and to neurological conditions such as Parkinson's disease (5). Importantly, recent work has also indicated that this may start at a young age, with important implications for both emotional and social functioning. However, childhood mind-microbiome literature is sparse and has primarily focused on patients with autism spectrum disorder (ASD), a condition characterized by atypical emotional, attentional, and social behaviors (6,7). Thus, little is known as to whether the microbiome is linked to adaptive psychological constructs in typically developing children. The current study will contribute to this gap in the literature and explore whether certain microbes are connected to prosocial behaviors and constructs in healthy toddlers.

Given the robust body of research examining bidirectional mind-microbiome relations in animals, we first explore this more established research to inform the current study. Early work indicates that social factors can shape microbial composition. For instance, symbiotic bacteria (i.e., *Lactobacillus*) in infant rhesus monkeys significantly decreased following maternal separation (8). Further, rodents placed with an aggressive cage mate resulted in decreased levels of the symbiotic bacteria *Bacteroides* and increased levels of the pathogenic bacteria *Clostridium* (9). However, numerous rodent studies have also shown that the gut microbiome regulates social behaviors (3). For instance, both rats and mice reared in the absence of microbial colonization (germ-free; GF) showed decreased social approach behaviors when introduced to novel rodents compared to conventionally colonized controls (10,11). Similarly, GF mice chose to spend more time in a solitary chamber over a social chamber and also showed abnormally low interest in unfamiliar mice compared to controls. Interestingly, microbial reconstitution helped to reverse some of the social deficits observed (12), providing preliminary evidence that certain social behaviors are linked to the gut microbiome. These studies point to the interesting possibility that microbial transmission is fostered by host sociality. Thus, some microbes may have evolved to promote positive social behaviors, for example, by decreasing anxiety (13) and encouraging prosocial behaviors such as the likelihood of exploration, interactions with novel individuals, and general increases in socialization (12).

Adult human studies complement the animal research by connecting microbial composition to psychological domains directly related to social behavior including stress responses, anxiety, and depression. For instance, a meta-analysis of seven human studies showed

improvement in depression, anxiety, and perceived stress in participants who ingested a probiotic compared to those given a placebo (14). Similarly, depression is positively linked to naturally occurring levels of *Bacteroidales*, *Lachnospiraceae,* and *Alistipes* (15). Research has also found greater microbial diversity in those with larger social networks, and lower diversity in those with high anxiety and stress (typically tied to reduced sociability) (16). Intriguingly the same pattern is found in married individuals, especially those with close relationships, who harbor microbial communities of greater diversity relative to those living alone (17).

Although sparse, research assessing mind-microbiome relations in healthy children is promising. For example, one study revealed links between happiness and microbial composition in a sample of typically developing children (18). Further, a study of 51 12-month-olds in China revealed associations between temperament and gut microbiota. Specifically, *Hungatella* (a member of the Clostridiaceae family) was negatively correlated with cuddliness while the ability to be easily soothed was positively correlated with *Bifidobacterium* (19). Another study of healthy toddlers revealed a connection between extraverted temperaments and greater microbial diversity. This study also showed that, in girls only, higher EC is tied to bacterial diversity within samples (20). Together, this growing body of research suggests that certain features of socially adaptive psychological constructs, including temperament, are bidirectionally associated with microbial composition. Further, there is a related body of work indicating that numerous environmental factors overlap to shape the microbiome from birth. From infancy to childhood, the composition of the gut microbiome becomes more complex and diverse and these changes coincide with dietary changes such as weaning and the introduction of solid foods (21). Other factors known to shape the gut microbiome in toddlers are mode of delivery (vaginal versus cesarean section) (22), sex (23), body composition (24), and breast-feeding duration (15).

While there is little work on social-microbiome connections in typically-developing children, we can draw from the more developed child ASD literature to identify autism linked microbes and assess whether these bacteria are *also related to* emotion regulation and social behaviors among a group of typically developing children. Given evidence that social deficits characteristic of ASD are continuously distributed across the population (25,26), gut microbiome composition may also be related to variation in sociability in the general population. This body of work points to a number of bacterial candidates. For example, young ASD patients have lower relative abundances of the bacterium *Dialister* (27), *Akkermansia* (28–30) and *Parabacteroides* (31) and higher relative abundances of *Lachnospiraceae* (32), and *Ruminococcus* (31,33) relative to healthy controls. Other bacteria frequently associated with ASD include *Bacteroides, Prevotella*, *Escherichia/Shigella*, and *Alistipes* (25,26). Specifically, research reveals increased levels of certain strains of *Bacteroides* in those with autism while other research indicates no difference compared to healthy controls (25,27).Similarly, some studies found elevated levels of *Prevotella* (29,30), *Escherichia/Shigella* (34), and *Alistipes* (29), while other studies revealed decreased levels of *Prevotella* (35), *Escherichia/Shigella* (30), and *Alistipes* (34) when compared to controls. Taken together, these mixed findings suggest that certain bacteria are symbiotic with the host within optimal parameters, that is, not excessively high or low in relative abundance compared to typically developing individuals.

Indeed, early childhood is a crucial time in which the microbiome becomes established and this coincides with a child's emerging temperament, making it a pivotal period in which to examine mind-microbiome connections. In the first years of life, researchers assess adaptive social temperaments via a child's ability to modify attention, emotion, and behavior (36). One example is the Effortful Control (EC) component of temperament which is composed of sub-categories assessing factors like child Cuddliness (i.e., joy and ease of being held) and Attentional Focusing (i.e., ability to hold focus and resist distraction) (37). The Cuddliness sub-category of EC is especially interesting in this context since it is the only EC category that effectively captures the features of many atypical socioemotional disorders (i.e., the ability/inability to engage in prolonged physical touch to emotion-regulate, presence/absence of prosocial behavior).

