The effect of different dietary structure on gastrointestinal dysfunction in children with cerebral palsy and epilepsy based on intestinal flora

Huang Congfu[1,*], Li Xiuyun[1], Peng Yuanping[4], Yang zhenyu[2], Ge Lan[3], Wu GenFeng[1], Huang Shuyuan[4], Wang Peiqin[1], Dai Wenkui[2], Lyu yansi[5], Wang Linlin[5], Zhang Anquan[6]

1. *Longgang District Maternity & Child Healthcare Hospital, Shenzhen, China, 518172
2. Yang zhenyu: School of Statistics and Data Science, Nankai University, Tianjin, China, 300000 ; Dai Wenkui: Department of Microbial Research, WeHealthGene Institute, Shenzhen, Guangdong, China, 518000；Joint Laboratory of Micro-ecology and Children's Health, Shenzhen Children's Hospital & Shenzhen WeHealthGene Co. Ltd., Shenzhen, Guangdong, China,518000
3. BGI Nutrition Precision Co., Ltd, Shenzhen, China, 518083
4. Longgang District Social Welfare Center, Shenzhen, China, 518116
5. Shenzhen University General Hospital, Shenzhen, China, 518000
6. Bao'an District Fuyong People's Hospital, Shenzhen, China, 510370

Corresponding author: Zhang Anquan, E-mail: 928572424@qq.com

Name of program: Longgang District Science and Technology Innovation Bureau (no. p020180720388406844161)

Primary author: Huang Congfu (1975–), associate chief physician on nerve and respiratory system diseases of children, 78333755@qq.com

Correspondence to: Zhang Anquan (1976–), associate chief physician on nerve and respiratory system diseases of children, 928572424@qq.com

Abstract

Background: Gastrointestinal (GI) difficulties are very common among children with cerebral palsy (CP) and comorbid epilepsy. GI function is influenced by dietary structure on intestinal flora. The aim of this study was to compare intestinal flora changes in two dietary groups of this population and examine whether such differences are related to GI dysfunction.

Methods: Forty children with CP and epilepsy were recruited from among patients being cared for at a social welfare center, including 23 consuming a (semi)fluid diet (liquid diet group) and 17 consuming a normal diet (general diet group). Bacterial DNA was extracted from feces, the V3-V4 region of the 16S rRNA gene was amplified from the DNA, and high-throughput sequencing of the amplified sequences was performed. Microbe prevalence levels were compared on multiple phylogenic levels.

Results: Gut microbial populations differed substantially between the liquid diet and general diet groups. The only two phyla that differed significantly between the two groups were *Bacteroidetes* (p = 0.034) and *Actinobacteria* (p = 0.013). Regarding representation of genera, *Prevotella* species were selectively predominant in the general diet group (25.849% vs. 3.612% in the liquid diet group, p < 0.001), while *Bifidobacterium* species were selectively predominant in the liquid diet group (24.929% vs. 12.947% in the general diet group, p = 0.013). The gut flora of children in the general diet group contained more butyric acid-producing flora which was also common in healthy people (e.g. *Lachnoclostridium, Dorea, Ruminococcus, Faecalibacterium, Roseburia,* and *Coprococcus*). The gut flora of children in liquid diet group however, were rich in symbiotic pathogenic bacteria (e.g. *Collinsella*, *Alistipes*, and *Eggerthella*).

Conclusion: The intestinal flora of children with CP and epilepsy consuming a liquid diet had elevated levels of symbiotic pathogens and diminished intestinal barrier protection bacteria, relative to a general diet group. These alterations in bacterial flora were associated with GI dysfunction symptoms.

Key words Obstipation; Cerebral palsy; Epilepsy; Intestinal flora; Gastrointestinal dysfunction

Background

Commonly, children with cerebral palsy (CP) experience multiple central nervous system dysfunctions. Epilepsy has been reported to be present in some 43% of pediatric CP patients (range, 35–62%) [1]. Quitadamo reported that nearly nine in ten children with CP experience digestive system problems—such as constipation, difficulty eating, salivation problems, dysphagia, gastroesophageal reflux, and among them constipation would affect 26%-74% children with cerebral palsy [2]. In our clinical experience, we find that almost every child with CP and comorbid epilepsy has some degree of gastrointestinal (GI) dysfunction, with the most bothersome one reported being obstipation, followed by bloating, vomiting, and recurrent GI bleeding [3].

