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Remnant Small Bowel Length in Pediatric Short Bowel Syndrome and the Correlation with Intestinal Dysbiosis and Linear Growth

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Abstract

Background: Pediatric Short Bowel Syndrome (SBS) is a malabsorptive state placing patients at risk for malnutrition, dehydration and bacterial overgrowth. These patients are often dependent on parenteral nutrition (PN) while intestinal adaptation is underway. The aim of this study was to characterize the effect of remnant small bowel length on the gut microbiome. Further, we sought to examine the contribution of clinical and nutritional variables to the gut microbiota and anthropometric growth.

Study Design: Clinical data, anthropometrics and fecal samples were collected from 14 SBS patients and 10 age and sex matched controls. Fecal bacterial DNA composition was analyzed using 16s ribosomal RNA gene sequencing. Statistical analysis was completed using the Mann-Whitney or Fisher's exact tests when applicable and linear mixed effect modeling.

Results: Distinct microbiota changes were found among those with the least remaining small bowel (<35 cm) compared to those with longer remaining bowel and controls. Those with <35 cm small bowel displayed an increased relative abundance of *Proteobacteria*, while those with longer remaining small bowel had a higher proportion of *Firmicutes*. Further, patients with less remaining bowel required more PN ($p<0.01$), and with a tendency to be shorter in height ($p=0.05$) and with a higher BMI ($p=0.05$).

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Conclusions: Remnant small bowel length appears to be a predictor of stunting with diminished linear growth, parenteral nutrition dependency, and a greater relative abundance of *Proteobacteria* in the gut. These findings suggest an integrated adaptive response predicted by remnant intestinal length. Further research is necessary to examine the effects of intestinal dysbiosis on clinical outcomes.

Precis

Adaptive growth, nutrition, and intestinal microbiota in pediatric short bowel syndrome patients are correlated with remnant small bowel length.

Introduction

Infantile short bowel syndrome (SBS) is a malabsorptive state occurring after significant intestinal resection or as the result of congenital bowel anomalies, such as gastroschisis or intestinal atresia.(1) These patients are at risk for malnutrition and growth failure as a consequence of insufficient absorptive surface area to support the absorption of fluids, electrolytes, and nutrients.(2) Following intestinal resection the remnant bowel undergoes a series of structural and functional changes known as adaptation in order to enhance digestion and absorption.(3)The time for complete adaptation occurs is variable and dependent on a variety of clinical features such as the site and length of remaining bowel, the delivery of intraluminal nutrients, and a number of growth factors, hormones and gastrointestinal secretions. (4, 5) If adaptation is incomplete, patients will require long-term supplemental parenteral nutrition (PN). The remaining small bowel length is a critical determinant of the ability to wean from PN.(5) However, there is no defined threshold of remaining small bowel length needed to predict PN-independence in infantile SBS. The ability to wean PN in these patients may be complicated by recurrent episodes of diarrhea related to small bowel bacterial overgrowth(6) or pathologic changes within the intestinal microbiome.(7) Prior studies have reported a decrease in bacterial diversity and an increase in the relative abundance of pathogenic bacteria in the intestinal microbial community associated with adverse clinical outcomes (7–9). However, no study has examined these factors in relation to remaining small bowel length.

The purpose of this study was to characterize the intestinal microbiome of pediatric SBS patients according to remaining small bowel length and to examine the interaction of clinical and nutritional variables on both the gut microbiota and anthropometric growth. We specifically sought to test the hypothesis that remnant small bowel length significantly perturbs the gut microbial community and body composition in our pediatric SBS population.

Methods

Patient Selection

Patients with SBS were recruited from the Pediatric Intestinal Rehabilitation Clinic at St Louis Children's Hospital (St Louis, MO). Patients were considered eligible for inclusion in the study if they were under 17 years of age and had been diagnosed with SBS during infancy (<2 years of life). The diagnosis of SBS was defined by having undergone any

intestinal resection rendering them dependent on PN for >90 days. Patients were excluded from the study if they had multiple congenital anomalies aside from an isolated bowel anomaly or had undergone a previous small bowel or liver transplant. Seventeen of the 18 patients in the Intestinal Rehabilitation Clinic were considered eligible and were recruited for this study. Three of the 17 patients were ultimately excluded from the final analysis due to insufficient samples obtained during the study period. Ten age and sex matched controls (ages 0.5 to 14 years) were recruited from the community. The controls had no chronic medical issues, diarrheal illnesses or oral antibiotic use in the past six months.