The current work uses data from a past study (20) assessing the relations between temperament and microbial composition in healthy toddlers. We build upon this research to examine whether microbiome characteristics typically tied to ASD related behavior are associated with emotion regulation and social behavior in a sample of typically developing children. Bacteria assessed include *Bacteroides*, *Prevotella*, *Ruminococcaeceae, Akkermansia* (38), *Escherichia/Shigella, Alistepes*, *Parabacteroides*, *Lachnospiraceae*, and *Dialister* (39) with a particular interest in the EC prosocial subcategory: Cuddliness. We predict that higher levels of *Akkermansia*, *Dialister* and *Parabacteroides* and lower levels of *Lachnospiraceae*, and *Ruminococcus* will be linked to higher levels of EC. In light of the mixed literature regarding *Bacteroides, Prevotella*, *Escherichia/Shigella*, and *Alistipes,* we test these associations in an exploratory manner.

Method

Participants

Participants included 79 mother-child dyads recruited from the general community of Columbus, Ohio. This sample size was based on a power analysis with power set at 0.95 and effect size set at 0.2. Participants were excluded if the parent reported that the child had a developmental delay, a major health condition, or if the child was already toilet-trained (due to sampling requirements). A more detailed report of recruitment of this sample is described by Christian and colleagues (20). Two microbial samples were removed due to low sequence count (< 5108) leaving a final sample of 77 toddlers for analyses (41 boys and 36 girls; *M* age = 23.14 months, *SD* = 2.00). Mothers were 87.0% White (*n* = 67), 9.1% Black (*n* = 7), and 3.9% Asian (*n* = 3). Mother's self-reported annual household income in U.S. dollars on a scale of 1–6 with 1 representing less than $15,000 per year and 6 representing $100,000 or above. The average annual household income was $50,000-$74,999, coded as 4 ($M_{=}$ 4.04, *SD* = 1.62). Parents education level was coded on a scale from 1–7 (less than a 7th grade education, junior high, some high school, high school graduate, some college, college graduate, some graduate school or higher). Mother's average education level was 'College graduate', coded as a 6 (*M* = 6.09, *SD* = .98). Father's average education level was 'Some college', coded as a 5 (*M* = 5.65, *SD* = 1.17). Mean maternal age at the time of delivery was 31.1 years (*SD*=5.43) and 87.0% of mothers (*n* = 67) were married. This study was approved by the Ohio State University Biomedical Institutional Review Board.

Mothers provided informed written consent on behalf of themselves and their children and were compensated for participation. Data were collected between May 2011 to December 2012.

Microbial Assessment

16S rRNA gene sequencing data, previously published by Christian and colleagues (20) were probed in the current study. Phylum and genus relative abundances are provided in Tables S1 and S2 (Supplemental Digital Content), and from these, a total of nine bacterial genera were selected for analysis based on previous literature commonly linking each genus with ASD. ASD-linked genera include: *Bacteroides*, *Prevotella*, *Ruminococcaeceae, Akkermansia* (38), *Escherichia/Shigella, Alistepes*, *Parabacteroides*, *Lachnospiraceae*, and *Dialister* (39). The Benjamini-Hochberg (B-H) method (40) was used to control for the false discovery rate. False discovery rates (q-values) in genome and metagenome (microbiome) research vary and include q-values such as .15 (41). In the current study we considered a p-value < .05 and a q-value < .15 significant. Preliminary analyses revealed that *Akkermansia, Dialister*, and *Alistipes* were the only select bacterial genera significantly associated with EC and EC sub-categories, thus, these 3 genera are the primary focus of the current study.

Procedure

After providing consent, mothers completed online questionnaires describing the child's diet, body composition, duration of breast-feeding, demographic characteristics and child temperament. Stool samples were collected from the toddler by the mother within 7 days of completing questionnaires.

Collection and Storage of Stool Samples—Toddler stool samples were collected by mothers from children's diapers using sterile wooden applicators and 50-milliliter plastic conical collection tubes. Samples were refrigerated at approximately 4°C for up to 24 hours until delivery by research personnel or the participant to the Ohio State University Wexner Medical Center (OSUWMC). Samples were transported in coolers with ice at which time samples were stored at −80°C until pyrosequencing was conducted.

Measures

Effortful Control—Effortful Control (EC) was measured with a composite of five temperament subscales assessed by the short form of the Early Childhood Behavior Questionnaire (ECBQ) (37). Mothers were asked to report the number of times in the last two weeks the child exhibited behavioral indicators of EC. Each subscale of EC consisted of 6 – 8 items and items were rated on a 7-point Likert scale (never, very rarely, less than half the time, half the time, more than half the time, almost always, always). Subscales included: Attentional Focusing; the toddler's ability to resist distraction and sustain focus on the object of concentration, Attentional Shifting; the ability to redirect focus from one target to another, Cuddliness; the degree to which the child expresses joy in and molds to the body of the caregiver when being held, Inhibitory Control; the ability to change behavior when instructed, Low Intensity Pleasure; the level of pleasure or satisfaction derived from low stimulus activities that involve novelty, complexity or incongruity. We examined each subscale for analyses as well as the composite score of all subscales to calculate overall EC.

Specifically, associations between the 7-point Likert scale ratings for each subscale and the composite score of EC (composite scores ranged from 3.74–5.98) were assessed in relation to relative abundances of select bacteria.

Body Composition and Delivery Mode—Delivery mode (1 = vaginal; 2 = cesarean section) and the child's sex (1 = boys; 2 = girls) were reported by mothers in the online questionnaire and included as covariates in analytic models. Additionally, mothers reported the child's height and weight percentile from the most recent well-child visit to the pediatrician to assess body composition based on weight-to-height ratio. Weight-to-height ratios were converted to decimal numbers (see Table 1) for statistical analyses.

