Characteristics of gut microbiota of term small gestational age infants within 1 week and their relationship with neurodevelopment at 6 months

Xiaona Chen†, Zheng Yan†, Lili Liu, Rui Zhang, Xiaojiao Zhang, Cheng Peng, Yuehang Geng, Faliang Zhou, Ying Han* and Xinlin Hou*

Department of Pediatrics, Peking University First Hospital, Beijing, China

Introduction: Small for gestational age (SGA) infants are at a higher risk of neurodevelopmental delay than infants appropriate for gestational age (AGA). Previous studies have confirmed that gut microbiota in early life influences subsequent neurodevelopment. However, few studies have reported corresponding data in SGA populations.

Objective: We aimed to evaluate the characteristics of the gut microbiota of term SGA infants and the associations between the gut microbiota in SGA infants and neurodevelopmental outcomes at 6 months of age.

Methods: Fecal samples were collected on days 1, 3, 5, and 7 from term SGA and AGA infants born between June 2020 and June 2021 at the Peking University First Hospital. 16S ribosomal deoxyribonucleic acid amplicon sequencing was used to analyze the fecal microbiota. We followed up for 6 months and used the Ages and Stages Questionnaires-3 (ASQ-3) to evaluate the neurodevelopmental outcomes among SGA infants.

Results: A total of 162 neonates were enrolled, with 41 SGA infants (25.3%) in the study group and 121 AGA infants (74.7%) in the control group. The gut microbial diversity in the SGA group was lower than that in the AGA group on days 1, 3, 5, and 7. Non-metric multidimensional scaling and analysis of similarities showed significant differences between the two groups. The SGA group had increased relative abundances of *Ralstonia* (3, 5, and 7 days) and *Clostridium* (3 and 7 days). The dominant microorganisms of the SGA group were *Ralstonia* on day 1, *Escherichia_Shigella* on days 3 and 7, and *Clostridia* on day 5. We found that the gut microbial diversity of SGA infants with poor communication scores was higher than that of SGA infants with good communication scores on day 3. Fine motor scores were negatively correlated with the relative abundance of *Bacteroides_fragilis* on day 1. A negative correlation was observed between gross motor scores and relative abundance of *Clostridium_saccharobutylicum* on day 1.
7. Bacteroidota, Bacteroidia, Bacteroides, and Bacteroides_fragilis were the dominant microorganisms in the good communication score group on day 7. Communication scores were positively correlated with the relative abundance of Bacteroidota, Bacteroides, and Bacteroides_fragilis on day 7.

Conclusion: The gut microbial diversity of term SGA infants was significantly lower in the first week of life than that of term AGA infants. Certain pathogenic and conditional pathogenic bacteria, such as Escherichia_Shigella, Ralstonia and Clostridium increased or formed the dominant microbiota in SGA infants. Alpha diversity, Bacteroidota, Bacteroides, Bacteroides_fragilis, and Clostridium_saccharobutylicum found in SGA infants may be associated with neurodevelopmental outcomes at 6 months of age, indicating possible therapeutic targets for clinical intervention.

KEYWORDS
gut microbiota, Bacteroides, SGA, neonates, neurodevelopment

Introduction

Small for gestational age (SGA) infants, defined as having a birth weight less than the 10th percentile of the birth weight of the same sex at the same gestational age, comprise a heterogeneous group (Chen et al., 2017; McCowan et al., 2018; Chawla, 2019). The incidence of SGA in China is ~ 6.5%, ranking fifth worldwide (Lee et al., 2013). The development of each SGA system is imperfect, and the incidence of neurodevelopmental delay is significantly higher than that in appropriate for gestational age (AGA) infants (Sharma et al., 2016; McCowan et al., 2018; Kesavan and Devskar, 2019). At present, the mechanisms leading to neurodevelopmental delays are unclear.

In recent years, gut microbiota has become a research hotspot in the fields of biology and medicine. Researchers have realized that the gut microbiota plays an important role in digestion, immune response, nutrient absorption, growth, and metabolism. The gut microbiota is involved in the regulation of many diseases such as inflammatory bowel disease, metabolic syndrome, and diabetes (Adak and Khan, 2019; Dabke et al., 2019; Ma et al., 2019; Mentella et al., 2020). Studies have found that microbiota plays a significant role in early neurological development (Carlson et al., 2018; Cohen Kadosh et al., 2021; Seki et al., 2021).

The so-called "gut–brain axis" represents a two-way communication network between the gut microbiota and the brain. The gut–brain axis theory proposes that the gut microbiota participates in the regulation of brain development and maturation, thus impacting brain functions, including anxiety-like behavior, locomotor behavior, social cognition, learning, and working memory (Al-Asmakh et al., 2012; Cryan et al., 2019; Long-Smith et al., 2020; Saurman et al., 2020). Compared with mice with normal gut microbiota, germ-free mice showed more obvious short-term cognitive and working memory impairments, whereas probiotic treatment prevented memory impairment after an inflammatory response in mice (Gareau et al., 2011). Gut microbiota affects various normal psychological processes and phenomena, participating in the pathophysiology of several psychological and neurological diseases (Li et al., 2018). It has been reported that the gut microbial composition is altered in children with autism (Finegold et al., 2010; Plaza-Diaz et al., 2019; Dan et al., 2020; Saurman et al., 2020; Wong et al., 2022) and adults with Parkinson’s disease (Vascellari et al., 2020; Hirayama and Ohno, 2020) and Alzheimer’s disease (Zhuang et al., 2018; Bostanciklioglu, 2019). Many studies have shown that probiotics are effective against anxiety, depression, autism spectrum disorder (ASD), and obsessive-compulsive disorder, and can also improve cognitive function, learning, and memory ability (Wang et al., 2016; Eastwood et al., 2021; Kim et al., 2021; Aleomhammad et al., 2022). The intake of probiotics may ameliorate neurodegenerative disorders, including Alzheimer’s disease, multiple sclerosis, Parkinson’s disease, and amyotrophic lateral sclerosis (Cheng et al., 2019; Roy Sarkar and Banerjee, 2019).

Research focusing on children has indicated associations between gut microbiota in the first year of life and subsequent early neurodevelopment (Carlson et al., 2018; Sordillo et al., 2019; Tamana et al., 2021). Researchers have found that the alpha diversity of the gut microbiota in 1-year-old children could predict cognitive function at 2 years of age (Carlson et al., 2018). A cohort study found strong evidence of positive associations between Bacteroidetes in late infancy and subsequent cognitive and language performance.
Another cohort study observed an association between the gut microbiome composition of infants aged 3–6 months and communication—personal and social—and fine motor skills at 3 years of age (Sordillo et al., 2019).