Clinical and demographic information were obtained from the medical records. The remaining small bowel length was obtained either by review of the operative note, surgical pathology report or intestinal rehabilitation clinic notes. The estimated percentage of remaining small bowel length controlling for age was calculated as previously described by Struijs et al(10). Stool samples from cases were collected quarterly from 2014 to 2016 and the controls each contributed one sample for analysis. Anthropometric measurements including weight, length, head circumference, and body mass index were obtained at the time of the last stool collection and converted to Z scores using age and sex specific World Health Organization (WHO) or Centers for Disease Control (CDC) reference charts when appropriate(11, 12). Tricep and subscapular skinfold thickness measurements were obtained using Harpenden Calipers by a trained member of the research staff for patients 1.5 to 17 years of age and were converted to percentiles using sex and age specific CDC reference curves(11). This study was approved by the Washington University Institutional Review Board (#201403122).

Specimen Collection, Microbial DNA and Cytokine Analytes

Fecal specimens were collected directly from ostomy output, diaper, or stool “hat” placed in the toilet. Samples were placed into a sterile cup and frozen at -80°C . After all the stools had been gathered they were sent to the Alkek Center for Metagenomics and Microbiome Research in the Department of Molecular Virology and Microbiology at Baylor University, Houston, Texas, for DNA extraction and 16S rRNA gene sequencing. Bacterial DNA was extracted using the MOBIO PowerSoil v3.4 DNA Isolation kit (MOBIO Laboratories, Inc.; Carlsbad CA) per the manufacturer’s protocol. Sequencing of bacterial 16S rRNA genes was performed using amplicons specific to the V4 hypervariable region (254 bp). Single-indexed sequencing libraries were pooled (100 ng per sample) prior to nucleotide sequencing on the Illumina MiSeq instrument, and paired-end sequences were generated using the 2×250 Miseq Reagent Kit V2 (Illumina). The sequence data produced were delivered to Washington University School of Medicine for taxonomic classification and further analysis.

Serum was collected from seven patients with SBS to measure the pro-inflammatory cytokines Tumor Necrosis Factor Alpha (TNF α), Interferon Gamma (IFN γ), Interleukin (IL) 1 Beta (IL1 β), IL-6, and IL-8. The analytes were processed using the Proinflammatory Panel 1 Human Kit (K15049G) V-PLEX Assay (Meso Scale Diagnostics, Rockville MD). Normal reference ranges for age were used for comparison.

16S rRNA gene amplicon classification

Taxonomic classification of 16S rRNA gene sequences was carried out with Mothur using the methods described by Kozich, et al.(13) Sequences were assigned into operational taxonomic units (OTUs) using Mothur's optclust method, with subsequent cluster threshold of 97% similarity. UCHIME(14) was used to remove chimeric sequences from analysis.

Statistical Analysis

Patients with SBS were compared according to their remaining small bowel length (<35 cm vs ≥35 cm remaining small bowel length) and to controls using the Mann-Whitney and Fisher's Exact Test when applicable. The 35 cm discriminant was chosen as this was the median bowel length for our SBS group. This allowed statistical comparisons of SBS patients with shorter (<35 cm) versus longer (≥35 cm) remnant bowel. There were insufficient numbers of patients and distribution of bowel length measurements to evaluate the data using bowel length as a continuous variable. Data are presented as median with interquartile ranges or mean with standard error of the mean (SEM) as appropriate. A cross sectional analysis was performed with the most recently collected stool sample and was used to determine the relative abundance of microbial taxa in all SBS patients. This was then compared with remaining small bowel length. The median time from surgery to collection of the index stool was 3.1 years (IQR 2 to 3.8 years) in the <35 cm group and 1.9 years (1.1 to 4.6 years) in the ≥35 cm group. The Shannon Diversity Index was calculated in all SBS patients, those with <35 cm remaining small bowel, ≥35 cm remaining small bowel and controls. The Shannon diversity index is commonly used to characterize species diversity within a community and accounts for both abundance and evenness of the species present. Calculations were performed using the IBM SPSS 24 statistical software package (Chicago, IL, USA). Values were considered statistically significant if the two-tailed p values were <0.05. An additional Bonferroni correction method was used to adjust for multiple comparisons using a p value of <0.017 as statistically significant.

