The Relationship Between the Preterm Infant Gut Microbiome and Later Childhood Behavior

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Research Article

Keywords: Behavior problems, Gastrointestinal microbiome, Gut, Child, Preschool, Microbiota, Diversity index

Posted Date: December 29th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-2180302/v2

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Abstract

Background and Objectives

Very Low Birth Weight (VLBW) infants, born weighing less than 1500 grams, are at risk for both gut dysbiosis and later neuropsychological developmental deficits. With gut dysbiosis there is a disequilibrium of the gut microbial community. The *Gammaproteobacteria* dominated gut dysbiosis in VLBW infants likely results from a combination of immaturity derived from interrupted intrauterine development and environmental exposures in the Neonatal Intensive Care Unit (NICU) after birth. The extent of later neurobehavioral consequences associated with such microbial dysbiosis have yet to be determined.

Methods

We explored associations between the infants’ gut microbiome richness, diversity, composition, and network and early childhood behavior at 2 and 4 years of age in 25 children who were previously preterm born and studied while hospitalized in the Neonatal Intensive Care Unit (NICU). Behavior was measured with the Child Behavior Checklist (CBCL) at home visits at 32.2+/-4.8 months, and again at 49 +3.6 months. We also measured children's microbiomes at 2 and 4 years of age.

Results

The diversity and richness of the gut microbiome in VLBW infants were associated with later parent-reported maladaptive behavioral and emotional symptoms, including symptoms associated with autism, anxiety, Attention-Deficit/Hyperactivity Disorder (ADD), attention problems, and aggressive behavior. Microbiome compositional signatures were also associated with later childhood behavior. Network analysis revealed that the microbiome networks differed in the children at 2 and 4 years of age and different amplicon sequence variants (ASVs) were associated with behavior at these timepoints.

Conclusions

These data provide preliminary support for relationships between both the VLBW and later child gut microbiome dysbiosis and childhood behavior.

Introduction

In 2019, over 50,000 infants weighing less than 1,500 grams were born in the U.S. ¹. These very low birth weight (VLBW) infants have interruptions in intrauterine development which cause immaturity at birth ². These infants are at risk for behavioral, neurodevelopmental and emotional problems ³,⁴. Immaturity, coupled with the intensive care that is necessary, predisposes these infants to gut dysbiosis ⁴,⁵ and delayed and impaired neurodevelopment as well as behavioral problems in childhood. Intestinal dysbiosis often includes reduced microbial alpha diversity and increased intestinal barrier permeability ⁶ which may be correlated with poorer health status ⁷,⁸. The VLBW infants in this study experienced gut microbial dysbiosis during the first six weeks of life in the NICU which was characterized by a dominant abundance of *Gammaproteobacteria*. The low stool microbial diversity was associated with lower gestational age, growth faltering and lower amounts of mother's own milk ⁹ and later delays in neurodevelopment ¹⁷. The roles of the dysbiotic infant gut microbiome in later neurodevelopment and behavior are understudied. Pathogens present during sensitive developmental periods are associated with anxiety-like behavior and cognitive impairment ¹⁰-¹³. Disruption of host-microbe interplay at an early age may have lifelong consequences for distal organs, such as the
Proteobacteria in the early life of infant rhesus monkeys predicted a slower growth trajectory and smaller brain volumes at one year of age. Proinflammatory bacterial metabolites from the gut can alter the blood brain barrier or cross into the brain, altering microglia, and contributing to the development of neurological injury which then translates into later neurodevelopmental and behavioral problems.

We have previously analyzed connections between the VLBW infant gut microbiome and neurodevelopment. Behavioral effects, while related to neurodevelopment, are often more subtle and difficult to measure. The use of a parent qualitative scale to describe child behavior is a nuanced approach used in the current study.

Methods

This study explored associations between richness, diversity and composition of the VLBW infant's gut microbiome and later behavioral symptoms through 4 years of age and analyzed microbial composition and gut microbial networks at 2 and 4 years old.