Child Diet—Mothers completed an online questionnaire reporting the age in months in which fruits/vegetables, cereals/grains, and meats were introduced to the toddlers' diet and the frequency in which each food type was consumed. For analyses, food frequency item ratings were included as covariates. Specifically, mothers reported fruit/vegetable and meat consumption on a scale from 1–8 (less than once per month, once a month, once every two weeks, once a week, twice a week, every other day, once a day, two or more times per day). Mothers also reported on the occurrence and duration of breastfeeding (age in months in which breastfeeding stopped); duration was also included in analyses.

bTEFAP

Bacterial tag-encoded FLX-Amplicon Pyrosequencing (bTEFAP) was performed (42,43). The 16s rrn 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 minutes, then 28 cycles of: 30 seconds at 94°C ; 40 seconds at 53°C, 1 minute at 72°C, with a single 5 minute cycle at 72°C for 5 minutes for elongation. Amplicons were pooled at equivalent concentrations and purified (Agencourt Bioscience Corporation, MA, USA). Sequencing was performed with the Roche 454 FLX Titanium system using manufacturer's guidelines.

Sequencing Analysis

The software package, Quantitative Insights Into Microbial Ecology (QIIME), v.1.8.0. (44) was used for filtering and analysis of attained sequences. Since the current study builds upon previous analyses that used QIIME v.1.8.0 we continued with the use of this version so results were comparable. Quality filtering and demultiplexing were performed using the provided sequence file (.fasta) and sequence quality file (.qual). Filtering was completed with the following parameters: quality score > 25, sequence length between 200bp-1000bp, 6 allowed ambiguous bases, maximum of 6 homopolymer run, and zero allowed primer mismatches. On average, 14862 sequences passed filtering per sample.

UClust (45) clustered sequences at 0.97 similarity into operational taxonomic units (OTUs). After representative sequence selection for each OTU, Greengenes v.13_8 was used for taxonomic assignment (46). PyNAST was used for sequence alignment (44) with the Greengenes core reference alignment database (47). Sequences from boys and girls were filtered and de-multiplexed using the above method together, but were separated

before OTU-picking. Next generation 454 pyrosequencing was used to identify bacterial communities. This approach was chosen due to its low error rate and ability to classify microbes at lower taxonomic levels. Relative abundances of phylum and genera were used to assess relations between EC, community structure and select bacterial genera.

Analytical Approach

Alpha diversity was measured with the Shannon Diversity Index (SDI) (48), which assesses bacterial abundance (richness) and equalness of these abundances (evenness) using QIIME. Beta diversity analyses were conducted in R statistical software using the adonis function in the vegan package to generate Bray-Curtis matrices (49) and perform permutational analysis of variance (PERMANOVA) and analysis of similarities (ANOSIM; uses the *R* statistic to compare means of groups that use rank variables) between high and low EC groups. Differences in bacterial relative abundances derived from QIIME were assessed using version 25 of SPSS. If participants were missing data for any of the variables used in our analyses, their data were excluded. Male and female participants were very similar, with the exception of *Akkermansia* levels (Table 1). We first completed preliminary correlational analyses to test whether abundances of select genera were associated with EC, as well as the subscales of EC. Pearson's Correlation Coefficients are reported in Table 2. Independent samples *t*-tests revealed that there were no significant sex differences in levels of Cuddliness ($p = 0.27$) or Attentional Focusing ($p = 0.56$). Descriptive statistics for key variables, overall and by child sex are reported in Table 1.

Preliminary analyses revealed associations between select bacterial genera and overall EC. To probe these findings further, we conducted a series of hierarchical regressions on the genera that correlated with EC and EC subscales while adjusting for sex, diet (frequency of fruit/vegetable and meat consumption), body composition (weight to height ratio), and breast-feeding duration (age in months in which breast-feeding stopped) to assess the relations between each subscale. Covariates were chosen based on prior research suggesting that sex (23), delivery mode (22), diet (50), body composition (24), and breast-feeding duration (51) influence gut microbial profiles. Although breast-feeding is linked to compositional changes in the microbiome (51), the mean age of the current sample is 23.14 months and microbial profiles resemble those found in adults by approximately two (52,53) to three years of age (54). Indeed, progression from infancy to childhood includes dietary changes such as weaning and an increase in solid food consumption both of which impact microbial composition (21). Since our sample falls within this nutritional and developmental transition, we conduct each analysis twice; once adjusting for breast-feeding duration and once excluding it from the statistical model. We report the adjusted R^2 and p-values in step 1 of each model and the R^2 change, unstandardized beta coefficient, standard error, and p-values in step 2 of each model.

Results

To determine whether EC was related to overall measures of microbial community composition, we conducted regression analyses between EC and diversity measures. Regression analyses revealed no significant association between levels of EC and alpha

diversity (r =.123, p =.293). Additionally, PERMANOVA analyses of Bray-Curtis (49) dissimilarities (used to quantify variation in genus and phylum between samples) revealed that there were no significant differences in community structure between high and low EC groups on the phylum level ($R^2 = 0.00372$, $p = 0.745$; see figure 1) or on the genus level ($R^2=0.01559$, $p=0.276$; see figure 2). ANOSIM analyses of Bray-Curtis (49) dissimilarities were also conducted. ANOSIM's R statistic measures dissimilarities between groups for rank variables (i.e., relative abundances) by comparing within a between group differences (55). Results of the ANOSIM revealed no significant differences between high and low EC groups on the phylum ($R = -0.0002$, $p = 0.545$) or on the genus level ($R = -0.012$, $p = 0.818$). Relative abundance data for phylum and genera are reported in Supplemental Tables S1 and S2.

Despite the lack of relationships between EC and measures of microbial diversity, EC was related to the relative abundances of 3 predicted bacterial taxa. Based on our a priori hypotheses, zero-order correlations among Effortful Control and select bacterial taxa (presented in Table 2) revealed that Effortful Control was significantly positively intercorrelated with *Akkermansia* $r(75) = 0.25$, $p = 0.027$ and *Dialister* $r(75) = .24$, $p = 0.034$ and negatively correlated with *Alistipes* $r(75) = -0.24$, $p = 0.035$. Follow up analyses of Effortful Control's five subscales revealed that significant findings were driven by Cuddliness and Attentional Focusing. Specifically, Cuddliness was positively associated with *Akkermansia* $r(75) = 0.36$, $p = 0.001$, and *Dialister* $r(75) = 0.23$, $p = 0.049$. Additionally, Attentional Focusing was negatively associated with *Alistepes* $r(75) = -0.27$, $p = 0.017$.