At present, there are many studies on the development and establishment of gut microbiota in healthy neonates. However, studies on the characteristics and evolution of the gut microbiota in SGA infants and their relationship with long-term neurodevelopmental outcomes remain scarce. Therefore, the objective of this study was to explore the characteristics of the gut microbiota of SGA infants during the first week of life using high-throughput sequencing technology. Additionally, this study aimed to further explore the potential relationship between gut microbiota and neurodevelopmental prognosis of SGA infants at 6 months of age. The discovery of the effects of specific microbiota on neural development would provide important insights into potential therapeutic targets for the clinical improvement of neurological development in SGA infants.

Subjects and methods

Subjects

Term SGA and AGA neonates hospitalized in the pediatric neonatal ward of Peking University First Hospital between June 2020 and June 2021 were recruited for this study.

Inclusion criteria

The following inclusion criteria were used: (a) neonates in the study group needed to meet the diagnostic criteria of SGA infants: newborns whose birthweight was less than the 10th percentile of the birth weight of the same sex at the same gestational age (1); (b) gestational age was defined as ≥ 37 and < 42 weeks; (c) neonates without asphyxia, neonatal hypoxic-ischemic encephalopathy, severe intracranial hemorrhage, cerebral infarction, cytomegalovirus infection, recurrent hypoglycemia, bilirubin encephalopathy, and genetic metabolic diseases were enrolled; (d) informed consent was provided by the legal guardian(s); and (e) neonates were only enrolled with the agreement of cooperation with the follow-up by the legal guardian(s).

Exclusion criteria

The following exclusion criteria were used: (1) critical clinical conditions, such as sepsis and multiple organ failure; (2) gastrointestinal malformation, abdominal distension, vomiting, diarrhea, bloody stool, necrotizing enterocolitis, and other gastrointestinal diseases within 1 week; and (3) the presence of diseases that might affect neurological development during the follow-up period, such as severe brain trauma, epilepsy, meningitis, and genetic metabolic diseases.

Methods

Data collection

Clinical data, including sex, gestational age, birth weight, mode of delivery, and antibiotic application within 1 week after birth, were collected. Feces produced on postnatal days 1, 3, 5, and 7 were collected. Fecal samples were stored in sterile freezing tubes (Haimen Morder Experimental Equipment Factory) at −20°C and subjected to microbiota analysis within 1 week.

Gut microbiota test and analysis

Microbiota sequencing

A biological information database was built using an Illumina TruSeq DNA PCR-Free Sample Preparation Kit. Quality was evaluated with the assistance of the Qubit® 2.0 and Agilent Bioanalyzer 2100 system. High-throughput sequencing was performed using an Illumina NovaSeq 6000 platform.

Bioinformatics analysis

The effective data were obtained by filtering the original data. The sequences were then clustered into operational taxonomic units (OTUs) with 97% identity, and the OTUs sequences were compared with the silva138 database for species annotation to obtain the basic analysis results of the OTUs and taxonomic pedigree for each sample. Finally, the analysis of OTUs, including alpha and beta diversity, was completed according to species annotation.

- Alpha diversity analysis: The richness and diversity of microbiota can be indicated by alpha diversity, wherein Observed species, Chao1, abundance-based coverage estimator (ACE), Shannon, Simpson, and goods coverage are major evaluation indices of alpha diversity. Observed species represents the actual number of OTUs in the sample. The Chao1 and ACE indices use different calculation methods to estimate the number of OTUs in a sample; the higher the number of OTUs, the higher the diversity of the sample. The abundance and uniformity of the gut microbiota can be expressed using the Shannon and Simpson indices. If all the OTUs contained in the sample were the same, the diversity was the lowest; if they were different, the diversity was the highest. The larger the values of the Shannon and Simpson indices, the higher the diversity of the samples. Good coverage index indicates the sequencing depth; the higher the value, the better the sequencing. The closer the value is to 1, the closer the sequencing depth is to cover all bacteria in the test sample. Rarefaction and rank variance curves are
mainly includes five parts, namely communication, gross motor, fine motor, problem-solving, and personal-social, with each part containing six specific assessment questions.

Statistical analysis
Statistical software (SPSS 25.0) was used to analyze the data. Measurement data consistent with a normal distribution are expressed as the mean ± standard deviation (x ± s). Student’s t-test was used to compare two groups, while analysis of variance (ANOVA) was used to compare three or more groups. The measurement data that were not in line with the normal distribution were expressed as median (IQR) or median (P25, P75). The Wilcoxon Mann-Whitney U test was used for the comparison between two groups, while the Kruskal-Wallis H test was used for the comparison of three groups and above. The enumeration data were expressed as the number of cases and percentages, and comparisons between groups were performed using the χ² test. If the total number n was < 40 or at least one actual frequency, t < 1, Fisher’s exact test method was applied. For the correlation analysis of two quantitative datasets, Pearson correlation analysis was adopted if it conformed to the bivariate normal distribution; otherwise, Spearman correlation analysis was adopted. Statistical significance was set at P < 0.05.

Ethical approval
The study was approved by the Ethics Committee of the Peking University First Hospital. The legal guardians of each participant provided written informed consent.

Results
Clinical characteristics of neonates in the small for gestational age and appropriate for gestational age groups
A total of 41 SGA neonates were enrolled in the SGA group, including 19 males (46.3%) and 24 neonates (58.5%) delivered via cesarean section. A total of 121 AGA neonates were enrolled in the AGA group, with 75 males (62.0%) and 33 neonates (27.5%) delivered via cesarean section. All neonates were born at a gestational age of 37–42 weeks and fed a mixed feed (breast milk + formula). The clinical characteristics of the enrolled neonates are presented in Table 1. A total of 31 neonates (75.6%) in the SGA group and 112 neonates (92.6%) in the AGA group had aspiration pneumonia or increased non-specific inflammatory indices. The proportion of ampicillin users in the AGA group was significantly higher than that in the SGA group. The gestational age and birth weight of the neonates in the SGA group were significantly lower than those in the AGA group, and the proportion of cesarean sections was significantly higher in the SGA group. The following clinical characteristics differed significantly between the SGA and AGA groups: infants from twin pregnancies, premature rupture of membranes, hospital
stay of neonates, chorioamnionitis, and maternal antibiotics. There were no significant differences in sex, Apgar scores at 1 and 5 min, region, siblings, mother’s pregnancy weight gain, pregnancy complications (diabetes or gestational hypertension), or pet ownership between the two groups. None of the enrolled neonates were infected with the novel coronavirus, and neither their mothers nor their family members showed the emergence of pandemic-related mental or personality disturbances.