The longitudinally collected stools included all SBS patients and were used to examine the impact of enteral nutrition on the intestinal microbiome. The median time from surgery to collection of the first stool for the longitudinal evaluation was 0.7 years (IQR 0.2 to 2.7 years). The 76 stool samples collected over the course of two years were grouped according to their enteral intake at the time of stool sample collection. Each sample was then catalogued according to their % enteral nutrition (EN) intake; those receiving <25% EN (29 stools), 25–50% EN (27 stools), 50–75% EN (12 stools) and >75% EN (8 stools). A single stool was collected from 10 control patients (100% EN) to serve as a comparison. We did not collect additional fecal samples in the control patients over time since there was minimal variability in their microbial community within the age span of this group and preliminary analysis established that their samples reflected a healthy gut microbiome. As nutritional intake changed for patients over time, some individual patients fell into more than one category. A mean relative abundance of each phyla was used to account for multiple stool samples from one patient within each group. SAS software version 9.4 (Cary, NC, USA) was used to generate a linear mixed effects model using the quarterly collected stools from the SBS subjects. In this model, the response variable was relative abundance of *Proteobacteria* and fixed effects included oral antibiotic use at time of stool sample

collection, remaining small bowel length and enteral nutrition intake. Relative abundance of *Proteobacteria* and enteral nutrition intake were transformed by taking the square root, which improved the model fit. Values were considered statistically significant if the p value was <0.05.

Results

Clinical Characteristics and Anthropometric Evaluation of SBS Patients

Fourteen patients with SBS followed in the Pediatric Intestinal Rehabilitation clinic at St Louis Children's Hospital were included in the final analysis. The SBS patients had a median age of 2.5 (IQR 1.3 to 4) years at time of last stool sample and equivalent numbers of males and females. Eleven patients had a congenital bowel anomaly including gastroschisis or intestinal atresia as their underlying cause of SBS, whereas only three patients had SBS due to either necrotizing enterocolitis or volvulus. One patient had long segment Hirschsprung's disease in addition to an intestinal atresia. The median remaining small bowel length was 35 cm in our cohort, with no significant differences in anatomical characteristics or complications between those who had <35 cm remnant small bowel and those with ≥35 cm remnant small bowel (**Table 1**).

Patients had anthropometric measurements recorded, including weight, length, body mass index (BMI) and nutritional characteristics at the time of the most recently collected stool sample (**Table 2**). When evaluating the SBS patients as a whole, all had anthropometric measurements within the normal range for their age and sex. However, when stratified by remaining bowel length, there tended to be differences in both height and BMI Z scores ($p=0.05$, $p=0.05$, respectively), but not in weight ($p=0.16$). Patients with <35 cm remaining small bowel tended to be shorter with a median height Z score of -1.8 and three out of the six patients were considered stunted with height Z scores < -2. Those with ≥35 cm remaining small bowel had a median height Z score of -0.5 and none of the patients fell into the stunted category.

Tricep and subscapular skinfold thickness measurements were obtained in 10 of the 14 patients. Three of the patients did not have skinfold thickness measurements taken because no reference values currently exist for their age group. One of the patients relocated during the study and therefore was unable to be assessed. There were three patients who had tricep and subscapular skinfold thickness percentiles ranging from the 60th to 97th percentile. These patients fell into the <35 cm remaining small bowel group and were the same patients who had stunted linear growth. The remaining patients had percentiles ranging from the 3rd to 25th percentile, regardless of their remaining small bowel length. Overall, the median tricep and subscapular skinfold thickness was significantly greater in the <35 cm group indicative of greater body fat stores.

Patients with <35 cm small bowel were nearly entirely dependent on PN for their energy intake. Conversely, they received less enteral nutrition and therefore fewer total calories than those with a greater degree of remaining small bowel (**Table 2**). During the study period, the median duration of PN was 3.2 years (IQR 2.1 to 3.8 years) for the <35 cm remaining small bowel group and 1.9 years (IQR 0.8 to 3.2 years) for the ≥35 cm remaining small bowel

group. Three patients in the 35 cm small bowel group had been weaned off PN at the time of the last stool collection, whereas all other patients had remained on PN since the time of their initial surgery, which occurred in the first three weeks of life for 13 of the 14 patients. One patient had surgery at seven weeks of life following a midgut volvulus and was subsequently dependent on PN.