Association between Cuddliness and *Akkermansia*.

After adjusting for sex, delivery route, diet, body composition and breast-feeding duration (adjusted $R^2 = -0.024$, $p = 0.646$), Cuddliness was positively associated with higher levels of *Akkermansia*, $\Delta R^2 = 0.116$, $b = .022$, $SE = .007$, $p = 0.003$. Next, we adjusted for sex, delivery route, diet, body composition but excluded breast-feeding duration from the model (adjusted $R^2 = -0.014$, $p = 0.558$). Cuddliness remained positively associated with higher levels of *Akkermansia*, $\Delta R^2 = 0.117$, $b = .022$, $SE = .007$, $p = 0.002$.

Association between Cuddliness and *Dialister*.

We then probed the relation between Cuddliness and *Dialister* while adjusting for sex, delivery route, diet, body composition and breast-feeding duration (adjusted $R^2 = -0.75$, $p = 0.994$). Cuddliness was no longer associated with higher levels of *Dialister* in step 2 of the model, $\Delta R^2 = 0.053$, $b = 0.036$, $SE = .018$, $p = 0.052$. Next, we conducted the analysis again using the same covariates but excluded breast-feeding duration (adjusted $R^2 = -0.065$, $p = 0.997$). Cuddliness was still not significantly associated with *Dialister* in step 2 of the model, $\Delta R^2 = 0.052$, $b = 0.035$, $SE = 0.018$, $p = 0.054$ at an alpha level of .05.

Association between Attentional Focusing and *Alistipes*.

Finally, we conducted another series of hierarchical regressions to examine the association between Attentional Focusing and the relative abundance of *Alistipes*. Results of the analyses revealed that after accounting for covariates (covariate adjusted $R^2 = -0.004$, $p =$

0.463), Attentional Focusing was negatively associated with levels of *Alistipes*, $\Delta R^2 = 0.061$, $b = -0.011$, $SE = .005$, $p = 0.031$. These results remained significant after, excluding breast-feeding duration from the model (covariate adjusted $R^2 = 0.005$, $p = 0.377$). Attentional Focusing was again negatively associated with levels of *Alistipes*, $\Delta R^2 = 0.062$, $b = -0.011$, $SE = .005$, $p = 0.028$.

Discussion

The current study showed that *Akkermansia* and *Dialister* were positively associated with EC and Cuddliness. Additionally, *Alistipes* was negatively related to Attentional Focusing. These findings complement past mind-microbiome research (27,28) and indicate that the microbiome is connected to social behaviors in a healthy, young, toddler sample. One possible explanation for links between *Akkermansia*, EC, and Cuddliness may be the bacterium's role in stimulating the production of mucous that lines the gut (56). Evidence suggests that low levels of *Akkermansia* are associated with compromised mucous barrier function resulting in the displacement of gut microbiota and/or their metabolic products into the serum (i.e., leaky gut) and heightened inflammation (28). This is thought to be a mechanism connecting the microbiome to mood and neurological disorders (57). Additionally, the current study also showed positive associations between *Dialister* and Cuddliness which supports our hypotheses and is in line with past work linking lower levels of *Dialister* to depression, anxiety (58) and ASD (34); three conditions commonly marked by social deficits. Interestingly, Cuddliness is the only sub-category of EC that exclusively uses prosocial behavior as a means by which children regulate emotion. Thus, one possibility for these mind-microbiome associations is that *Akkermansia* and *Dialister* promote social behaviors in humans, however, future experimental and longitudinal research is needed to test this suggestion. Finally, given the connection between *Alistipes* in tryptophan metabolism (a precursor of serotonin) (59), there is a mechanistic reason to support this bacterial group playing a role in mood-related functioning which is also supported by past work connecting *Alistipes* levels to ASD symptoms (29).

Contrary to our hypothesis, the remaining six bacterial taxa were not related to adaptive host behaviors in this sample. It is possible that, of the select nine bacteria that we chose to test, the non-significant six only influence psychological processes on the disease-disorder end of the continuum. Additionally, much of the research on ASD-microbiome links are conducted among a wide age range of youth, ages 2–18 years. It could be that the inverse associations we predicted would not be evident until later into childhood or adolescence.

This study is among the first to examine whether there is a prosocial, self-regulating counterpart to ASD behavior-microbiome associations in typically developing toddlers. Existing research examining mind-microbiome relations is primarily geared to investigate pathology. While understanding pathological associations is vitally important, the subsequent interventions or therapies developed would only be designed to treat those exhibiting symptoms at a diagnosable severity. Clearly, the majority of the population is not diagnosed with ASD, yet, many typically developing individuals also experience some level of suffering linked to problems with self-regulation and prosocial behaviors. The current study may guide the development of evidence-based interventions that improves EC

for both typical and atypical children alike. That is, future researchers could build upon this work to discover whether it is possible to calibrate the gut microbiome to promote emotion regulation and sociality; two features of wellbeing that affect the quality of life across the lifespan, regardless of any diagnoses. If studies in larger cohorts replicate these findings, possible interventions could include the use of a probiotic containing *Akkermansia* or dietary interventions that promote the growth of *Akkermansia*. This work may also help to set parameters of what constitutes an "optimal range" of *Akkermansia* and *Alistipes* since research has still not identified what defines excessively high versus low relative abundances of these bacterium in relation to wellbeing.

Limitations.

The current study has many strengths; however, it is important to note that this work is limited by cross-sectional design, self-reporting of predictor variables, and a lack of ethnic diversity. Further, the frequency of physical contact with parents, siblings, other children, and microbial composition of the home environment were not measured but may have influenced results. Additionally, the current study used multiple comparison procedures to minimize type 1 error, however, replication work in larger cohorts is needed to undergird these findings. Finally, while research on the relations between a single bacterial taxa and psychological processes has revealed important mind-gut connections, future researchers should also consider how these associations may vary in the presence and abundance of other microbes. Thus, we underscore the need for future research examining relations between EC and microbial composition with the aforementioned factors considered.