Gut microbiota analysis of the small for gestational age and appropriate for gestational age groups

In this study, an average of 76,816 tags were measured per sample, and an average of 75,108 valid data points was obtained after quality control. The sequence was clustered into OTUs with 97% identity and 13,747 OTUs were obtained.

Sequencing depth and rationality

After obtaining all the OTUs, a rarefaction curve was drawn to evaluate whether the current sequencing depth of each sample could fully reflect the microbial diversity in the community samples. When the dilution curve tended to be flat (Supplementary Figure 1), the sequencing data gradually became reasonable. The coverage index of these samples fluctuated between 0.974 and 1, indicating that the sequencing depth was close to covering all bacterial communities in the tested samples.

Alpha and beta diversities

A comparison of the alpha diversity of fecal microbiota in the SGA group on different days in the first week of life revealed no statistical difference in the Chao1, ACE, Observed species, Simpson, and Shannon indices, indicating that there was no significant difference in the richness and diversity of gut microbiota within the SGA group after postnatal days 1, 3, 5, and 7 (Supplementary Table 1).

A comparison of alpha diversity of fecal microbiota between the SGA and AGA groups revealed that the SGA group’s gut microbial diversity was significantly lower than that of the AGA group in the Chao1, ACE, Observed species, Simpson, and Shannon indices on the first day (< 0.05). On days 3, 5, and 7, the Chao1, ACE, and Observed species indices of fecal microbiota of the SGA group remained significantly lower than the AGA group (P < 0.05), whereas there was no significant difference in the Simpson and Shannon indices (Supplementary Figure 2 and Supplementary Table 2).

PCoA showed significant differences in the gut microbiota on days 1, 3, 5, and 7 between the two groups. The R-value > 0, and statistical analysis between groups showed significant differences (P < 0.05) (Supplementary Figures 3A–H and Supplementary Table 3). The NMDS analysis (Supplementary Table 4) indicated that the gut microbiota of the two groups differed significantly on days 1, 3, 5, and 7 (stress score < 0.2) (Figure 1).

Analysis of differential gut microbiota

Differential relative abundance

The main microbiota in the AGA group at the phylum level were Firmicutes, Proteobacteria, Actinobacteria, and Bacteroidetes (Supplementary Figures 4, 5); at the family level, Enterococcaceae, Streptococcaceae, Enterococcales, Staphylococcaceae, Vibrionaceae (Supplementary Figures 6, 7); and at the genus level, Enterococcus, Streptococcus, Escherichia-Shigella, Staphylococcus, and Vibrio (Supplementary Figures 8A, 9).

On day 1, the SGA group showed a decreased relative abundance of Cyanobacteria, Vibrionaceae, Parabacteroides, Lactiplantibacillus, Serratia, Citrobacter, and Catibacterium. However, the relative abundance of Ileibacterium was higher in the SGA group than in the AGA group (Table 2, Figure 2, and Supplementary Figures 10, 11).

On day 3, the SGA group showed decreased relative abundances of Actinobacteria, Streptococcaceae, Vibrionaceae, Streptococcus, Vibrio, Pseudalteromonas, Uruburuella, Parabacteroides, and Lactiplantibacillus, whereas Burkholderiaceae, and Ralstonia were higher in the SGA group than in the AGA group (Table 2, Figure 2, and Supplementary Figures 10, 11).

On day 5, the SGA group showed decreased relative abundances of Streptococcaceae, Lysinibacillus, Streptococcus, Lactiplantibacillus, Catibacterium, Serratia, and Citrobacter, while those of Campylobacteria, Verrucomicrobiota, Burkholderiaceae, Erysipelotrichaceae, Micrococcaceae, Helicobacteraceae, Ileibacterium, and Akkermansia were higher in the SGA group than in the AGA group (Table 2, Figure 2, and Supplementary Figures 10, 11).

On day 7, the SGA group showed decreased relative abundances of Actinobacteria, Cyanobacteria, Streptococcaceae, Lysinibacillus, Lactiplantibacillus, and Serratia, whereas those of Burkholderiaceae, Erysipelotrichaceae, Ralstonia, Ileibacterium, Akkermansia, Halomonas, and Rhodococcus were higher in the SGA group than in the AGA group (Table 2, Figure 2, and Supplementary Figures 10, 11).

Differential dominant microorganisms

LEfSe was used to analyze differentially dominant microorganisms, and the LDA value was set to 4. On day 1, the dominant microorganisms in the SGA group were g-Ralstonia and s-Ralstonia_pickettii, while s-Streptococcus_sp_FDAARGOS_192, f-Vibrionaceae, and g-Vibrio were dominant in the AGA group. On day 3, the dominant microorganisms in the SGA group were s-Ralstonia_pickettii and g-Escherichia_Shigella, while f-Streptococcaceae, g-Streptococcus, and s-Streptococcus_sp_FDAARGOS_192...
were dominant in the AGA group. On day 5, the dominant microorganisms in the SGA group were *c-Clostridia*, *g-Rothia*, *s-Bacteroides fragilis*, and *o-Clostridiales*, while *f-Streptococcaceae*, *g-Streptococcus*, and *s-Streptococcus_sp_FDAARGOS_192* were dominant in the AGA group. On day 7, the dominant microorganisms in the SGA group were *s-Ileibacterium_valens*, *g-Ileibacterium*, *f-Enterobacteriaceae*, *g-Helicobacter*, and *g-Escherichia_Shigella*, while *s-Streptococcus_sp_FDAARGOS_192* were dominant in the AGA group (Figure 3 and Supplementary Figures 12A–D).