Study Design and Participants

Upon approval by the university Institutional Review Board (IRB), parents of VLBW infants admitted to the NICU of a large Florida tertiary care hospital were invited to be in the initial cohort (IRB#Pro00003468, R21 NR013094). Parents gave written informed consent to participate in the study and in additional follow up studies. Eighty-three VLBW infants were measured during the first six weeks of their NICU admission. Parents who consented were contacted for the follow-up study (IRB#Pro00019955, NIH grant R01NR015446) that explored relationships between the gut microbiome and later health, growth, and development. A total of 25 VLBW infants were followed from birth to four years of age. Home visits were done, and multiple types of data were collected. In the current paper we report on data collected in the NICU, including stool microbiome data, and later behavioral outcomes at two and four years of age. In addition, stools sequenced at 2 and 4 years of age were analyzed for relationships of the microbiome and the behavioral outcomes.

Sample processing for measurement of infant and childhood follow up of stool microbiome

Infant stool samples were collected weekly from diapers during the first six weeks of life and aliquots were stored at -80°C prior to use. Microbial genomic DNA was extracted using the PowerSoil DNA Isolation Kit (MoBio). The microbial content was profiled by one contiguous region of 16S rRNA V3-V4 sequencing on an Illumina MiSeq that generated ~100,000 250 bp paired end reads per sample. Sequencing quality was assessed, errors corrected, Amplicon Sequence Variants (ASVs) were generated, and their taxonomic annotation were obtained against Silva v138 using the DADA2 pipeline. Amplicon Sequence Variants (ASVs) were used to calculate alpha diversity richness and diversity, specifically.

At the 2 and 4 year visits the investigators collected stool samples from the children and their mothers. Stool was collected from the diaper or from the toilet using the ALPCO Easy Sampler® Stool Collection kit. The stool was delivered to the lab and immediately frozen at -80°C until processing for DNA extraction. The samples were sequenced in the investigator's laboratory using the Illumina MiSeq, as above.
Behavioral Measures

Parents completed the CBCL at home visits. The CBCL is a standardized instrument used to assess behavioral problems in children between 18 and 71 months old. It contains 99 items, and each is rated on a three-point Likert scale. There are five problem domains: affective, anxiety, pervasive developmental, attention deficit/hyperactivity, and oppositional defiant; and eight syndrome scales: emotionally reactive, anxious/depressed, somatic complaints, withdrawn, sleep problems, attention problems, aggressive behavior, and stress problems. A total Internalizing score (anxiety/depression, withdrawn, emotional reactivity, somatic problems) and an Externalizing score (aggression, attention/hyperactivity) are also computed. The scale is a first level screening reporting symptoms aligned with diagnostic areas such as autism spectrum disorder (ASD), attention deficit disorder (ADHD), depression, and oppositional defiant disorder. The results are T-scores with a mean of 50. A previous publication provides the CBCL descriptive statistics for this sample with means and standard deviations.

Statistical Analysis

IBM SPSS Statistics version 25 (IBM Inc., Armonk, NY, USA) was used to calculate descriptive and frequency statistics for demographic and clinical data. Scores from the CBCL were analyzed using Spearman correlations because of non-normal bivariate distributions. All alpha diversity indices including Chao, Shannon, Simpson and Inverse Simpson were calculated for each sample using R package vegan. The effective number of species (Hill numbers) was computed from read counts.

Microbiome data from the second- and third-week stools were averaged to represent the early NICU stool diversity profile; and the fourth, fifth-, and sixth-week samples were averaged to represent the later, more stable NICU diversity stool profile. Canonical correlation analyses were performed to identify correlations between infant microbiome and later behavior.