Emerging findings linking gut microbes to social processes in humans open numerous avenues for further inquiry. Future work should further consider possible origins of bacterial population differences (e.g., hygiene hypothesis, overexposure to antibiotics at an early age, microbial composition of the home/family environment) and how this translates into later differences in social processes. The gut microbiome has been well-described as an ecosystem and as such, the stability of this ecosystem may be influenced by the presence or abundance of cohabitating organisms, an individual's gene expression, and the environment in which the host lives. Each of these variables could influence the stability of the gut microbial community. As this field advances, the challenge for future researchers will be to design methodologies that capture these dynamic and sensitive systems. Despite challenges, emerging research linking microbes to social behavior is promising and opens a myriad of possible ways in which typical and atypical psychological processes may improve. Studies like this may illuminate numerous pathways by which microbes are linked to other highly adaptive psychological constructs in healthy children.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Glossary

ASD	autism spectrum disorder
EC	effortful control
bTEFAP	Bacterial tag-encoded FLX-Amplicon Pyrosequencing

References

1. Shreiner AB, Kao JY, Young VB. The gut microbiome in health and in disease. Current Opinion in Gastroenterology 2015;31:69–75. [PubMed: 25394236]
2. Mayer EA. Gut feelings: the emerging biology of gut–brain communication. Nature Reviews Neuroscience 2011;12:453–66. [PubMed: 21750565]
3. Archie EA, Tung J. Social behavior and the microbiome. Current Opinion in Behavioral Sciences 2015;6:28–34.
4. Kelly JR, Kennedy PJ, Cryan JF, Dinan TG, Clarke G, Hyland NP. Breaking down the barriers: the gut microbiome, intestinal permeability and stress-related psychiatric disorders. Frontiers in Cellular Neuroscience 2015;9.
5. Ghaisas S, Maher J, Kanthasamy A. Gut microbiome in health and disease: Linking the microbiome–gut–brain axis and environmental factors in the pathogenesis of systemic and neurodegenerative diseases. Pharmacology & Therapeutics 2016;158:52–62. [PubMed: 26627987]
6. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders American Psychiatric Association; 2013.
7. Ashwood P, Wills S, Van de Water J. The immune response in autism: a new frontier for autism research. Journal of Leukocyte Biology [Internet] 2006 [cited 2020 Oct 19];80:1–15. Available from: 10.1189/jlb.1205707
8. Bailey MT, Coe CL. Maternal separation disrupts the integrity of the intestinal microflora in infant rhesus monkeys. Developmental Psychobiology 1999;35:146–55. [PubMed: 10461128]
9. Bailey MT, Dowd SE, Galley JD, Hufnagle AR, Allen RG, Lyte M. Exposure to a social stressor alters the structure of the intestinal microbiota: Implications for stressor-induced immunomodulation. Brain, Behavior, and Immunity 2011;
10. Arentsen T, Raith H, Qian Y, Forssberg H, Heijtz RD. Host microbiota modulates development of social preference in mice. Microbial Ecology in Health & Disease 2015;26.
11. Crumeyrolle-Arias M, Jaglin M, Bruneau A, Vancassel S, Cardona A, Daugé V, Naudon L, Rabot S. Absence of the gut microbiota enhances anxiety-like behavior and neuroendocrine response to acute stress in rats. Psychoneuroendocrinology 2014;42:207–17. [PubMed: 24636517]
12. Desbonnet L, Clarke G, Shanahan F, Dinan TG, Cryan JF. Microbiota is essential for social development in the mouse. Molecular Psychiatry 2014;19:146–48. [PubMed: 23689536]
13. Messaoudi M, Lalonde R, Violle N, Javelot H, Desor D, Nejdi A, Bisson J-F, Rougeot C, Pichelin M, Cazaubiel M, Cazaubiel J-M. Assessment of psychotropic-like properties of a probiotic formulation (*Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175) in rats and human subjects. British Journal of Nutrition 2011;105:755–64. [PubMed: 20974015]
14. McKean J, Naug H, Nikbakht E, Amiet B, Colson N. Probiotics and Subclinical Psychological Symptoms in Healthy Participants: A Systematic Review and Meta-Analysis. The Journal of Alternative and Complementary Medicine 2017;23:249–58. [PubMed: 27841940]
15. Naseribafrouei A, Hestad K, Avershina E, Sekelja M, Linløkken A, Wilson R, Rudi K. Correlation between the human fecal microbiota and depression. Neurogastroenterology & Motility 2014;26:1155–62. [PubMed: 24888394]