Correlation analysis between the top six microbiota at the genus and species levels in the small for gestational age group and ASQ-3 scores at 6 months of age

Neonates in the SGA group were followed up to 6 months of age, of which, 38 (92.7%) infants completed the follow-up and three infants were lost to follow-up (7.3%). The fine motor scores of ASQ-3 were negatively correlated with the relative abundance of *s-Bacteroides fragilis* on day 1 (\( r = -0.412, P = 0.041 \)). On day 7, the communication scores were positively correlated with the relative abundances of *g-Bacteroides* (\( r = 0.875, P = 0.004 \)) and *s-Bacteroides fragilis* (\( r = 0.886, P = 0.003 \)), whereas a negative correlation was observed between gross motor scores and the relative abundance of *s-Clostridium_saccharobutylicum* (\( r = -0.736, P = 0.037 \); Table 3).

Analysis of gut microbiota of neonates in small for gestational age group with different neurological prognosis (communication)

We followed SGA infants until 6 months of age, and 38 of them completed the follow-up. A communication score \( \geq 40 \) was considered normal. There were 31 (81.6%) infants with normal communication scores and 7 (18.4%) infants with poor communication scores in the SGA group. The two subgroups showed no significant differences in sex, gestational

### TABLE 1 Clinical characteristics of the SGA and AGA groups.

| Descriptive variable                  | SGA group \( n = 41 \) | AGA group \( n = 121 \) | Statistic value | \( P \) |
|--------------------------------------|-------------------------|--------------------------|-----------------|------|
| Male                                 | 19 (46.3%)              | 75 (62.0%)               | 3.076           | 0.079|
| Gestational age (weeks)              | 37.6 (1.3)              | 39.3 (1.6)               | -4.946          | < 0.001|
| Birthweight (grams)                  | 2352.7 ± 300.6          | 3282.3 ± 331.7           | 15.66           | < 0.001|
| Infants from twin pregnancy          | 10 (24.4%)              | 2 (1.7%)                 | 19.887          | < 0.001|
| Cesarean                             | 24 (58.5%)              | 33 (27.5%)               | 12.872          | < 0.001|
| Premature rupture of membranes       | 2 (4.9%)                | 36 (29.8%)               | 10.553          | 0.001|
| Apgar score at 1 min                 | 10 (0)                  | 10 (0)                   | 0.428           | 0.669|
| Apgar score at 5 min                 | 10 (0)                  | 10 (0)                   | 0.852           | 0.394|
| Mixed fed (formula + breast-feeding) | 41 (100%)               | 121 (100%)               | -               | > 0.999|
| Ampicillin to neonates (first week)  | 31 (75.6%)              | 112 (92.6%)              | 6.942           | 0.008|
| Hospital stay of neonates (days)     | 7 (2)                   | 6 (1)                    | -3.712          | < 0.001|
| Sibling                              | 14 (34.1%)              | 30 (24.8)                | 1.354           | 0.245|
| Mother’s age (years)                 | 33.0 ± 3.6              | 32.2 ± 3.8               | -0.343          | 0.732|
| Mother’s pregnancy weight gain (kg)  | 12.0 (4.8)              | 13.6 (5.0)               | -1.273          | 0.203|
| Maternal smoking                     | 0 (0%)                  | 0 (0%)                   | -               | > 0.999|
| Gestational hypertension             | 8 (19.5%)               | 10 (8.3%)                | 2.866           | 0.090|
| GDM or DM                            | 10 (24.4%)              | 36 (29.8%)               | 0.433           | 0.511|
| Antenatal TG (mmol/L)                | 2.22 (1.82)             | 2.56 (1.67)              | -0.286          | 0.775|
| Antenatal TCHO (mmol/L)              | 5.87 (2.38)             | 5.93 (2.40)              | -0.485          | 0.628|
| Antenatal HDL (mmol/L)               | 1.63 (1.00)             | 1.69 (0.00)              | -0.080          | 0.936|
| Antenatal LDL (mmol/L)               | 3.14 (1.40)             | 2.82 (1.54)              | -1.115          | 0.265|
| Chorioamnionitis                     | 11 (26.8%)              | 16 (13.2%)               | 4.082           | 0.043|
| Antibiotics to mother                | 8 (19.5%)               | 48 (39.7%)               | 5.501           | 0.019|
| Antenatal corticosteroids            | 0 (0%)                  | 1 (0.8%)                 | -               | > 0.999|
| Inclusion site-countryside           | 2 (4.9%)                | 6 (5.0%)                 | 0.00            | > 0.999|
FIGURE 1
NMDS analysis between the SGA and AGA groups on days 1, 3, 5, and 7. (A) NMDS analysis on day 1. (B) NMDS analysis on day 3. (C) NMDS analysis on day 5. (D) NMDS analysis on day 7. NMDS, non-metric multi-dimensional scaling. The red points represent belonging to the AGA group, and the green points represent belonging to the SGA group. S11, gut microbiota of the SGA group on day 1; A11, gut microbiota of the AGA group on day 1; S13, gut microbiota of the SGA group on day 3; A13, gut microbiota of the AGA group on day 3; S15, gut microbiota of the SGA group on day 5; A15, gut microbiota of the AGA group on day 5; S17, gut microbiota of the SGA group on day 7; A17, gut microbiota of the AGA group on day 7.

age, birth weight, mode of delivery, feeding pattern, or antibiotic application within 1 week after birth (Table 4).

Alpha and beta diversities
A comparison of the alpha diversity of fecal microbiota between the good and poor communication score groups revealed no statistical difference in the Chao1, ACE, Observed species, Simpson, and Shannon indices on days 1, 5, and 7. On day 3, the gut microbial diversity of the poor communication score group was significantly higher than that of the good communication score group in the Chao1, ACE, and Observed species indices (P < 0.05), whereas there was no significant difference in the Simpson and Shannon indices (Figure 4 and Supplementary Table 5).

PCoA and Anosim showed no significant differences in the gut microbiota on days 1 and 7 between the good and poor communication score groups; although the R-value was > 0, but the statistical analysis between the groups showed no significant difference (P > 0.05). There were no significant differences in gut microbiota on days 3 and 5 in the good and poor communication score groups; the R-value was < 0, but the statistical analysis within groups showed no significant difference (P > 0.05) (Supplementary Figures 13A–D and Supplementary Table 6). However, NMDS analysis indicated that the gut microbiota of the two groups differed significantly on days 1, 3, 5, and 7 (stress score < 0.2) (Supplementary Figures 14A–D).