Microbiota Changes According to Remaining Small Bowel Length

A total of 88 stool samples were collected from the 14 SBS patients. A median of 36,339 reads were identified per specimen and 2 samples with <9,000 reads were excluded from analysis. Stools were further excluded if they were collected at the time of bacteremia with an identified organism by blood culture. A total of 76 stool samples from 14 patients were therefore studied. There were 332 operational taxonomic units (OTU) identified among all specimens. Bacteria identified in the fecal samples of SBS patients over the study period and healthy controls included those from the six dominant phyla of the intestinal microbiome including *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Fusobacteria* and *Verrucomicrobia*. However, the relative abundance of each phyla was significantly different when compared with controls. All patients with SBS displayed a perturbed microbiota signature with increased relative abundance of bacteria (% $\pm$ SEM) considered to be pathogenic, including *Proteobacteria* (33.8% $\pm$ 8.1% SBS vs 1.5% $\pm$ 0.5% Control; $p=0.001$) and *Fusobacteria* (2.1% $\pm$ 1% SBS vs 0% Control; $p=0.01$). Further, we detected a significant decrease in phyla considered to be beneficial in the SBS cohort, specifically *Bacteroidetes* (9.5% $\pm$ 4.5% SBS vs 33% $\pm$ 7.3% Control; $p=0.01$) and *Verrucomicrobia* (3.4% $\pm$ 2.9% SBS vs 4.4% $\pm$ 1.6% Control; $p=0.01$). Finally, we found that the composition of the intestinal microbiome differed among SBS patients according to their remaining small bowel length (**Figure 1**). Specifically, in patients with <35 cm remaining small bowel, *Proteobacteria* dominated the microbiome with a mean relative abundance of 57.8% $\pm$ 12.3%, compared with 15.7% $\pm$ 4.9% and 1.5% $\pm$ 0.5% mean relative abundance in those with 35 cm remaining small bowel ($p=0.02$) and healthy controls ($p<0.01$), respectively (**Figure 2**). Patients with 35 cm remaining small bowel also significantly differed from healthy controls in terms of their relative abundance of *Proteobacteria* ($p=0.01$). Among the *Proteobacteria* phylum the ten predominant genera or families were selected for further analysis (**Figure 3**). *Enterobacteriaceae* dominated a large proportion of the *Proteobacteria* in both the <35 cm remaining small bowel group (33.5% $\pm$ 17.9%) and the 35 cm remaining small bowel group (39.1% $\pm$ 14.4%), but only made up 5% $\pm$ 2.9% of the *Proteobacteria* in the controls. The control group had a more diverse makeup of *Proteobacteria*. Conversely, those in the <35 cm group appeared to have a less diverse composition of *Proteobacteria*. Interestingly, the genus *Escherichia/Shigella* composed 77–99% of the *Proteobacteria* and 53–96% of the total microbiota in three patients with <35 cm remaining small bowel. *Escherichia* and *Shigella* are often indistinguishable with 16s rRNA analysis(15). There was a median of 33 unique bacterial taxa identified at the genus level in all patients with SBS, compared to a median of 58 taxa identified among the controls ($p=0.002$). There were statistically significant differences between the taxa that dominated those with <35 cm remaining small bowel compared to controls, whereas those with 35 cm remaining small bowel had unique taxa in addition to sharing similar taxa to both healthy controls and those with <35 cm (**Figure 4**). Additionally *Bifidobacteria* and *Veillonella*, which are both

considered to be ubiquitous inhabitants and early colonizers of the normal gastrointestinal tract(16), occupied <0.01% of the intestinal microbiota in the patients with <35 cm remaining small bowel. In marked contrast, *Bifidobacteria* made up $15.5\% \pm 7.5\%$ ($p<0.01$) and *Veillonella* comprised $16\% \pm 5.6\%$ ($p<0.01$) of the microbiota those patients with 35 cm remaining small bowel. There were no significant differences in richness defined as the number of species present in a sample when the patients with SBS were grouped according to bowel length (<35 cm SB median richness 31.5, IQR 30.3–35; 35 cm SB median richness 34, IQR 30.8–40; $p=0.52$). Bacterial diversity was determined by calculating the Shannon Diversity Index (SDI) at the genus level. All patients with SBS had a median SDI of 1.47 (IQR 1.13–1.87). When segregated by remnant small bowel length, those with <35 cm remaining small bowel had a median SDI of 1.19 (IQR 1.02–1.4) and those with 35 cm remaining small bowel had a median SDI of 1.69 (IQR 1.48–2.15). Those with the least remaining small bowel length were less diverse than the control group (Median SDI 2.51, IQR 1.99–2.64, $p=0.005$), but were not significantly different from those with 35 cm remaining small bowel ($p=0.07$). There was no significant difference in diversity between the healthy controls and those with greater remaining small bowel length ($p=0.06$). Detailed information regarding individual patients and their remnant small bowel length, estimated percent remaining small bowel length and key clinical factors including their nutritional status, anthropometric data and relative abundance of *Proteobacteria* are provided in **Table 3**.