GI function can be modulated by dietary influences on intestinal flora [4, 5, 6, 7, 8]. The present study included a group of children diagnosed with CP and epilepsy who ate a fluid or semifluid diet and a similarly diagnosed reference group of children consuming a normal diet. We compared GI microbial diversity between the two groups, and examined whether the children's GI difficulties could be related to their intestinal flora characteristics.

Methods

Patients and Study Design

A cohort of 40 children being cared for at the Longgang District Social Welfare Center who had CP and epilepsy diagnoses were enrolled in this study, including 23 on a (semi)fluid diet (liquid diet group) and 17 who were consuming a normal diet (general diet group). (Semi) fluid diets consisted predominantly of powdered milk as well as rice (paste) and soup. Normal diets included primarily

plant-based solid foods (i.e. cereals, potatoes, beans, fruits, vegetables, and products derived from these ingredients), as well as modest amounts of animal proteins and fats. The CP and epilepsy diagnoses were made by clinicians in the neurology departments of the three major hospitals in the region. Patients who had been diagnosed with a metabolic disease or a severe infection and those taking antibiotics or probiotics within 2 weeks before sample collection were not eligible for inclusion in the study. Informed consent was obtained from the legal guardian of each enrolled child.

Fecal sample collection and processing

Fecal specimens were collected from all participating patients; ~5 g was taken from the middle and center of each sample as a study specimen. Each 5-g specimen was frozen after collection at -80 °C in 30 min, and transported in dry ice to Beijing Nuohe Zhiyuan Technology Co., Ltd. for high-throughput sequencing. Microbial whole DNA was extracted from the specimens with MoBio's PowerSoil® DNA Isolation Kit, and then the V3-V4 region of the 16S rRNA gene was amplified by polymerase chain reaction. Universal primers (338F and 806R) were used with following condition: initial denaturation at 95°C for 3 min, followed by 27 cycles of denaturation at 95°C for 30 sec, annealing at 55°C for 30 sec, and extension at 72°C for 30 sec. The amplified sequence was subjected to high throughput sequencing in the Illumina Miseq Platform.

Analysis of sequencing data and species annotation

The sequencing data were filtered by a bioinformatics tool developed in our laboratory (available upon request), and the sequences were spliced with Flash software (v1.2.11, http://ccb.jhu.edu/software/flash/index.shtml). The spliced sequences were aggregated in USEARCH into operational taxonomic units, which were compared and annotated based on a bacterial library (Greengene v201305) to yield bacterial composition data for each specimen. The bacterial abundances

of the specimens were analyzed on the level of phylum, class, order, family, and genus.

Statistical analysis

Statistics were conducted in R software (v3.3.3) with the ade4 software package. Principle component analysis (PCA) was performed based on the composition and relative abundance of genera and the overall flora distributions of samples by group were plotted. Wilcoxon rank-sum test was used to detect inter-group differences in the presence of phyla and genera. The Benjamini-Hochberg method was applied to adjust the difference analysis results. Means are reported with standard deviations (SDs). P values < 0.05 and false discovery rates (FDRs) < 0.05 were considered statistically significant.

Results

GI symptoms

The incidence of GI dysfunction in the liquid diet group was significantly higher than that in the general diet group ($p < 0.05$). With the exception of diarrhea, each examined symptom was found significantly more frequently in the former group than in the latter (Table 1).

Phylum analysis of dominant bacteria and microbial composition

The percentages of the top-five most abundant bacterial phyla found in each group are reported in Table 2. A histogram of the relative abundance levels of bacterial phyla, by group, based on bacterial structure analysis is shown in Figure 1. The five most abundant bacterial phyla found in the general diet group were *Bacteroidetes*, *Firmicutes*, *Actinobacteria*, *Proteobacteria*, and *Fusobacteria*. The five most abundant bacterial phyla found in the liquid diet group were *Firmicutes*, *Actinobacteria*,

Bacteroidetes, *Proteobacteria*, and *Verrucomicrobia*. Only the *Bacteroides* (p = 0.034) and *Actinobacteria* (p = 0.013) phyla differed significantly between the two groups.