Analysis of differential gut microbiota
Differential relative abundance
The main microbiota in the good communication score group included Firmicutes, Proteobacteria, Actinobacteria, and...
| Taxonomy       | Days | Microbiota                  | SGA group | AGA group | P   |
|---------------|------|-----------------------------|-----------|-----------|-----|
| **Phylum**     |      |                             |           |           |     |
| (P < 0.05)    |      |                             |           |           |     |
| D1            |      | Cyanobacteria                | $3.22 \times 10^{-4}$ | $1.18 \times 10^{-2}$ | 0.005 |
| D3            |      | Actinobacteria               | $3.32 \times 10^{-2}$ | $6.69 \times 10^{-2}$ | 0.030 |
| D5            |      | Campylobacteris              | $3.31 \times 10^{-4}$ | $7.20 \times 10^{-6}$ | 0.010 |
| D7            |      | Verrucomicrobiota            | $8.33 \times 10^{-4}$ | $1.62 \times 10^{-5}$ | 0.017 |
|                |      | Actinobacteria               | $1.45 \times 10^{-2}$ | $5.12 \times 10^{-2}$ | 0.011 |
|                |      | Cyanobacteria                | $4.09 \times 10^{-5}$ | $4.72 \times 10^{-3}$ | 0.005 |
| **Family** (P < 0.05) |      |                             |           |           |     |
| D1            |      | Vibrionaceae                 | $4.95 \times 10^{-3}$ | $4.37 \times 10^{-2}$ | 0.021 |
| D3            |      | Streptococaceae              | $7.23 \times 10^{-2}$ | $1.74 \times 10^{-1}$ | 0.003 |
|                |      | Burkholderiaceae             | $8.85 \times 10^{-2}$ | $7.22 \times 10^{-4}$ | 0.002 |
|                |      | Vibrionaceae                 | $7.40 \times 10^{-4}$ | $6.88 \times 10^{-3}$ | 0.003 |
| D5            |      | Streptococaceae              | $1.18 \times 10^{-1}$ | $2.58 \times 10^{-1}$ | 0.002 |
|                |      | Burkholderiaceae             | $9.37 \times 10^{-3}$ | $4.69 \times 10^{-4}$ | 0.022 |
|                |      | Erysipelotrichaceae          | $1.40 \times 10^{-3}$ | $9.85 \times 10^{-5}$ | 0.048 |
|                |      | Micrococcaceae               | $2.85 \times 10^{-2}$ | $1.94 \times 10^{-3}$ | 0.016 |
|                |      | Helicobacteriaceae           | $3.22 \times 10^{-4}$ | $5.85 \times 10^{-6}$ | 0.016 |
| D7            |      | Streptococaceae              | $9.06 \times 10^{-2}$ | $2.85 \times 10^{-1}$ | 0.027 |
|                |      | Burkholderiaceae             | $1.29 \times 10^{-2}$ | $7.39 \times 10^{-5}$ | 0.003 |
|                |      | Erysipelotrichaceae          | $6.98 \times 10^{-2}$ | $1.69 \times 10^{-4}$ | 0.017 |
| **Genus** (P < 0.01) |      |                             |           |           |     |
| D1            |      | Parabacteroides              | $5.46 \times 10^{-5}$ | $1.02 \times 10^{-2}$ | 0.001 |
|                |      | Lactiplantibacillus          | $3.15 \times 10^{-6}$ | $4.84 \times 10^{-4}$ | 0.001 |
|                |      | Serratia                     | $2.31 \times 10^{-5}$ | $1.17 \times 10^{-3}$ | 0.005 |
|                |      | Citrobacter                  | $2.10 \times 10^{-6}$ | $1.29 \times 10^{-3}$ | 0.008 |
|                |      | Citrobacteriace              | $2.04 \times 10^{-4}$ | $2.93 \times 10^{-3}$ | 0.009 |
|                |      | Ileibacterium                | $1.04 \times 10^{-3}$ | $5.75 \times 10^{-6}$ | 0.008 |
| D3            |      | Streptococcus                | $7.19 \times 10^{-2}$ | $1.73 \times 10^{-1}$ | 0.003 |
|                |      | Vibrio                       | $7.40 \times 10^{-4}$ | $6.88 \times 10^{-3}$ | 0.003 |
|                |      | Pseudoalteromonas            | $1.74 \times 10^{-4}$ | $1.50 \times 10^{-3}$ | 0.004 |
|                |      | Ureaplasma                   | $3.28 \times 10^{-5}$ | $2.60 \times 10^{-4}$ | 0.004 |
|                |      | Parabacteroides              | $1.75 \times 10^{-4}$ | $2.41 \times 10^{-3}$ | 0.006 |
|                |      | Lactiplantibacillus          | $9.74 \times 10^{-6}$ | $1.86 \times 10^{-4}$ | 0.006 |
|                |      | Ralstonia                    | $8.85 \times 10^{-2}$ | $6.61 \times 10^{-4}$ | 0.001 |
| D5            |      | Lysinibacillus               | $0.00$     | $2.52 \times 10^{-3}$ | 0.001 |
|                |      | Streptococcus                | $1.18 \times 10^{-1}$ | $2.58 \times 10^{-1}$ | 0.002 |
|                |      | Lactiplantibacillus          | $4.36 \times 10^{-6}$ | $1.68 \times 10^{-3}$ | 0.002 |
|                |      | Citrobacteriace              | $2.29 \times 10^{-5}$ | $1.88 \times 10^{-4}$ | 0.008 |
|                |      | Serratia                     | $2.18 \times 10^{-6}$ | $2.11 \times 10^{-5}$ | < 0.001 |
|                |      | Citrobacter                  | $0.00$     | $1.75 \times 10^{-5}$ | < 0.001 |
|                |      | Ileibacterium                | $9.00 \times 10^{-4}$ | $1.35 \times 10^{-6}$ | 0.001 |
| D7            |      | Lysinibacillus               | $0.00$     | $1.01 \times 10^{-3}$ | 0.009 |
|                |      | Lactiplantibacillus          | $6.30 \times 10^{-6}$ | $4.74 \times 10^{-3}$ | 0.005 |
|                |      | Serratia                     | $0.00$     | $1.44 \times 10^{-3}$ | 0.001 |
|                |      | Ralstonia                    | $1.28 \times 10^{-2}$ | $5.57 \times 10^{-5}$ | 0.003 |
|                |      | Ileibacterium                | $6.45 \times 10^{-2}$ | $1.01 \times 10^{-6}$ | 0.002 |
|                |      | Akkermansia                  | $2.09 \times 10^{-3}$ | $8.10 \times 10^{-6}$ | 0.001 |
|                |      | Halomonas                    | $2.71 \times 10^{-4}$ | $0.00$       | 0.001 |
|                |      | Rhodococcus                  | $5.29 \times 10^{-4}$ | $1.01 \times 10^{-6}$ | 0.002 |
FIGURE 2
Results of heatmap analysis of species with significant differences between the SGA and AGA groups at the genus level. S11, gut microbiota of the SGA group on day 1; A11, gut microbiota of the AGA group on day 1; S13, gut microbiota of the SGA group on day 3; A13, gut microbiota of the AGA group on day 3; S15, gut microbiota of the SGA group on day 5; A15, gut microbiota of the AGA group on day 5; S17, gut microbiota of the SGA group on day 7; A17, gut microbiota of the AGA group on day 7.