16. Johnson KV-A. Gut microbiome composition and diversity are related to human personality traits. Human Microbiome Journal 2020;15:100069.
17. Dill-McFarland KA, Tang Z-Z, Kemis JH, Kerby RL, Chen G, Palloni A, Sorenson T, Rey FE, Herd P. Close social relationships correlate with human gut microbiota composition. Scientific Reports 2019;9:703. [PubMed: 30679677]
18. Michels N, Van de Wiele T, Fouhy F, O'Mahony S, Clarke G, Keane J. Gut microbiome patterns depending on children's psychosocial stress: Reports versus biomarkers. Brain, Behavior, and Immunity 2019;80:751–62. [PubMed: 31112792]
19. Wang Y, Chen X, Yu Y, Liu Y, Zhang Q, Bai J. Association between Gut Microbiota and Infant's Temperament in the First Year of Life in a Chinese Birth Cohort. Microorganisms 2020;8:753.
20. Christian LM, Galley JD, Hade EM, Schoppe-Sullivan S, Kamp Dush C, Bailey MT. Gut microbiome composition is associated with temperament during early childhood. Brain, Behavior, and Immunity 2015;45:118–27. [PubMed: 25449582]
21. Ku H-J, Kim Y-T, Lee J-H. Microbiome Study of Initial Gut Microbiota from Newborn Infants to Children Reveals that Diet Determines Its Compositional Development. Journal of Microbiology and Biotechnology 2020;30:1067–71. [PubMed: 32270658]
22. Reyman M, van Houten MA, van Baarle D, Bosch AATM, Man WH, Chu MLJN, Arp K, Watson RL, Sanders EAM, Fuentes S, Bogaert D. Impact of delivery mode-associated gut microbiota dynamics on health in the first year of life. Nature Communications 2019;10:4997.
23. Jašarević E, Morrison KE, Bale TL. Sex differences in the gut microbiome–brain axis across the lifespan. Philosophical Transactions of the Royal Society B: Biological Sciences 2016;371:20150122.
24. O'Sullivan A, Farver M, Smilowitz JT. Article Commentary: The Influence of Early Infant-Feeding Practices on the Intestinal Microbiome and Body Composition in Infants. Nutrition and Metabolic Insights 2015;8s1:NMI.S29530.
25. Constantino JN, Todd RD. Autistic Traits in the General Population. Archives of General Psychiatry 2003;60:524. [PubMed: 12742874]
26. Ruzich E, Allison C, Smith P, Watson P, Auyeung B, Ring H, Baron-Cohen S. Measuring autistic traits in the general population: a systematic review of the Autism-Spectrum Quotient (AQ) in a nonclinical population sample of 6,900 typical adult males and females. Molecular Autism 2015;6:2. [PubMed: 25874074]
27. Liu F, Li J, Wu F, Zheng H, Peng Q, Zhou H. Altered composition and function of intestinal microbiota in autism spectrum disorders: a systematic review. Translational Psychiatry 2019;9:43. [PubMed: 30696816]
28. Wang L, Christophersen CT, Sorich MJ, Gerber JP, Angley MT, Conlon MA. Low Relative Abundances of the Mucolytic Bacterium Akkermansia muciniphila and Bifidobacterium spp. in Feces of Children with Autism. Applied and Environmental Microbiology 2011;77:6718–21. [PubMed: 21784919]
29. De Angelis M, Piccolo M, Vannini L, Siragusa S, De Giacomo A, Serrazzanetti DI, Cristofori F, Guerzoni ME, Gobbetti M, Francavilla R. Fecal Microbiota and Metabolome of Children with Autism and Pervasive Developmental Disorder Not Otherwise Specified. PLoS ONE 2013;8:e76993. [PubMed: 24130822]
30. Zou R, Xu F, Wang Y, Duan M, Guo M, Zhang Q, Zhao H, Zheng H. Changes in the Gut Microbiota of Children with Autism Spectrum Disorder. Autism Research 2020;13:1614–25. [PubMed: 32830918]
31. Xu M, Xu X, Li J, Li F. Association Between Gut Microbiota and Autism Spectrum Disorder: A Systematic Review and Meta-Analysis. Frontiers in Psychiatry 2019;10.
32. Ding X, Xu Y, Zhang X, Zhang L, Duan G, Song C, Li Z, Yang Y, Wang Y, Wang X, Zhu C. Gut microbiota changes in patients with autism spectrum disorders. Journal of Psychiatric Research 2020;129:149–59. [PubMed: 32912596]
33. Wang L, Christophersen CT, Sorich MJ, Gerber JP, Angley MT, Conlon MA. Increased abundance of Sutterella spp. and Ruminococcus torques in feces of children with autism spectrum disorder. Molecular Autism 2013;4:42. [PubMed: 24188502]

34. Strati F, Cavalieri D, Albanese D, De Felice C, Donati C, Hayek J, Jousson O, Leoncini S, Renzi D, Calabrò A, De Filippo C. New evidences on the altered gut microbiota in autism spectrum disorders. Microbiome 2017;5:24. [PubMed: 28222761]
35. Andreo-Martínez P, García-Martínez N, Sánchez-Samper EP, Martínez-González AE. An approach to gut microbiota profile in children with autism spectrum disorder. Environmental Microbiology Reports 2020;12:115–35. [PubMed: 31713352]
36. Kochanska G, Murray KT, Harlan ET. Effortful control in early childhood: Continuity and change, antecedents, and implications for social development. Developmental Psychology 2000;36:220–32. [PubMed: 10749079]
37. Putnam SP, Gartstein MA, Rothbart MK. Measurement of fine-grained aspects of toddler temperament: The Early Childhood Behavior Questionnaire. Infant Behavior and Development 2006;29:386–401. [PubMed: 17138293]
38. Ding HT, Taur Y, Walkup JT. Gut Microbiota and Autism: Key Concepts and Findings. Journal of Autism and Developmental Disorders 2017;47:480–89. [PubMed: 27882443]
39. Hughes HK, Rose D, Ashwood P. The Gut Microbiota and Dysbiosis in Autism Spectrum Disorders. Current Neurology and Neuroscience Reports 2018;18:81. [PubMed: 30251184]
40. Benjamini Y, Hochberg Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. Journal of the Royal Statistical Society: Series B (Methodological) 1995;57:289–300.
41. Cole CB, Nikpay M, Lau P, Stewart AFR, Davies RW, Wells GA, Dent R, McPherson R. Adiposity significantly modifies genetic risk for dyslipidemia. Journal of Lipid Research 2014;55:2416–22. [PubMed: 25225679]
42. Dowd SE, Callaway TR, Wolcott RD, Sun Y, McKeehan T, Hagevoort RG, Edrington TS. Evaluation of the bacterial diversity in the feces of cattle using 16S rDNA bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP). BMC Microbiology 2008;8:125. [PubMed: 18652685]
43. Dowd SE, Wolcott RD, Sun Y, McKeehan T, Smith E, Rhoads D. Polymicrobial Nature of Chronic Diabetic Foot Ulcer Biofilm Infections Determined Using Bacterial Tag Encoded FLX Amplicon Pyrosequencing (bTEFAP). PLoS ONE 2008;3:e3326. [PubMed: 18833331]
44. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R. QIIME allows analysis of high-throughput community sequencing data. Nature Methods 2010;7:335–36. [PubMed: 20383131]
45. Edgar RC. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 2010;26:2460–61. [PubMed: 20709691]
46. McDonald D, Price MN, Goodrich J, Nawrocki EP, DeSantis TZ, Probst A, Andersen GL, Knight R, Hugenholtz P. An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. The ISME Journal 2012;6:610–18. [PubMed: 22134646]
47. DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, Huber T, Dalevi D, Hu P, Andersen GL. Greengenes, a Chimera-Checked 16S rRNA Gene Database and Workbench Compatible with ARB. Applied and Environmental Microbiology 2006;72:5069–72. [PubMed: 16820507]
48. Shannon CE. The mathematical theory of communication. 1963. M.D. computing : computers in medical practice 14:306–17.
49. Bray JR, Curtis JT. An Ordination of the Upland Forest Communities of Southern Wisconsin. Ecological Monographs 1957;27:325–49.
50. David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, Ling AV, Devlin AS, Varma Y, Fischbach MA, Biddinger SB, Dutton RJ, Turnbaugh PJ. Diet rapidly and reproducibly alters the human gut microbiome. Nature 2014;505:559–63. [PubMed: 24336217]
51. Kashtanova DA, Popenko AS, Tkacheva ON, Tyakht AB, Alexeev DG, Boytsov SA. Association between the gut microbiota and diet: Fetal life, early childhood, and further life. Nutrition 2016;32:620–27. [PubMed: 26946974]