Genus analysis of dominant bacteria and microbial composition

The proportions of the top 15 flora, by abundance, were determined for each group and compared, as reported in Table 3. Two of the top-three genera for the general diet group (*Prevotella, Bifidobacteria*, and *Bacteroides*) were also in the top-three genera for the liquid diet group (*Bifidobacteria, Bacteroides*, and *Enterococcus*). There were numerous genera whose representations differed significantly between the two groups, the 15 most significant of which are reported in Table 4. Notably, *Prevotella, Roseburia, Latobacillus*, and *Faecalibacterium* were markedly more abundant in the general diet group than in the liquid diet group, whereas *Bifidobacterium* and *Collinsella* were markedly more abundant in the liquid diet group than in the general diet group.

Further analysis of gut microbiota exhibiting relative abundance levels greater than 0.1% together with significant inter-group differences at the genus level showed a pronounced enrichment of *Prevotella* in the general diet group (25.85%) relative to levels in the liquid diet group (3.61%), as well as a near two-fold enrichment of *Bifidobacterium* representation in liquid diet group (24.93%) compared with levels in the general diet group (12.95%). Notably, genera generally considered to be components of functional flora in a healthy GI tract (e.g. *Lachnoclostridium, Dorea, Ruminoccus, Faecalibacterium, Roseburia,* and *Coprococcus*) were significantly more abundant in the general diet group, whereas symbiotic pathogenic bacteria (e.g. *Collinsella, Alistipes,* and *Eggerthella*) were more abundant in the gut flora of children in the liquid diet group (all $p < 0.05$). These results suggest that the different dietary structures of the two groups led to significant differences in gut flora (Table 4).

PCA of inter-group bacterial differences

As shown in Figure 2, PCA showed a significant difference between the structures of intestinal flora in the liquid diet group compared to that in the general diet group ($p = 0.002$).

Discussion

In the present study, we found distinctively different gut microbial populations between patients with CP comorbid with epilepsy who consume a diet rich in plant-based solid foods and those fed a liquid diet, with the latter group exhibiting more GI dysfunction symptoms. *Prevotella* species were predominant in the general diet group, whereas *Bifidobacterium* species were predominant in the liquid diet group. The gut flora of children in the general diet group contained more butyric acid-producing flora, whereas the gut flora of children in liquid diet group were rich in symbiotic pathogenic bacteria.

The connection among dietary, intestinal flora and constipation

A multitude of factors can influence the composition and evolution of infant intestinal flora, including gestational age at birth, mode of delivery, mode of feeding, prenatal diet, prenatal use of antibiotics, and living environment. By the age of 3 years old, children should have a mature and stable gut flora, and diet composition becomes the most important factor in determining gut flora. Diet affects the constitution and metabolism of GI microbiota, and nutrient intake is important to the maintenance of gut flora [4]. Compared to European children who were fed with a higher fat diet, African children eating a fiber-rich diet have been reported to have higher level of the *Bacteroidetes,* especially *Prevotella*, and *Xylanibacter* genera, which are related to cellulose and xylan hydrolysis, with relatively low levels of *Firmicutes*, as well as more short-chain fatty acids (SCFAs) in their GI tracts [5]. High-fat diets have been linked to elevated levels of potentially pathogenic bacteria, especially *Fusobacterium* species [6].

Dietary interventions have been shown to improve the composition and functional status of gut flora [7], which may play an important role in the occurrence and development of constipation [8, 9]. A gut flora imbalance may lead to an insufficiency of probiotics, which impairs digestion and absorption functions, resulting in the accumulation of oligosaccharides in the intestines [10, 11, 12]. It can also disrupt normal intestinal flora composition and metabolite levels—such as by reducing SCFAs, stimulating neurotransmission (e.g. serotonergic transmission), and elevating of neurotoxic metabolites (e.g. lactic acid and ammonia). These changes alter related signaling pathways, leading to dysfunction of the gut-brain axis and impaired intestinal motility, which can cause constipation[10, 11, 12]. Park et al. postulated that colonal motor nerve dysfunction may be a major cause of constipation based on the finding that children with CP often show delayed emptying of the proximal colon and rectal sigmoid colon [13]. Altogether, this convergence of findings suggests that dietary structure is closely related to intestinal flora, and that insufficient fiber intake, reduced intestinal flora metabolites (e.g. SCFAs), and elevated levels of neurotransmitters and toxic metabolites may lead to constipation.