Microbiome composition and Later Behavior

We adapted a method for selecting compositional balances to identify signatures within the microbiomes of the VLBW that best explain the associated behavior. The selecting balance algorithm (selbal) parsimoniously identifies a compositional microbial signature, or compositional balance, that is associated with a continuous or categorical variable of interest. Our extension (selbalMM) uses mixed effect models instead of ordinary least squares regression to account for correlations from clustered or longitudinal data when identifying microbial signatures (https://github.com/dmcskim/selbalmm).

Network analysis

A microbial network analysis explored connection and structure of microbial ecosystems. This approach was used to further analyze the influential microbes and networks in the samples collected from two- and four-year-old children. The microbial network construction (NetCoMi) was drawn with ‘sparcc’ method which addresses compositional nature. The R package NetCoMi (Network Construction and comparison for Microbiome data), v1.0.2 was used here. It allowed the construction and visualization of microbial networks in a fast and reproducible manner. In this analysis a phyloseq object (useful for microbiome analysis in R) of 34 samples (17 each for two and four years) was made and grouped according to the age. To handle the excess number of zeroes, a pseudo count of one was used. To normalize the read counts, rarefaction was used and ‘sparcc’ method was used to compute the associations while addressing for the compositional nature of data.
Results

Demographics

The 25 children were born early and at very low birth weight (Table 1). They were followed by home visits at 32.2 +/- 4.8 months of age, and again at 49 +/- 3.6 months. Most were born by Caesarean section, received courses of antibiotics and other medications, were fed varying amounts of mothers’ own milk and experienced multiple illnesses associated with prematurity.

Table 1 Sample characteristics (N = 25) included in this study:
<table>
<thead>
<tr>
<th>Demographics</th>
<th>Count (N=25)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>12</td>
<td>48%</td>
</tr>
<tr>
<td>Female</td>
<td>13</td>
<td>52%</td>
</tr>
<tr>
<td>Race</td>
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<td></td>
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<tr>
<td>White</td>
<td>16</td>
<td>64%</td>
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<tr>
<td>Black or African American</td>
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<tr>
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<td>4%</td>
</tr>
<tr>
<td>Native American</td>
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<td>4%</td>
</tr>
<tr>
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<td>8%</td>
</tr>
<tr>
<td>Ethnicity</td>
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<td>Household income</td>
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<tr>
<td>Under $4,999</td>
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<td>16%</td>
</tr>
<tr>
<td>$5,000-$14,999</td>
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<td>24%</td>
</tr>
<tr>
<td>3.00 $15,000-$24,999</td>
<td>4</td>
<td>16%</td>
</tr>
<tr>
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<tr>
<td>$40,000-$69,999</td>
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<td>4%</td>
</tr>
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<td>24%</td>
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<td>0%</td>
</tr>
<tr>
<td>Maternal education</td>
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<td></td>
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<td>Grammar/Elementary School</td>
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<tr>
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<tr>
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<td>Delivery method</td>
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<tr>
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<td>C-Section</td>
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<tr>
<td>Sepsis</td>
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<td>Yes</td>
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<td>24%</td>
</tr>
<tr>
<td>Seizures</td>
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<td></td>
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<tr>
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<td>8%</td>
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<td>Respiratory Distress Syndrome</td>
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<td>Bronchopulmonary Dysplasia, Chronic lung</td>
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<tr>
<td>No</td>
<td>23</td>
<td>92%</td>
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</tbody>
</table>
### Disease Associations