% Enteral Nutrition (EN) is a Predictor of Intestinal Microbiota Signature

Using a univariate mixed model to account for the repeated measure design, we assessed the relationship of EN to the relative abundance of *Proteobacteria*. We found that the % EN was inversely related to the relative abundance of *Proteobacteria* ($p<0.001$). In a multivariate model examining the effect of EN, antibiotic use, remaining small bowel length, and time, we found that again the % EN was significantly inversely related to the relative proportion of *Proteobacteria* ($p<0.01$), with no additional significant contribution from antibiotic use, remaining small bowel length, or time. Therefore, in our population the % EN was the single most significant factor for predicting of the relative abundance of *Proteobacteria*. As patients tolerated increasing enteral nutrition, the relative abundance of *Proteobacteria* declined (**Figure 5**).

Proinflammatory Cytokines in SBS Patients

Blood was collected from seven of the 14 SBS patients. None of the patients displayed elevated circulating concentrations for TNF- α , IL-1 β or IL-6 (**Table 4**). There was nearly a 2-fold increase in IFN- γ in five of the patients, while all patients exhibited a 1.5 to 25 fold increase in IL-8. The two patients with the greatest increase in IL-8 also exhibited the greatest relative abundance of *Proteobacteria* (98.6 and 78.6%), had <35 cm remaining small bowel, and only tolerated <5% EN. One of the patients was a term infant who developed SBS secondary to malrotation with midgut volvulus on the second day of life and had 8 cm of remaining small bowel. The other patient was born at 35 weeks gestation following an in-utero volvulus secondary to complex gastroschisis with a resultant 30 cm of remaining small bowel. Both of the patients were maintained on PN since the time of their initial surgery (3.1 and 1.8 years, respectively).

Discussion

To our knowledge, this is the first study to establish a relationship between gut microbial profiles, linear growth, body composition, and remnant small bowel length in children with short bowel syndrome. Significant differences in the intestinal microbiome of pediatric SBS patients has previously been reported (7–9, 17). However, our data suggests that functional differences can be discerned among SBS patients related to their intestinal length and gut microbial signature. Children with the shortest intestinal length tended to have stunted linear growth with enhanced body fat stores. In addition, those with the shortest remnant bowel had less tolerance for enteral nutrition, a greater relative abundance of the pathogenic bacteria, *Proteobacteria*, and significantly decreased bacterial diversity. Accounting for clinical and nutritional variables using mixed models, we found that the amount of enteral nutrition was the most important determinant of *Proteobacteria* abundance. These findings suggest that while remnant small bowel length is related to the ability to tolerate enteral nutrition, the amount of enteral feeding directly affects the composition of the intestinal microbiota.

Proteobacteria composes only a minor proportion of the healthy human gut flora, which is normally composed primarily of the *Firmicute* and *Bacteroidetes* phyla(18). A higher prevalence of *Proteobacteria* has been found in the intestine of hosts who consume a high fat, high carbohydrate and low fiber diet,(19) chronically malnourished children in developing countries(20, 21), and in certain adult disease states such as obesity(22), inflammatory bowel disease(23) and type 2 diabetes mellitus(24). Therefore, the intestinal microbiome composition is closely linked to the enteral diet. It is considered that *Proteobacteria* and other proinflammatory bacteria may play a role in disrupting the intestinal epithelial barrier allowing for bacterial translocation and upregulation of pro-inflammatory molecules contributing to a chronic low-level state of inflammation(18, 25, 26). Enhanced systemic inflammation in association with *Proteobacteria* were revealed in our population, as the two patients who displayed elevated IL-8 levels and had >75% of their microbiota occupied by *Proteobacteria*. These patients had the most severely shortened remnant bowel, and each received <5% of their calories enterally. We identified evidence of linear stunting within the group with <35 cm remaining small bowel. We also identified a significant difference in caloric intake between our two groups, with those with 35 cm remnant small bowel receiving more calories. This difference in caloric intake may help explain the evidence of linear stunting, however there was no difference in weight Z scores between the two groups indicating adequate calories to maintain a normal weight for their age and sex. Further, anthropometric and skinfold thickness measurements indicated that weight maintenance is at least in part accounted for by increased fat deposition in these subjects. Also, those with <35 cm were almost entirely dependent on PN. Parenteral nutrition has lower caloric targets when compared with enteral nutrition due to factors such as diet-induced thermogenesis and incomplete absorption of luminal nutrient. There may be multiple factors that contribute to linear growth failure, including critical illness or patients unable to receive adequate nutrition in the newborn time period (27). Prolonged nutritional deficit will eventually affect linear growth and deplete lean muscle mass. This phenotype is strikingly similar to a PN-independent murine SBS model in which body fat is replaced