The influence of dietary fiber and SCFAs on the body

Dietary fiber is an indigestible form of carbohydrate that acts as a substrate for gut microbial fermentation. A carbohydrate-based diet has been associated with a *Prevotella*-dominant enterotype, with a higher fiber intake increasing the abundance of *Prevotella* in the gut [14,15]. Dietary fiber promotes the secretion of intestinal mucus, reduces oxygen levels in the gut, and helps to maintain normal physiological intestinal functions. Thus, long-term fiber insufficiency can change the intestinal flora structure, leading to a thinning of the mucus layer, which increases susceptibility to pathogenic bacterial infections and chronic inflammatory diseases. SCFA metabolites can promote differentiation of regulatory T cells and induce acetylation of histone-H3 in a G-protein-coupled receptor 43-dependent manner, while a high-fat diet has been associated with delayed maturation of the thymus,

reduced numbers of thymocytes, and apoptosis of developing T cells; the health-promoting characteristics of dietary fiber appear to depend on microbial metabolite (e.g. SCFA), regulation of physiological changes (such as lowering pH) and protection of the mucosal barrier [16,17]. Gut microbe-produced SCFAs (especially butyrate), which are highly concentrated in the cecum and proximal colon, serve as an energy source for colon cells. The major gut SCFAs (acetate, propionate and butyrate) influence the liver and peripheral tissues when they are transported into peripheral circulation through the portal vein. In this way, microbial products play a key role in regulating metabolism, immune function, and cell proliferation.

SCFAs are produced in the colon mainly through fermentation of dietary fiber, digestion-resistant starch, oligosaccharides, and non-digestible sugars by beneficial genera of bacteria, such as *Lactobacillus* and *Bifidobacteria*. The physiological effects of SCFAs include: 1) providing energy and regulating electrolytes; 2) protecting the intestinal mucosal barrier (by mechanical and chemical strengthening); 3) influencing pH and inhibiting the growth of pathogenic bacteria and of conditional pathogens, such as *Escherichia coli* and *Shigella*; 4) intestinal immune regulation to resist pathogens; 5) an anti-tumor effect; 6) regulation of intestinal motility; and 7) promotion of intestinal mucosa growth [18-20]. Intake of foods high in carbohydrates can provide the gut with dietary fiber, and thus support the production of SCFAs. Both dietary fiber and SCFAs can help stabilize intestinal flora, thereby strengthening gut barrier protection and supporting immune system development.

Intestinal flora in children in the general diet group

Prevotella, Roseburia, Lactobacillus, and *Faecalibacterium* species were found to be significantly higher in children in the general diet group than in the liquid diet group. *Prevotella*, which represented the most divergent genus between the two groups, can degrade broad-spectrum plant polysaccharides and support carbohydrate-rich food digestion. Besides, it can contribute to the biosynthesis of vitamin

B1 and support the metabolism of fish oil, both of which are beneficial for brain development [5,14, 21, 22, 23]. The relatively low incidence of GI dysfunction among children in our general diet reference group, who were able to eat normally and participate in group activities, could be related to their fiber-supplying, carbohydrate-based diet which lead to a high abundance of protective functional bacteria for intestinal barrier such as *Prevotella, Lactobacillus,* and *Faecalis* in the intestinal flora. R*oseburia* species, which accounted for the 10th most prevalent genus in the general diet group, can break down a variety of carbohydrates into SCFAs, including beneficial butyrate, while inhibiting secretion of the proinflammatory cytokine interleukin-17 and promoting the differentiation and maturation of mucosal regulatory T cells, thereby having an anti-inflammaotry influence on the colon [24,25]. *Faecalibacterium* can also increase butyrate and exert an anti-inflammatory effect [26]. Butyrate, which is absorbed and used by intestinal epithelial cells, can regulate the growth and development of epithelial cells and act as an energy source. In this way, butyrate can lessen inflammatory colitis and Crohn's disease, and also inhibit proliferation of colon cancer cells [27]. *Lactobacillus* species are beneficial to host health; they ferment sugar, produce lactic acid, and regulate immune function [28]. As the main energy source for intestinal epithelial cells, butyrate supports the mucosal barrier of the colon and promotes the production of antibacterial peptides. Butyrate can also promote the repair of injured gastric mucosa and provide protection from ulcers by increasing cell proliferation [29]. Our finding of enriched butyrate-producing flora in the intestinal flora of children in the general diet group supports the notion that the low incidence of GI disorders in these children may be consequent to gut flora mediated protection of the intestinal mucosal barrier and maturation of immune function.