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<th>No</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinopathy of Prematurity</td>
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<td>20</td>
<td>8%</td>
</tr>
<tr>
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<td>1</td>
<td>16%</td>
</tr>
<tr>
<td>Stage 2 of ROP</td>
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<td></td>
<td>4%</td>
</tr>
<tr>
<td>Stage 3 of ROP</td>
<td>0</td>
<td></td>
<td>0%</td>
</tr>
<tr>
<td>Stage 4 of ROP</td>
<td>0</td>
<td></td>
<td>0%</td>
</tr>
<tr>
<td>Stage 5 of ROP</td>
<td>0</td>
<td></td>
<td>0%</td>
</tr>
<tr>
<td>Anemia</td>
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<td>1</td>
<td>48%</td>
</tr>
<tr>
<td>Blood transfusion</td>
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<td>15</td>
<td>40%</td>
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<tr>
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<td></td>
<td>88%</td>
</tr>
<tr>
<td>IVH 1 degree</td>
<td>2</td>
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<td>8%</td>
</tr>
<tr>
<td>IVH 2 degree</td>
<td>1</td>
<td></td>
<td>4%</td>
</tr>
<tr>
<td>IVH 3 degree</td>
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<td></td>
<td>0%</td>
</tr>
<tr>
<td>Necrotizing Enterocolitis</td>
<td>23</td>
<td>2</td>
<td>92%</td>
</tr>
<tr>
<td>Yes</td>
<td>13</td>
<td></td>
<td>52%</td>
</tr>
<tr>
<td>No</td>
<td>10</td>
<td>15</td>
<td>40%</td>
</tr>
<tr>
<td>No</td>
<td>22</td>
<td>1</td>
<td>88%</td>
</tr>
<tr>
<td>No</td>
<td>2</td>
<td></td>
<td>8%</td>
</tr>
</tbody>
</table>

**Associations between diversity and the CBCL at two and four years of age**

The sample size for data from home visits varied according to the information available for the type of analysis: 25 had the necessary information for (1) diversity indices analysis, 16 for the (2) Hill numbers/Canonical Correlation analysis -both analysis related to alpha diversity-, 13 for the compositional balances at (3) infancy with behavior during early childhood, and 17 for both the (4) compositional balances at two and four years of age and behavior (5) a network analyses of these data. Considering the variability of sample size in different analyses, we performed a cross-comparison of the significant results between different alpha diversity correlations analyses and models. Commonalities among significant findings across the different analysis and models is provided in the supplementary material (Supplementary Table S1). The various diversity indices were inversely associated with CBCL domains and scales. There was an inverse association between the domain of anxiety problems with microbiome diversity of stool samples collected at both one-three weeks (chao rho= -0.564, p= 0.01) and four-six weeks (Shannon, rho= -4.76, p= 0.025; Simpson, rho= -0.469, p= 0.028; InvSimpson, rho= -0.539, p=0.010). Lower Chao diversity from the earlier stool samples showed relationship with higher scores in the syndrome scales of emotionally reactive (Chao, rho= -.514, p=0.021), attention problems (chao, rho = -0.467, p= 0.038), and total problems (Chao, rho= -0.494, p=0.027) at four years of age. The Shannon, Simpson, and InvSimpson of later infant stool samples were related to the sleep problems scale (Shannon, rho= -0.456, p = 0.033; Simpson, rho = -0.455, p= 0.033; InvSimpson, rho= -0.496, p=0.019). Figure 1 shows heat plots for correlation between microbiome diversity and CBCL scores for the children at four years old. File S2 (Figure) shows scatterplots of the significant diversity associations with different CBCL domains and scales.

Hill numbers/Canonical Correlation Analysis (CCA) of infant microbiome and CBCL
The Canonical Correlation Analysis (CCA) performed with the Hill numbers (used for alpha diversity), controlling for gestational age, showed correlations between the infant microbiome and CBCL scores at years two and four. At the two-year comparison, 15 samples had the necessary information and showed strong correlation in the first four dimensions between infant gut microbiome and CBCL syndrome scales and problem domains. The pairwise combination of canonical variable plots at two years of age can be examined in Figure 2. Variables names that are close to one another are more closely correlated with each other and distance from the center provides strength of the correlation between a variable and the canonical variate.

At two years old, pairwise comparison of the first two dimensions showed all values of the infant's gut microbiome and total CBCL scores within the interior of the circle, except for gestational age (Figure 2).

This indicates a strong and significant association between the infant gut microbiome alpha diversity and total CBCL scores. At four years old, none of the dimensions for the cumulative tests showed strong correlations (Figure 3).