52. Koenig JE, Spor A, Scalfone N, Fricker AD, Stombaugh J, Knight R, Angenent LT, Ley RE. Succession of microbial consortia in the developing infant gut microbiome. Proceedings of the National Academy of Sciences 2011;108:4578–85.

53. Palmer C, Bik EM, DiGiulio DB, Relman DA, Brown PO. Development of the Human Infant Intestinal Microbiota. PLoS Biology 2007;5:e177. [PubMed: 17594176]

54. Arrieta M-C, Stiemsma LT, Amenyogbe N, Brown EM, Finlay B. The Intestinal Microbiome in Early Life: Health and Disease. Frontiers in Immunology 2014;5.

55. Clarke KR, Somerfield PJ, Gorley RN. Testing of null hypotheses in exploratory community analyses: similarity profiles and biota-environment linkage. Journal of Experimental Marine Biology and Ecology 2008;366:56–69.

56. Zhou K Strategies to promote abundance of Akkermansia muciniphila, an emerging probiotics in the gut, evidence from dietary intervention studies. Journal of Functional Foods 2017;33:194–201. [PubMed: 30416539]

57. Maes M, Kubera M, Leunis J-C. The gut-brain barrier in major depression: intestinal mucosal dysfunction with an increased translocation of LPS from gram negative enterobacteria (leaky gut) plays a role in the inflammatory pathophysiology of depression. Neuro endocrinology letters 2008;29:117–24. [PubMed: 18283240]

58. Taylor AM, Thompson SV., Edwards CG, Musaad SMA, Khan NA, Holscher HD. Associations among diet, the gastrointestinal microbiota, and negative emotional states in adults. Nutritional Neuroscience 2020;23:983–92. [PubMed: 30794085]

59. de Theije CGM, Wopereis H, Ramadan M, van Eijndthoven T, Lambert J, Knol J, Garssen J, Kraneveld AD, Oozeer R. Altered gut microbiota and activity in a murine model of autism spectrum disorders. Brain, Behavior, and Immunity 2014;37:197–206. [PubMed: 24333160]

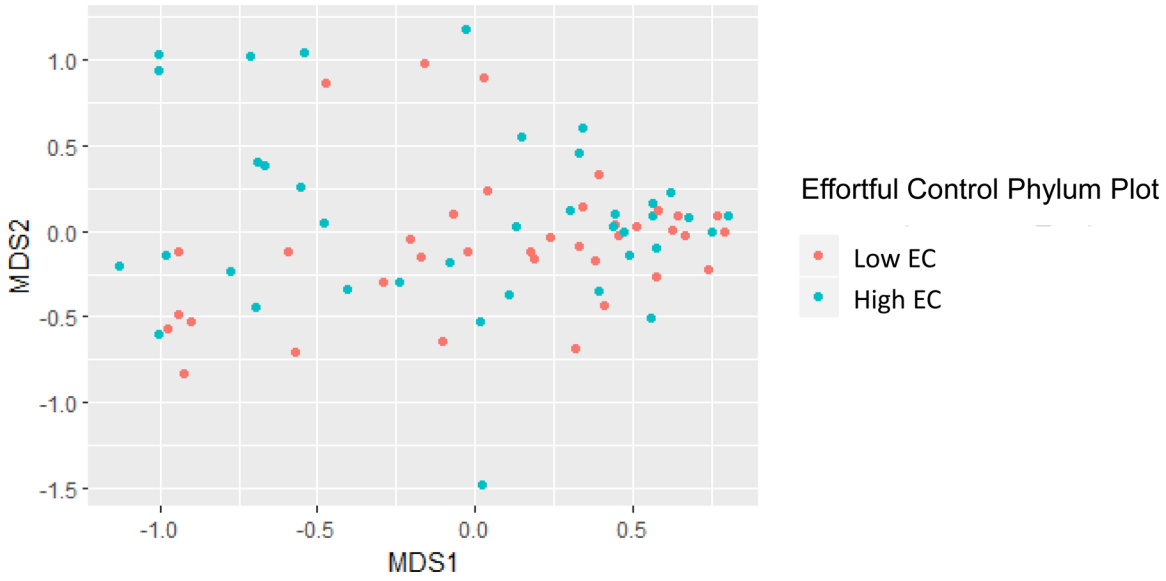

Figure 1:
Nonmetric multidimensional scaling (NMDS) ordination using Bray-Curtis dissimilarity to calculate distances between samples on the phylum level.