FIGURE 3
LEfSe comparison between the SGA and AGA groups. (A) LDA score histogram of differential microbiota of the two groups on day 1. (B) LDA score histogram of differential microbiota of the two groups on day 3. (C) LDA score histogram of differential microbiota of the two groups on day 5. (D) LDA score histogram of differential microbiota of the two groups on day 7. p_ represents phylum level, c_ represents class level, o_ represents order level, f_ represents family level, g_ represents genus level, and s_ represents species level. The length of the column represents the LDA score, and the greater the score, the greater the influence of the dominant microbiota. LDA, linear discriminant analysis; LEfSe, linear discriminant analysis effect size. S11, gut microbiota of the SGA group on day 1; A11, gut microbiota of the AGA group on day 1; S13, gut microbiota of the SGA group on day 3; A13, gut microbiota of the AGA group on day 3; S15, gut microbiota of the SGA group on day 5; A15, gut microbiota of the AGA group on day 5; S17, gut microbiota of the SGA group on day 7; A17, gut microbiota of the AGA group on day 7.
### TABLE 3  Correlation analysis between the top six microbiota at genus and species level in the SGA group and ASQ-3 scores at 6 months postnatal age.

| Days and microbiota | ASQ-3 scores | Correlation index (<sup>r</sup>) | <p>

#### Bacteroidetes at the phylum level (Supplementary Figure 15); Enterococaceae, Burkholderiaceae, Enterobacteriaceae, Streptococcaceae, and Staphylococcaceae at the family level (Supplementary Figure 16); Enterococcus, Streptococcus, Escherichia-Shigella, Staphylococcus, Bacteroides, and Ileibacterium at the genus level (Supplementary Figure 17); and Ralstonia_pickettii, Streptococcus_sp_FDAARGOS_192, Bacteroides_fragilis, Ileibacterium_valen, Rothia_mucilaginosa, and Clostridium_saccharobutylicum at the species level (Supplementary Figure 18). On day 1, the poor communication score group showed decreased relative abundances of Enterobacteriaceae, Streptococcaceae, and Streptococcus (Supplementary Table 7 and Supplementary Figures 19A–D). On day 3, the main microbiota between the good and poor communication score groups showed no significant differences in the phylum, family, genus, and species levels (Supplementary Table 7 and Supplementary Figures 20A–D). On day 5, the poor communication score group showed decreased relative abundances of Staphylococcaceae and Staphylococcus, whereas the relative abundance of Enterococcus was higher in the poor communication score group (Supplementary Table 7 and Supplementary Figures 21A–D).

#### Differential dominant microorganisms

On day 1, the dominant microorganisms in the poor communication score group were f-Erysipelotrichaceae, f-Carnobacteriaceae, and g-Allobaculum. On day 3, they were p-Actinobacteria, c-unidentified Actinobacteria, and f-Peptostreptococcaceae. There were no dominant microorganisms in the poor communication score group on day 5. On day 7, the dominant microorganism in the poor communication score group was f-Peptostreptococcaceae. p-Bacteroidota, c-Bacteroidia, o-Bacteroidales, f-Bacteroidiaceae, g-Bacteroides, and s_Bacteroides_fragilis formed the dominant microorganisms in the good communication score group on day 7 (LDA score > 3). There were no dominant microorganisms in the good communication score group on days 1, 3, and 5 (Figure 5 and Supplementary Figure 23).

#### Analysis of differential microbiota and communication scores at 6 months of age

We analyzed the correlation between differentially abundant microbiota and communication scores at 6 months postnatal, and found that p-Bacteroidota, g-Bacteroides, and s_Bacteroides_fragilis were positively correlated with communication scores, on day 7. Moreover, there was no correlation between the rest of the
differentially abundant microbiota and communication scores (Supplementary Table 8).

**Effect of delivery mode on gut microbiota of small for gestational age neonates and ASQ-3 scores**

A total of 41 SGA neonates were enrolled, including 24 neonates (58.5%) delivered by cesarean section and 17 neonates (41.5%) through vaginal delivery. The two subgroups showed no significant differences in sex, gestational age, birth weight, feeding patterns, or antibiotic application within 1 week after birth (Supplementary Table 9).

**Alpha and beta diversities**

A comparison of alpha diversity of fecal microbiota between the cesarean birth and vaginal delivery groups revealed no statistical difference in the Chao1, ACE, Observed species, Simpson, and Shannon indices on days 1, 3, and 5 (Supplementary Table 10 and Supplementary Figure 24).

PCoA and Anosim showed no significant differences in gut microbiota between the cesarean birth and vaginal delivery groups; the R-value was > 0, but the statistical analysis between the groups showed no significant difference ($P > 0.05$; Supplementary Figures 25A–C, 26A–C and Supplementary Table 11). However, NMDS analysis indicated that the gut microbiota of the two groups differed significantly on days 1, 3, and 5 (stress score < 0.2; Supplementary Figures 27A–C).

**Differential relative abundance and dominant microorganisms**

Between the two groups, there were differences in the relative abundances of *Campylobacterota*, *Bacteroidota*, and *Actinobacteria* at the phylum level (Supplementary Figures 28A–C and Supplementary Table 12); *Bacteroidaceae* and *Tannerellaceae* at the family level (Supplementary Figures 29A–C and Supplementary Table 12); and Bacteroides, *Eubacteriumhallii* group, and *Ileibacterium* at the genus level (Supplementary Figures 30A–C and Supplementary Table 12). On day 1, the dominant microorganisms in the vaginal delivery group were *g-Ileibacterium* and *s-Ileibacterium_valens* (Supplementary Figure 31A); *f-Bacteroidaceae*, *g-Bacteroides*, and *s-Bacteroides_fragilis* on day 3 (Supplementary Figure 31B); and *p-Bacteroidota*, *c-Bacteroidia*, *o-Bacteroidales*, *f-Bacteroidaceae*, *g-Bacteroides*, and *s-Bacteroides_fragilis* on day 5. The dominant microorganisms in the cesarean birth group were *o-Staphylococcales*, *f-Staphylococcaceae*, and *g-Staphylococcus* (Supplementary Figure 31C).