Longitudinal variance analysis of infant Microbiome Compositional Balances

Microbiome compositional balances provides groups of microbial signatures that are specifically predictive of a phenotype. The microbiome compositional balances were analyzed by identifying groups of microbial signatures in VLBW infants that were associated with later childhood behavior, using the CBCL scores as continuous variables of interest.

While the Supplemental Figure S2a show longitudinal upward global balances in depression, anxiety, ADHD scores, and the anxiety/depression syndrome scale, Figure S2b graphs shows a general longitudinal downward trend in the oppositional problem domain, withdrawn, attention, aggressive, sleep syndrome and externalizing scales. Other balances are presented in Supplemental Figure S2c and S2d, respectively.

Table 1 summarizes the top and bottom microbiome genera balances with the lowest dispersion from the mean (coefficient of variation (c.v.) <30%) in infant’s stool. A characteristic of the VLBW infant microbiome was the presence of Gammaproteobacteria, particularly Enterobacteriaceae, in the group of microbial signatures associated with later childhood syndrome scales of withdrawn, sleep problems, and the depression problem domain. The microbial groups, including Firmicutes, particularly Clostridium and Veillonella, were associated with the affective problems domains, and the withdrawn and emotional reactivity syndromes. The microbial groups including genus Bifidobacterium were associated with the affective and pervasive developmental domains, and the somatic and anxiety/depression syndrome scales. Finally, a final balance without much dispersion from the mean was genus Staphylococcus, associated with attention deficit/hyperactivity syndrome scale. The supplementary Table S2 presents the microbiome balances’ c.v.s per CBCL domains and scores.

Analysis of microbiome composition at 2 and 4 years old

A microbiome compositional balances analysis was also performed. The relative abundance of the organisms at the top and bottom were associated with multiple domains and scores. The supplement Table S3 summarizes the major bacterial taxa associated with the CBCL domains while Supplementary Figure S3 display the corresponding accuracy of the model for the different CBCL domains.

The microbial signature including Clostridiales was associated with multiple domains and scores (pervasive developmental and oppositional defiant scores). The microbial groups including Bacteroidetes, particularly from the family Barnesiellaceae, were associated with children's different domains (anxiety and ADHD) and
Desulfovibrionaceae with oppositional behaviors. We detected commensals such as Paraprevotella, and Fusobacterium associated with the anxiety and attention deficit/hyperactivity domains.

Network analysis results

Figure 4 shows the microbial association network for stool samples collected at two and four years with the relative position of the nodes (ASVs grouped into genus level) same in both. The size and color of the node indicates its importance and its affiliation to a particular cluster of the node. The color of the lines (green indicates positive and red indicates negative) shows significant association between the ASVs while the thickness indicates the strength of association. The ASV belonging to Faecalibacterium (g_066) is influential at two years while the ASV belonging to Ruminococcaceae (g_003) is most important at four years. To compare if microbial networks for the two age groups were statistically similar, we calculated the Adjusted Rand index (ARI) implemented in the netCompare function in NetCoMi with 100 permutations. The ARI (ARI = 0.027, p=0.338) indicates that the microbial networks for the two and four years were not significantly related. Lachnospiraceae abundance at two years old was replaced by Ruminococcaceae at four years old, and both were associated with changes in behavior. Christensenellaceae was present only at four years old and the genus Blautia had considerably lower influence in the network at four years of age. The microbial network construction at two and four years of age shows differences of key players with red nodes overpowering the cluster identified at two years old.

Findings from this preliminary research suggest associations between VLBW infant gut microbiome richness, diversity, composition, and networks and various early childhood maladaptive behaviors and emotional problems. Additional findings are that gut microbial taxa at two and four years of age were also associated with behavior. There were distinctive dimensions of microbiome and behavior that were correlated, and microbial signatures associated with childhood behavior. The microbial networks differed at two and four years, reflecting successional development towards the adult enterotype, but some consistencies in relationship with behavior were discovered.