Intestinal flora in children from liquid diet group

Bifidobacterium, Collinsella, Alistipes, and *Eggerthella* species were found to be significantly higher in the GI tracts of children in the liquid diet group than in those of children in the general diet group, while bacteria related to the digestion of dietary fiber and carbohydrates, such as *Prevotella* and *Lactobacillus* species, were markedly decreased. *Bifidobacterium* is a functional genus of flora found in high abundance in the intestines of breast-fed infants, and its abundance decreases as whole foods are introduced. Thus, intestinal enrichment of *Bifidobacterium* in childhood reflects an immaturity of intestinal flora [30]. Children in liquid diet group with milk-based liquid diet for a long-term might result in *Bifidobacteria* mainly in adolescent and infant types, and a lack of *Bifidobacterium breve*. As a result, the concomitant lack of butyrate-producing flora may compromise immune function and intestinal mucosal integrity. An elevated abundance of *Collinsella* species has been associated with low dietary fiber intake [31]. Conversely, increasing *Collinsella* bacteria can reduce the expression of tight junction proteins in intestinal cells, enabling intestinal leakage and, consequently, symptoms of metabolic endotoxemia [32, 33]. An abundance of *Alistipes* has been related to abdominal pain frequency in patients with irritable bowel syndrome and thus has been speculated to cause intestinal inflammation [34]. *Eggerthella lenta* is an important human pathogen associated with severe gastrointestinal disorders [35].

Low GI levels of SCFA in the children in our liquid diet group can be attributed to their limited intake of dietary fiber and carbohydrates. Such a SCFA deficiency can lead to a compromised state of intestinal barrier protection, which is normally associated an adequate presence of *Clostridium* and *Lactobacillus* species. Conversely, an elevated presence of potentially pathogenic bacteria, including *Collinsella, Alistipes,* and *Eggerthella*, can lead to chronic inflammation in the intestinal tract and a weak immune system function in the GI tract. As a result, proliferation of potentially pathogenic species, such as *Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Escherichia coli*, can lead to endogenous infection of the intestinal tract. Repeated antibiotic treatment to reduce the presence of

these pathogenic microbes can, eventually, lead to drug-resistant strains and a consequent transition from an endogenous infection to an exogenous infection, resulting in recurrent GI infections requiring repeated hospitalizations [36]. Moreover, serious intestinal flora imbalances which caused by the factors above can aggravate GI dysfunction symptoms, which further limits the absorption of essential nutrients, thereby forming a vicious cycle. Our findings showing deficiencies in butyrate- and lactate-producing gut bacteria in the liquid diet group suggest that these children have an immature complement of symbiotic pathogenic bacteria that does not support maintenance of the protective intestinal barrier or immune function, and these conditions may underlie their increased prevalence of GI dysfunction.

Research summary and further research ideas

Children with CP and epilepsy who eat a diet rich in plant-based solid foods were found to have abundant levels of beneficial bacteria, including *Roseburia, Lactobacillus,* and *Faecalibacterium*, in their intestinal flora, which is conducive to the digestion of dietary fiber and enables the production of SCFAs, which protect the intestinal mucosa while promoting immune development and maturation. Relatively decreased levels of protective functional bacteria with elevated levels of pathogenic bacteria in children fed a (semi)liquid diet may impede development of immune function. This underdeveloped immune function can lead to chronic gut inflammation and GI dysfunction. The most common GI dysfunction symptom, obstipation, can reduce quality of life severely. Although the *Bifidobacterium* genus was abundant in this group of children, the functionally important species *Bifidobacterium breve* was lacking.

In the present study, our gene sequence analysis was limited to the 16SrRNA gene. Thus there is a need for a macro factor analysis in future research. In addition, it will be important to examine the effects of dietary fiber and *Lactobacillus* and *Tyrobacter* intervention on changes in gut flora and GI

dysfunction symptoms in children with CP and epilepsy who eat a liquid or semi-liquid diet. Additionally, because our study population was limited to children cared for at the Longgang District Social Welfare Center, it is not known whether similar diet-related disparities in gut flora and GI dysfunction would be found among children with CP and epilepsy who are raised in their family homes. Thus, larger-sample, multi-center and clinical intervention studies are needed to determine the generalizability of the findings obtained here.