preferentially over lean body mass following a 50% proximal small bowel resection(28). This observation would suggest that independent of parenteral nutrition, intestinal resection alone is an important contributor to the pathogenesis of perturbed body composition.

Despite supplementation with PN, pediatric SBS patients may be at risk for chronic malnutrition due to the inability to tolerate enteral nutrition which plays a key role in structural, protective and metabolic functions in the host intestine. Enteral nutrition importantly acts as a stimulus for the release of growth hormones and signaling molecules that help to influence adaptation along with anabolic growth.(29) The microbiota also plays a role in neuroendocrine functions which influences nutrient metabolism, appetite, and intestinal transit time.(30) Piper et al(31) identified microbial differences among SBS patients who were classified as having good or poor weight gain. Similar to our patients with <35 cm remnant small bowel, those with poor weight gain in their patient population were depleted in *Firmicutes*. This was hypothesized to affect metabolic enzymes needed for nutrient utilization, but there was no difference in the relative abundance of *Proteobacteria* between the two groups. Linear growth failure has been reported in pediatric patients with SBS,(32, 33) but has not previously been linked to changes in either the intestinal microbiome or remnant small bowel length. Additional studies are needed to further evaluate this association and explore potential mechanisms that may involve both lack of enteral nutrition and alterations within the intestinal microbiome.

The remnant small bowel length in infants with SBS directs the need for prolonged PN and reduced enteral nutrient during a critical time of normal growth and development. We propose that this creates a pseudo-starvation environment within the intestine, which then become colonized with pathogenic bacteria that may disrupt the epithelial barrier, thus promoting a chronic inflammatory state. This is associated with decreased gut hormonal signaling that further disrupts normal anabolic growth contributing to poor linear growth and decreased lean muscle mass. However, we further suggest that with the gradual increase in intestinal adaptation and enteral tolerance, the microbiome may return to normal which in turn may mitigate these metabolic consequences.

There are a number of limitations to this study. The sample size in our patient cohort is small, but given the relative rarity of SBS, our patient cohort is similar in size to other studies published from other large pediatric centers.(7, 8, 17) We admit to the somewhat arbitrary cut off value of 35 cm for purposes of creating two groups within our SBS patients. It was chosen since it was the median small bowel length and afforded statistical comparisons with relative equal distribution between SBS patients with shorter and longer remnant bowel. The literature reports critical lengths ranging from 15 to 50 cm of remnant small bowel length as being important for gaining enteral autonomy(34, 35). Larger studies are needed to identify the precise cut off for the critical length of the remaining small bowel. We further recognize that it is difficult to establish cause and effect in regard to the alterations of the intestinal microbiome and the presence of linear stunting. Our population was studied after linear stunting was already established, since stunting occurs during the first two years of life. It is also unclear for how long colonization with pathogenic bacteria results in downstream effects of certain disease states such as metabolic diseases or systemic inflammation. Additional genetic factors such as parental height could have contributed to

the growth potential of these children. Unfortunately, we did not have parental height data available to assess whether this relationship. This study lends support for further research to characterize the longitudinal changes in the intestinal microbiome, linear growth and metabolic effects in patients with SBS diagnosed in the newborn period. As more of these children are surviving into adulthood, more studies will be essential to understanding the pathogenic changes that occur in the intestinal microbiome of pediatric SBS patients over time and the clinical health effects of intestinal dysbiosis.

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Figure 1.

The proportion of phyla within the microbiota in patients with either <35 cm remaining small bowel (n=6), 35 cm remaining small bowel (n=8), or in healthy, age and sex matched controls (n=10).

Figure 2.
The median and interquartile ranges of the relative abundance of each phyla according to remaining small bowel (SB) length and controls. *p 0.017, <35 cm SB vs 35 cm SB, †p 0.017, <35 cm SB vs control; ‡p 0.017, 35 cm SB vs control.