In summary, the gut microbiota with differences between the two delivery modes mainly included *g-Bacteroides*, *g-Staphylococcus*, and *s-Ileibacterium*, whereas the gut microbiota with differences between the SGA and AGA groups mainly included *g-Escherichia_Shigella*, *g-Ralstonia*, *g-Clostridium*, and *g-Streptococcus*.

**Effect of delivery mode on ASQ-3 scores of small for gestational age infants at 6 months of age**

The ASQ-3 scores of SGA neonates at 6 months of age were not associated with the delivery mode (Supplementary Table 13).

**Effect of antibiotic application on gut microbiota of small for gestational age neonates and ASQ-3 scores**

A total of 41 SGA neonates were enrolled, including 31 neonates (75.6%) treated with antibiotics and 10 neonates (24.4%) treated without antibiotics, according to their condition. The two subgroups showed no significant differences in sex, gestational age, birth weight, feeding patterns, or delivery mode (Supplementary Table 14).

**Alpha and beta diversities**

A comparison of the alpha diversity of fecal microbiota between the group with antibiotics and the group without antibiotics revealed no statistical difference in the Chao1, ACE, Observed species, Simpson, and Shannon indices on days 1, 3, and 5 (Supplementary Table 15 and Supplementary Figure 32).
The R- and P-values were > 0.05 on days 1 and 5, indicating that there was no statistical difference between the two groups on days 1 and 5. On day 3, R-value < 0 and P > 0.05, indicating that there was no statistical difference between the two groups (Supplementary Figures 33A–C, 34A–C and Supplementary Table 16). However, NMDS analysis indicated that the gut microbiota of the two groups differed significantly on days 1, 3, and 5 (stress score < 0.2; Supplementary Figures 35A–C).

Differential relative abundance and dominant microorganisms

Between the two groups, there was a difference in the relative abundance of Gemmatimonadota at the phylum level (Supplementary Figures 36A–C and Supplementary Table 17); Carnobacteriaceae, Enterococaceae, Enterobacteriaceae, and Burkholderiaceae at the family level (Supplementary Figures 37A–C and Supplementary Table 17); Aminobacter, Georgenia, and Loigolactobacillus at the genus level (Supplementary Figures 38A–C and Supplementary Table 17). On day 1, the dominant microorganisms in the antibiotic-treated group were f-Micrococcaceae, g-Rothia, o-Micrococcales, and s-Rothia_mucilaginosa (Supplementary Figure 39A); s-Clostridium_saccharobutylicum, g-Staphylococcus, o-Staphylococcales, f-Staphylococcaceae, s-Clostridium_perfringens, s-Enterococcus_faecalis, s-Clostridioides_difficile, and g-Clostridioides on day 3 (Supplementary Figure 39B); and o-Lactobacillales, f-Enterococaceae, g-Enterococcus, and c-Bacilli on day 5. The dominant microorganisms in the group without antibiotics were o-Enterobacterales, f-Enterobacteriaceae, g-Escherichia_Shigella, g-Clostridium_sensu_stricto_1, f-Peptostreptococcaceae, g-Clostridioides, and s-Clostridioides_difficile (Supplementary Figure 39C).

In summary, the gut microbiota with differences between the two groups with different applications of antibiotics mainly included g-Rothia, g-Clostridioides, g-Staphylococcus, g-Enterococcus, g-Escherichia_Shigella, and g-Clostridium; while the gut microbiota with differences between the SGA and AGA groups mainly included g-Escherichia_Shigella, g-Ralstonia, g-Clostridium, and g-Streptococcus.
Effect of application of antibiotics on ASQ-3 scores of small for gestational age infants at 6 months of age

The ASQ-3 scores of SGA neonates at 6 months of age were not associated with the use of antibiotics (Supplementary Table 18).

Discussion

SGA infants exhibit more significant long-term health issues, including a variety of major and subtle neurodevelopmental delays, than their appropriate gestational age counterparts (Sharma et al., 2016; McCowan et al., 2018; Kesavan and Devaskar, 2019). At present, the mechanisms underlying neurodevelopmental delay in SGA infants are unclear. A growing number of studies have indicated that the gut microbiota plays an important role in early neural development (Carlson et al., 2018; Cohen Kadosh et al., 2021; Seki et al., 2021). Colonization and maturation of the gut microbiota overlap with the critical period of early brain development, and an imbalance in gut microbiota during the early postnatal period may disrupt the developmental programming of the brain through the gut–brain axis, leading to brain injury and long-term neurodysplasia later in life (Al-Asmakh et al., 2012; Cryan et al., 2019; Seki et al., 2021). Therefore, it is of great significance to study the association between gut microbiota and neural development in SGA infants and to explore the impact of specific microbiota on neural development.

In our study, the alpha diversity of gut microbiota in the SGA group was significantly lower than that in the AGA group on days 1, 3, 5, and 7, consistent with the findings of a previous study (Zhang et al., 2019). At the phylum level, the main microbiota of the SGA group were Firmicutes, Proteobacteria, Actinobacteria, and Bacteroidota, similar to the results of previous studies (Martí et al., 2021; Zhang et al., 2021). With respect to the differential abundance of gut microbiota between the SGA and AGA groups, Actinobacteria (3, 7 days) and Cyanobacteria (1, 7 days) were significantly lower in the SGA group. Furthermore, another study found that pigs with intrauterine growth restriction had a lower relative abundance of Actinobacteria (Che et al., 2019), consistent with observations of our study. Cyanobacteria have been reported to exhibit good anti-inflammatory, antioxidant, cholesterol-lowering, and antimicrobial activities (Ferrazzano et al., 2020). Campylobacterota, considered to be associated with intestinal and extraintestinal infections (Fitzgerald, 2015; Same and Tamma, 2018), were more abundant on day 5 and were the dominant microbiota on day 7 in the SGA group.