Conclusions

The present study explored the effect of different dietary structure which including the liquid diet and the general diet on gastrointestinal dysfunction in children with cerebral palsy and epilepsy. And it demonstrated that there was a huge difference on gut microbial populations between the two groups. The intestinal flora of children with CP and epilepsy consuming a liquid diet had elevated levels of symbiotic pathogens and diminished intestinal barrier protection bacteria, while for children in the general diet group the intestinal flora contained more butyric acid-producing flora which was also common in healthy people. Even though there were some limitations in the study, it inspires clinical staffs who are working on improving the quality of life among the children with cerebral palsy and epilepsy and provides theoretical basis for the following intervention.

Abbreviations

CP: Cerebral palsy

FDR: false discovery rates

GI: Gastrointestinal

PCA: Principle component analysis

SCFA: short-chain fatty acids

SD: standard deviations

Declarations

Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of the Longgang District Maternity & Child Healthcare Hospital in Shenzhen, China (registration number LGFY2017005). Written informed consent was obtained through Longgang District Social Welfare Center which supports scientific research and investigation of the children in its care.

Consent for publication

Not applicable

Availability of data and materials

The dataset generated for this study can be viewed in the NCBI sequence archive under biological project number PRJNA530084.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Huang Congfu, Zhang Anquan, Yang Zhenyu, and Ge Lan managed the project. Peng Yuanping, Huang Shuyuan, Wang Peiqin, and Li Xiuyun took fecal samples and collected information. Huang Congfu, Zhang Anquan, and Yang Zhenyu prepared DNA. Yang Zhenyu and Dai Wenkui were

responsible for the bioinformatics analysis. Huang Congfu, Ge Lan, and Li Xiuyun analyzed and interpreted the results and wrote the manuscript. Yang Zhenyu, Zhang Anquan, and Wu Genfeng optimized the graphics and statistical analyses. Dai Wenkui directed the project design and revised the article. Lyu Yansi and Wang Linlin helped with the writing of the manuscript. All of the authors reviewed the manuscript before submission.

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Figure legends

Fig 1. Histogram of relative abundance of bacterial phyla based on bacterial structure analysis. Phylum percentages are reported in Table 2. Only *Bacteroides* and *Actinobacteria* percentages differed significantly (see Results text) between the liquid diet (N = 23) and general diet (N = 17) groups.

Fig 2. PCA comparison between study groups of intestinal flora. Each specimen (one per patient) is represented by a data point (red, general diet; blue, liquid diet). PCA revealed a significant inter-group structural difference ($p = 0.002$).

Tables

Table 1 Inter-group comparison of the prevalence (N) of GI dysfunction symptoms in pediatric patients with CP and epilepsy.

Symptom	General diet group N = 17	Liquid diet group N = 23	*P*
Obstipation	1	23	<0.001
Bloating	1	8	<0.05
Diarrhea	2	0	>0.05
Vomiting	2	10	<0.05
GI bleeding	0	6	<0.05

Table 2 Top five most prevalent bacterial phyla in each group.

General diet group		Liquid diet group	
Top 5 phyla	**Mean ± SD %**	**Top 5 phyla**	**Mean ± SD %**
Bacteroidetes	41.83 ± 27.12	*Firmicutes*	35.25 ± 17.71
Firmicutes	34.28 ± 15.00	*Actinobacteria*	30.99 ± 18.42
Actinobacteria	16.68 ± 20.69	*Bacteroidetes*	25.45 ± 17.92
Proteobacteria	4.96 ± 3.94	*Proteobacteria*	4.96 ± 4.21
Fusobacteria	0.94 ± 1.43	*Verrucomicrobia*	2.07 ± 2.49

Table 3 Top 15 genera of gut microbes in terms of representation by group.

General diet group		Liquid diet group	
Top 15 genera	**Mean ± SD (%)**	**Top 15 genera**	**Mean ± SD (%)**
Prevotella	25.85 ± 26.11	*Bifidobacterium*	24.93 ± 15.60
Bifidobacterium	12.95 ± 17.04	*Bacteroides*	12.13 ± 11.31
Bacteroides	8.45 ± 8.91	*Enterococcus*	6.15 ± 16.59
Parabacteroides	3.92 ± 7.47	*Parabacteroides*	5.13 ± 4.92
Streptococcus	2.79 ± 3.24	*Collinsella*	4.51 ± 3.96
Faecalibacterium	2.32 ± 2.19	*Prevotella*	3.61 ± 7.38
Collinsella	2.15 ± 4.05	*Streptococcus*	2.71 ± 3.65
Sutterella	1.68 ± 1.94	*Akkermansia*	2.02 ± 2.51
Acidaminococcus	1.35 ± 3.95	*Megasphaera*	1.79 ± 3.10
Roseburia	1.35 ± 1.78	*Blautia*	1.48 ± 2.79
Megasphaera	1.33 ± 3.87	*Alistipes*	1.45 ± 2.29
Alloprevotella	1.32 ± 1.82	*Eubacterium*	1.40 ± 4.45
Enterococcus	1.13 ± 2.78	*Faecalibacterium*	1.16 ± 2.24
Catenibacterium	1.02 ± 1.71	*Desulfovibrio*	0.87 ± 1.97
Megamonas	1.02 ± 3.13	*Sutterella*	1.68 ± 1.94