Figure 3.
The 10 predominant genera or families within the Proteobacteria phylum. The chart fractions represent the proportion each genus or family (if genus could not be identified) occupies within each cohort. SB, small bowel

Figure 4.

Heat map representing the 20 predominant taxa of the intestinal microbiome of patients with <35 cm remaining small bowel, >35 cm remaining small bowel and healthy controls.*p 0.017, <35 cm SB vs >35 cm SB, †p 0.017, <35 cm SB vs control; ‡p 0.017, >35 cm SB vs control.

Figure 5.

Composition of the intestinal microbiota according to the proportion of PN intake at the time of the stool sample collection. A total of 76 stools from 14 short gut syndrome patients were collected over two years and 10 stool samples from healthy, age and sex matched controls on 100% EN are included for comparison.

Table 1.

Demographic Information for Patients with <35 cm and ≥35 cm Small Bowel Remaining

Patient characteristic	All SBS patients (n=14)	Small bowel < 35 cm (n=6)	Small bowel ≥ 35 cm (n=8)	p Value
Demographic				
Age, y, median (IQR)	2.5 (1.3 to 4)	3.2 (1.7 to 7.2)	1.9 (1.1 to 3.9)	0.3
Male sex, n (%)	7 (50)	2 (33)	5 (63)	0.59
Gestational age, weeks, median (IQR)	35.4 (33.2 to 37.2)	34.9 (32.2 to 36.3)	37 (33.4 to 39)	0.47
Length of initial hospitalization, d, median (IQR)	120 (91 to 163)	116.5 (93 to 174)	120 (83 to 175)	0.77
Race/ethnicity, n (%)				
Black	3 (21)	3 (50)	0	0.06
Caucasian	11 (79)	3 (50)	8 (100)	0.06
Primary diagnosis, n (%)				
Necrotizing enterocolitis	2 (14)	2 (33)	0	0.17
Malrotation/Volvulus	4 (28)	1 (17)	3 (38)	0.58
Complex gastroschisis *	6 (44)	3 (50)	3 (38)	1.00
Intestinal atresia	2 (14)	0	2 (25)	0.47
Anatomical characteristic				
Residual small bowel length, cm, median (IQR)	35 (24.5 to 55)	24.5 (13.8 to 30)	55 (37.5 to 85)	<0.01
Estimated residual small bowel remaining, %, median (IQR)	22 (20 to 30)	20 (15 to 21)	33 (25 to 65), n=6	0.01
Ileocecal valve present, n (%)	4 (28)	0	4 (50)	0.09
Ostomy present, n (%)	5 (36)	4 (67)	1 (13)	0.09
Bowel lengthening procedure, n (%)	2 (14)	1 (17)	1 (13)	1.00
Partial or total colectomy, n (%)	7 (50)	5 (83)	2 (25)	0.10
Complication				
Culture proven sepsis per 1000-line days, median (IQR)	2.5 (1.5 to 6.3)	4.9 (1.2–9.5)	2.3 (1.3 to 3)	0.28
History of small bowel bacterial overgrowth, n (%)	6 (43)	2 (33)	4 (50)	0.63
Current oral antibiotic use, n (%)	1 (7)	1 (17)	1 (13)	1.00
History of cholestasis, n (%)	7 (50)	5 (83)	2 (25)	0.10

Age at time of stool collection.

* Complex gastroschisis defined as gastroschisis with 1 or more intestinal complications such as atresia, volvulus, perforation or necrosis.

SBS, short bowel syndrome

Table 2.

Growth and Nutrition Information for Patients with <35 cm and ≥35 cm Small Bowel Remaining