Our data corroborates other studies. Clostridium abundances in infant stool were associated with later behavioral problems and cognitive impairment risks. Antibiotics and formula feeding may contribute to the increase of Clostridium. Veillonella presence in the infant gut microbiome was reported in the literature to be associated with fear behavior and with higher infant stress scores from the Neonatal Infant Stress Scale (NISS). Lower abundances of Bifidobacterium has been associated with negative social behavior, leading to the administration of probiotic with Bifidobacterium in various studies. The interventions demonstrated reduction of anxiety-behavior in mice and a significant reduction in lethargy in the combination treatment of bovine colostrum product and Bifidobacterium probiotic.

The gut microbiome compositional balances in at two and four years were found to be related to multiple behavioral problem domains such as depressive, autism, oppositional, and emotional behaviors. These findings corroborate previous studies in which Bacteroidales and Clostridiales correlated with altered social behavior in mice exposed to early life stressors, which lingered to adolescence in mice. Clostridiales synthesize neurotoxins, so the association of this taxa with multiple behavioral domains and scales indicate potential for future research.

In network analysis of the two- and four-year olds’ stool microbiome, the Lachnospiraceae family had the strongest scoring nodes at two years while Ruminococcaceae was strongest at four years. Enrichment of both bacterial families is associated with increased levels of highly permeable metabolites, like cresol. Cresol changes gene expression and myelination, resulting in social avoidance and depressive-like behaviors. Ruminococcaceae is also associated with anxiety levels and is more abundant in persons with ADHD.
The mechanism for these effects are unknown, but the gut microbiome influences behavioral changes in three ways: epigenetic mechanisms, metabolites production, and altering brain structure and function \(^{36}\). The gut microbiome may modulate epigenetic mechanisms and lead to behavioral changes \(^{37}\), because gene expression and protein content throughout different parts of the brain are linked to behavioral changes \(^{38}\). Additionally, gut microbial metabolites, such as short-chain fatty acid, folates, biotin and trimethylamine-N-oxide, can regulate epigenetic modifications \(^{39}\).

Besides the epigenetic mechanism and metabolites production, the gut microbiome affects the amygdala \(^{40}\), increasing its volume in germ-free mice who have altered stress signaling and display anxiety-like behaviors \(^{41}\). We found an association of decreased abundance of commensal bacteria with behavioral problems. Reduced commensal bacteria are found in children with autism, who lack a \textit{Prevotella}-like enterotype and \textit{Fusobacteria} \(^{42-44}\).

Other factors aside from gestational age are potentially important in these later childhood relationships and were not controlled in the analyses, due to the small sample size. These include human milk, illnesses, growth and development characteristics after discharge, socioeconomic status, diet, illnesses, parenting and many other influences. The study is very preliminary due to the limited sample size. Regardless, our findings warrant further exploration of the infant gut microbiome relationship with later behavioral health.

**Abbreviations**

CBCL (Child Behavior Checklist); VLBW (Veery Low Birth Weight); ASV (Amplicon Sequence Variant); NICU (Neonatal Intensive Care Unit)

**Declarations**

**Conflict of Interest**

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**Funding**

This work was supported by National Institutes of Health under grants R21 NR013094 and R01NR015446 (M. Groer, P.I.).

**Acknowledgement**

Samia Valeria Ozorio Dutra acknowledges support from the Brazilian Federal Agency for Support and Evaluation of Graduate Education (CAPES) for graduate education

**Ethics approval and consent to participate:**

Upon approval by the university Institutional Review Board (IRB), parents of VLBW infants admitted to the NICU of a large Florida tertiary care hospital were invited to be in the initial cohort (IRB#Pro00003468). Parents gave written informed consent to participate in the study and in additional follow up studies. Eighty-three VLBW infants were measured during the first six weeks of their NICU admission. Parents who consented were contacted for the follow-up study (IRB#Pro00019955) that explored relationships between the gut microbiome and later health, growth, and development.