Table 4 Intestinal flora genera with significantly differing levels between the general diet and liquid diet groups.

Bacteria（Genus）	Mean Value		P Value
	The General Diet Group（%）	The Liquid Diet Group（%）	
Eggerthella	0.007	0.065	0.001
Prevotella	25.849	3.612	0.001
Lachnoclostridium	0.236	0.763	0.001
Dorea	0.160	0.053	0.001
Coprococcus	0.258	0.053	0.002
Ruminococcus	0.388	0.232	0.002
Faecalibacterium	2.324	1.156	0.002
Lactobacillus	0.582	0.060	0.003
Roseburia	1.347	0.093	0.004
Turicibacter	0.008	0.005	0.006
Collinsella	2.153	4.510	0.007
Corynebacterium	0.150	0.089	0.011
Alistipes	0.351	1.453	0.013
Bifidobacterium	12.947	24.929	0.013
Anaerococcus	0.001	0.013	0.014

Difference screening conditions: average relative abundance > 0.1%, $p < 0.005$, and FDR < 0.05.

Supplementary material

Supplementary materials associated with this article are available online at:

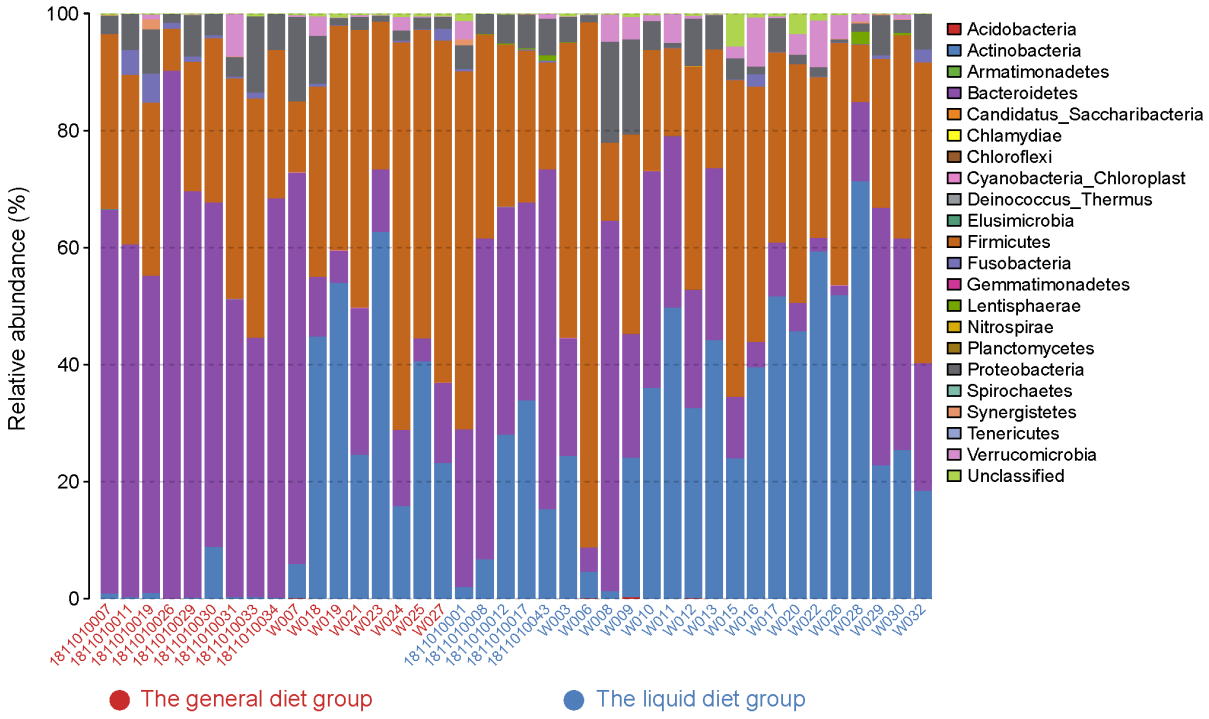
https://www.frontiersin.org/articles/10.3389/fped.2019.00394/full#

Supplementary Document 1 | Background information of registered subjects.