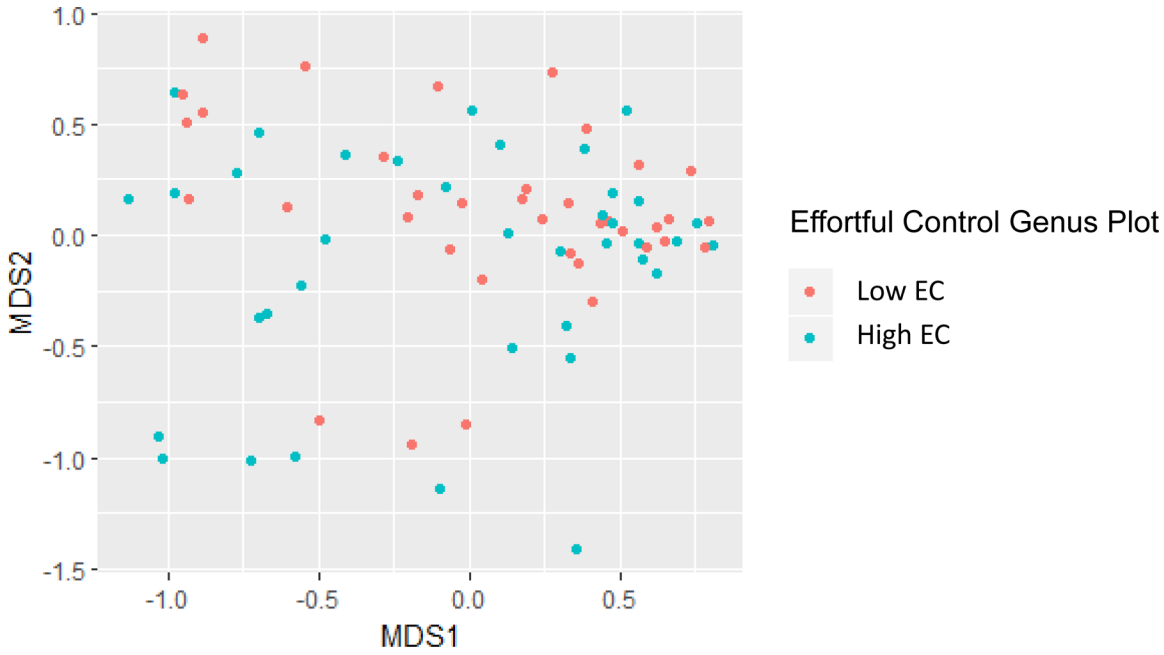

Figure 2:
Nonmetric multidimensional scaling (NMDS) ordination using Bray-Curtis dissimilarity to calculate distances between samples on the genus level.

Table 1

Descriptive Statistics of Key Variables by Children's Sex (n = 77)

Measures	**Total *N* = 77 *M (SD)***	**Boys *n* = 41 *M* (*SD*)**	**Girls *n* = 36 *M* (*SD*)**	***Sex Differences***
				t
Age in Months	23.14(2.00)	23.20(2.04)	23.08(1.99)	−.24
Body Composition	.93(.68)	1.04(.87)	.80(.34)	−1.63
Breast Feeding Duration	10.06(7.23)	8.87(6.34)	11.42(8.00)	1.54
Vegetable/ Fruit Consumption	7.00(1.41)	6.76(1.45)	7.28(1.34)	1.63
Meat Consumption	6.52(1.80)	6.41(1.73)	6.64(1.85)	.55
Effortful Control	4.72(.47)	4.61(.40)	4.86(.52)	2.33
Cuddliness	4.36(.75)	4.27(.65)	4.46(.84)	1.10
Attentional Focus	4.89(.75)	4.84(.68)	4.94(.83)	.60
Attentional Shifting	5.09(.69)	5.02(.69)	5.17(.68)	1.00
Inhibitory Control	4.19(.96)	3.94(.91)	4.47(.95)	*2.49
Low Intensity Pleasure	5.11(.77)	4.98(.71)	5.26(.81)	1.57
Dialister	.06(.11)	.07(.13)	.06(.10)	−.25
Alistipes	.01(.03)	.02(.04)	.01(.02)	−.76
Akkermansia	.01(.05)	.004(.01)	.02(.07)	*1.26

Note: Sex Coding: 1 = boys, 2 = girls; Body Composition: weight/height ratio; Breast Feeding Duration: age in months when breastfeeding stopped; Vegetable/Fruit/Meat Consumption: reported on a 1–8 Likert scale (1= never; 8= two or more times per day); Cuddliness, Attentional Focus, Attentional Shifting, Inhibitory Control, Low Intensity Pleasure: rated on a 7-point Likert scale (1= never, 8 = always). Effortful Control: composite scores of all EC subscales with values ranging from 3.74 – 5.98; Bacterial genera (*Dialister*, *Alistipes*, and *Akkermansia*): relative abundance/percentage of each bacterium. Mothers were 87.0% White (*n* = 67), 9.1% Black (*n* = 7), and 3.9% Asian (*n* = 3) and mean maternal age at the time of delivery was 31.1 years (*SD*=5.43).

*$p < .05$.

Table 2

Correlation Matrix for Key Variables (n = 77)

Variables	1	2	3	4	5	6	7	8	9
1. Body Composition	--								
2. Breastfeeding Duration	−.01	--							
3. Vegetable/Fruit Consumption	−.20	−.15	--						
4. Meat Consumption	.06	−.11	.41**	--					
5. Effortful Control	−.08	−.10	.07	.12	--				
6. Cuddliness	.04	−.08	.01	.08	.65**	--			
7. Attentional Focus	.06	−.02	.10	.07	.52**	.07	--		
8. *Dialister*	−.08	.04	−.03	.00	.24*	.23*	.16	--	
9. *Alistipes*	.14	−.03	−.11	−.25*	−.24*	−.02	−.27*	.02	--
10. *Akkermansia*	.02	.11	−.14	−.03	.25*	.36**	.00	.31**	−.07

Note: Sex Coding: 1 = boys, 2 = girls;

* $p < .05$.

** $p < .01$