**Consent for publication**
All authors have approved the manuscript and given consent for publication.

**Availability of data and material**

The study was registered in dbGaP under accession number phs001578.v1.p1. Deidentified metadata and raw forward and reverse sequence reads were associated with each sample via SRA. Raw sequence reads are available through the Sequence Read Archive under accession number SRP171050 (BioProject number PRJNA449987).

**Competing interests**

Not applicable.

**Funding**

This work was funded through two NIH grants: R21 NR013094 and R01NR015446.

**Authors’ contributions**

Dr. Samia Dutra collected some of the data, prepared the data for analysis, participated in some of the statistical analysis, drafted early versions of the manuscript, and critically reviewed and revised the manuscript.

Dr. Daniel McSkimming led the bioinformatics, drafted the results, edited the paper, and critically reviewed and revised the manuscript.

Dr. Anujit Sarkar worked with Dr. McSkimming on the bioinformatics, prepared figures, edited the paper and critically reviewed and revised the manuscript.

Dr. Ming Ji worked on preparing the data for analysis and basic statistical analyses.

Dr. Emily Shaffer contributed expertise to the instruments used in the study, their scoring and analyses. She also reviewed the interpretations and descriptions in the manuscript.

Dr. Ji Youn Yoo helped in the early data analyses and critically reviewed and revised the manuscript.

Dr. Jessica Gordon led in the collection of behavioral data and stool samples, prepared data for analysis, and critically reviewed and revised the manuscript.

Dr. Maureen Groer conceptualized and designed the study, acquired funding, and coordinated and supervised data collection, revised the manuscript based on coauthors suggestions, reviewed statistical approaches and techniques, and verified accuracy of all reports.

**Acknowledgements**

The authors acknowledge the NICU nurses, parents and children who generously gave of their time to participate in this research.

**Conflict of Interest Disclosures:** None of the authors have conflicts to disclose.

**Funding/Support:** All phases of the study were supported by grants R21 NR013094 and R01NR015446. Samia Valeria Ozorio Dutra acknowledges support from the Brazilian Federal Agency for Support and Evaluation of Graduate Education (CAPES) for graduate education.
**Article Summary:** This study describes the relationship between the early very low birth weight infant dysbiotic gut microbiome and later behaviors in these children at 2 and 4 years of age.

**References**


17. Sarkar A PS, Dutra S, Youn Yoo J, Gordon J, Shaffer E, McSkimming D, Groer M. Relationships of the very low birth weight infant microbiome with neurodevelopment at 2 and 4 years of age. Developmental Psychobiology 2022;64(7):e22317. DOI: http://dx.doi.org/10.1002/dev.22317


Figures
Figure 1

Spearman correlations between microbiome diversity measures and CBCL scores for the children at 4 years. Greater correlations are indicated by with dark purple color.
Figure 2

Canonical Correlation Analysis (CCA) between early infant microbiome and behavioral cumulative scales at 2 years of age. GA indicates gestational age while IS and ES represents Internalizing and Externalizing scores, respectively. EI and LI indicate early and late infancy and q0, q1 and q2 represents the microbiome diversity indices. Here, q0, q1 and q2 represents species richness, Shannon diversity and Simpson diversity respectively.
Figure 3

CCA between later infant microbiome and cumulative scales at 4 years of age. GA indicates gestational age while IS and ES represents Internalizing and Externalizing scores, respectively. EI and LI indicate early and late infancy and q0, q1 and q2 represents the microbiome diversity indices. Here, q0, q1 and q2 represents species richness, Shannon diversity and Simpson diversity respectively.
Figure 4

Comparison between 2 and 4 years old microbial network. The network is drawn separately for 2 and 4 years. The color of the nodes indicates the cluster a bacterium belongs to, and its size indicates the relative abundance. The color of the line indicates positive (green) and negative (red) association. The thickness of the line describes the strength of association.

Supplementary Files

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