Supplementary Document 2 | Phylum and genus distributions of genetically modified organisms in all study subjects.

Supplementary Document 3 | Functional distribution of gut microbiota of study subjects according to the KEGG database.

Fig. 1


Relative abundance (%)
100
80
60
40
20
0
Acidobacteria
Actinobacteria
Armatimonadetes
Bacteroidetes
Candidatus_Saccharibacteria
Chlamydiae
Chloroflexi
Cyanobacteria_Chloroplast
Deinococcus_Thermus
Elusimicrobia
Firmicutes
Fusobacteria
Gemmatimonadetes
Lentisphaerae
Nitrospirae
Planctomycetes
Proteobacteria
Spirochaetes
Synergistetes
Tenericutes
Verrucomicrobia
Unclassified
1811010007
1811010011
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1811010030
1811010031
1811010033
1811010034
W007
W018
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W021
W023
W024
W025
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1811010001
1811010008
1811010012
1811010017
1811010043
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W026
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W029
W030
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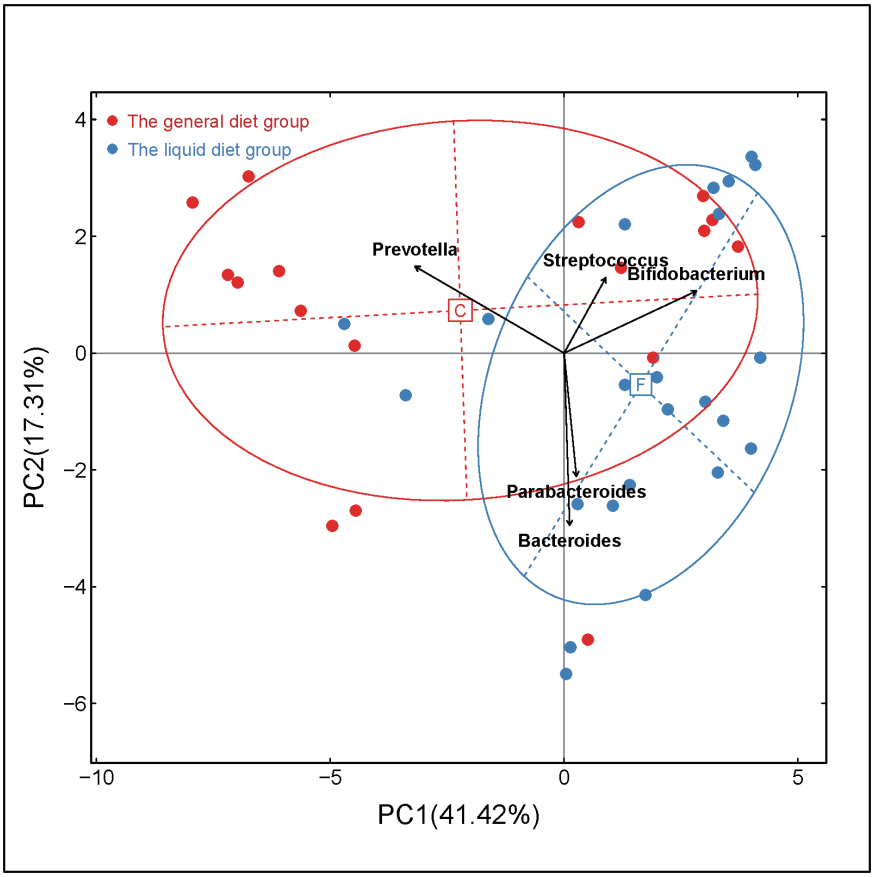
The general diet group
The liquid diet group

Fig 2.


The general diet group
The liquid diet group
Prevotella
Streptococcus
Bifidobacterium
Parabacteroides
Bacteroides
C
F
PC2(17.31%)
PC1(41.42%)
4
2
0
−2
−4
−6
−10
−5
0
5