Growth and nutrition characteristic	All SBS Patients (n=14)	Small bowel < 35 cm (n=6)	Small bowel ≥ 35 cm (n=8)	p Value
Anthropometric measurement, median (IQR)				
Weight Z score	−0.3 (−0.6 to 0.5)	0.1 (−0.6 to 0.8)	−0.5 (−0.6 to −0.2)	0.16
Height Z score	−1.2 (−1.6 to −0.1)	−1.8 (−2.5 to −0.9)	−0.5 (−1.2 to −0.1)	0.05
BMI Z score	0.5 (−0.1 to 1)	0.8 (0.5 to 2.4)	0 (−0.6 to 0.8)	0.05
Tricep skinfold thickness, %	20 (8.3 to 82.5), n=10	80 (20 to 92.5), n=5	10 (3 to 17.5), n=5	0.02
Subscapular skinfold thickness, %	10 (6 to 63.8), n=10	60 (12.5 to 86), n=5	7 (3 to 10), n=5	0.02
Nutritional characteristic, median (IQR)				
Total energy intake, kcal/kg/day	68 (56.5 to 72.5)	56.5 (49.8 to 66)	72 (68 to 100)	0.01
Parenteral energy intake	37 (3.8 to 78.3)	97.5 (84 to 100)	33 (0 to 59.5)	<0.01
Enteral energy intake	63 (21.8 to 96.2)	2.5 (0 to 16.3)	67 (40.5 to 100)	<0.01
Protein intake, gm/kg/day	2.4 (2 to 2.5)	2.5 (2.3 to 2.6)	2 (2 to 2.5)	0.26
Lipid intake, gm/kg/day	1 (0 to 1)	1 (0.8 to 1.1)	0.5 (0 to 1)	0.16
Glucose infusion rate, mg/kg/min	12 (11 to 18)	16 (10.8 to 22.3)	12 (11 to 15)	0.36
Time on parenteral nutrition, y	2.5 (1.4 to 3.8)	3.2 (2.1 to 3.8)	1.9 (0.8 to 3.2)	0.25
Liver biochemistry, median (IQR)				
Peak direct bilirubin, gm/dL	2.9 (0.9 to 4.4)	4 (2.8 to 6.7)	1.3 (0.2 to 2.9)	0.09
Peak aspartate aminotransferase	236.5 (108 to 466.8)	405.5 (236.8 to 672.5)	128 (91.5 to 286.5)	0.04
Peak alanine aminotransferase	300.5 (235.8 to 398)	300.5 (263.5 to 494.5)	305 (172.8 to 366.3)	0.61

Characteristics obtained at time of stool collection.

IQR, interquartile range; SBS, short bowel syndrome

Table 3.

Key Clinical Factors for Each Short Bowel Syndrome Patient

SBS patient *	Age, y	Diagnosis	Remnant small bowel length, cm	Estimated proportion of remnant small bowel, %	Proportion of enteral nutrition intake, %	Weight for age Z score	Height for age Z score	<i>Proteobacteria</i> relative abundance, %
<35 cm remnant small bowel								
Subject 1	3.5	Volvulus	10	8	0	0.7	-2.2	97
Subject 2	2.9	Necrotizing enterocolitis	15	22	0	1	-2.5	68
Subject 3	1.3	Complex gastroschisis	22	14	10	0.5	0.2	58
Subject 4	16.9	Necrotizing enterocolitis	27	23	35	-1.2	-2.6	23
Subject 5	1.8	Complex gastroschisis	30	20	5	-0.4	-1.5	79
Subject 6	4	Complex gastroschisis	30	20	0	-0.2	-1.3	22
35 cm remnant small bowel								
Subject 7	3.1	Complex gastroschisis	35	--	100	-0.4	-1.5	7
Subject 8	1.8	Intestinal atresia	37.5	21	30	0.7	0	28
Subject 9	1.2	Volvulus	40	23	100	-0.6	-0.7	4
Subject 10	0.9	Volvulus	55	74	39	-0.6	-1	26
Subject 11	5.9	Complex gastroschisis	55	36	71	-0.3	-0.2	37
Subject 12	1.1	Volvulus	85	--	100	-0.2	-1.3	5
Subject 13	4.2	Intestinal atresia, Hirschprung's	130	81	45	-0.6	-0.2	<0.5
Subject 14	2.1	Complex gastroschisis	140	98	63	-1	0.6	19

* Information was obtained at the time of the cross-sectional analysis using the most recently collected stool as a time reference.

SBS, short bowel syndrome

Table 4.

Serum Inflammatory Cytokines in 7 of 14 Patients with Short Bowel Syndrome

Short bowel syndrome patient	TNF- α (<22 pg/mL)	IFN- γ (<3 pg/mL)	IL-1 β (<36 pg/mL)	IL-6 (<5 pg/mL)	IL-8 (<8 pg/mL)
Sample 1	19.05	4.97	0.08	2.65	194.9
Sample 2	4.51	7.62	0.02	0.45	102.9
Sample 3	3.22	1.38	0	0.2	15.67
Sample 4	3.25	1.37	0.75	0.86	10.17
Sample 5	3.56	5.85	0	0.38	11.56
Sample 6	2.85	2.19	0	0.28	15.42
Sample 7	3.47	4.93	0	0.55	13.13

Normal serum values are depicted in parentheses below each cytokine.