At the genus level, the main microbiota of the SGA group included Enterococcus, Ralstonia, Staphylococcus, Streptococcus, Escherichia-Shigella, and Bacteroides, consistent with findings of previous research (Bäckhed et al., 2015; Gabriel et al., 2018; Zhang et al., 2021). Among the differential microbiota between the SGA and AGA groups, Ralstonia and Ralstonia_picketti were the dominant microorganisms in the SGA group on day 1, and Ralstonia was higher in the SGA group on
In summary, large differences were observed in the gut microbiota between the SGA and AGA groups in the first 6 months, with changes in alpha diversity and functional connectivity between 1 year of age and 2 years of age (Carlson et al., 2018). Gut microbiota is associated with increased alpha diversity in AGA infants, which is beneficial for subsequent neurocognitive outcomes (Abrahamsson et al., 2014; Kostic et al., 2015; Aatsinki et al., 2015). Our findings are consistent with recent evidence that increased gut microbiota diversity in infancy usually has a high level of beneficial response in a mature gut microbiota community in infancy usually has a high level of beneficial response (Cox et al., 2019). After a 6 month follow-up, we found an association between the SGA and AGA groups in the first year of life, which can be summarized as follows: (1) increased alpha diversity of gut microbiota in AGA infants, the alpha diversity of gut microbiota was significantly lower; (2) certain pathogenic bacteria increased or formed a community in AGA infants; (3) certain beneficial bacteria decreased or formed a community in AGA infants; and (4) gut microbiota diversity and health conditions in children is mixed.
in the good communication score group on day 7. In addition, we observed correlations between increased abundances of \textit{p-Bacteroidota}, \textit{g-Bacteroides}, and \textit{s-Bacteroides_fragilis} on day 7 and improved communication scores at 6 months, consistent with findings of previous studies on the association between gut microbiota in late infancy and subsequent neurodevelopment (Carlson et al., 2018; Tamana et al., 2021). We found that the relative abundance of \textit{s-Bacteroides_fragilis} on day 1 was negatively correlated with the fine motor scores, similar to observation of a previous study on the association between \textit{Bacteroides}-dominant gut microbiota at 3–6 months and subsequent delayed fine motor skills (Sordilho et al., 2019). \textit{Bacteroides_fragilis} is a gram-negative obligate anaerobe with two subtypes. Enterotoxigenic \textit{B. fragilis} (ETBF), identified as a common opportunistic pathogen in clinical infections, mainly causes colitis and systemic inflammation with the stimulation of toxins or lipopolysaccharides. The second subtype, non-toxigenic \textit{B. fragilis} (NTBF), has been suggested as a potential probiotic in recent studies because of its ability to produce immunomodulatory substances such as polysaccharide A (PSA) and SCFAs (Sun et al., 2019; Qu et al., 2022). Non-toxigenic \textit{B. fragilis} was found to be capable of protecting mice from central nervous system demyelination; this protective mechanism depended on the production of IL-10 (Ochoa-Repáraz et al., 2010). In addition, it was found to improve communication, stereotyped movement, anxiety-like behavior, and sensorimotor behavior in ASD model mice, as well as reduce the increased expression of IL-6 (Hsiao et al., 2013). Decreased levels of intestinal \textit{Bacteroides} are also characteristic of children diagnosed with ASD (Dan et al., 2020; Iglesias-Vázquez et al., 2020). \textit{Clostridium_saccharobutylicum} has a strong ability to produce butyrate (Huang et al., 2018; Miguel et al., 2019), and butyrate, as a microbial metabolite, can indirectly affect host metabolism through the gut–brain axis (Stilling et al., 2016). It has been reported that butyrate can reduce the inflammatory response of microglia and the hippocampus, inhibit inflammatory activities, promote the production of BDNF, and repair injured nerves (Kundu et al., 2019). What puzzled us was the negative correlation between the relative abundance of \textit{s-Clostridium_saccharobutylicum} on day 7 and the gross motor scores at 6 months postnatal. This finding was unexpected and it is unclear why there was such a connection. Thus, we believe further investigation is required to confirm these results.

Briefly, gut microbial characteristics associated with neurological prognosis in SGA infants can be summarized as follows: (1) higher alpha diversity on day 3 was associated with poor communication performance in SGA infants at 6 months of age; (2) \textit{Bacteroidota}, \textit{Bacteroides}, \textit{Bacteroides_fragilis}, and \textit{Clostridium_saccharobutylicum} may be related to the neurodevelopmental outcomes of SGA infants at 6 months of age.

The development of neonatal gut microbiota is affected by several factors (Bäckhed et al., 2015; Rutayisire et al., 2016; Kapourchali and Cresci, 2020; Vanddenplas et al., 2020). Most early colonists in the gut of neonates are maternal, and the mode of delivery strongly affects the formation of the early gut microbiota in term infants (Bäckhed et al., 2015; Rutayisire et al., 2016; Kapourchali and Cresci, 2020; Coelho et al., 2021). Studies have shown that vaginally-delivered neonates have more abundant \textit{Bacteroides}, \textit{Bifidobacterium}, and \textit{Lactobacillus} (Bäckhed et al., 2015; Rutayisire et al., 2016; Coelho et al., 2021), whereas neonates delivered by cesarean section (CS) are enriched in \textit{Staphylococcus}, \textit{Streptococcus}, and \textit{Clostridium} (Coelho et al., 2021). In our study, there was no difference in the alpha diversity of SGA neonates born by vaginal delivery and CS on days 1, 3, and 5, while the alpha diversity of SGA neonates delivered by CS showed a decreasing trend on day 7. Some researchers also found lower microbial diversity in the gut of infants delivered by CS than in vaginally-delivered infants in the first week of life (Bäckhed et al., 2015; MacIntyre et al., 2015; Rutayisire et al., 2016; Shi et al., 2018). LEfSe showed that the dominant microorganisms in vaginally-delivered SGA neonates were \textit{g-Bacteroides} and \textit{g-Bifidobacterium}. \textit{Bacteroides} seem to increase in abundance in vaginally-delivered infants compared with CS-delivered infants (Gronlund et al., 1999; Kabeerdoss et al., 2013; Hesla et al., 2014; Jakobsson et al., 2014; Rutayisire et al., 2016; Coelho et al., 2021). In addition, \textit{Bifidobacterium} and \textit{Lactobacillus} found in the vaginal delivery group did not increase significantly, in contrast to other findings (Bäckhed et al., 2015; Rutayisire et al., 2016; Coelho et al., 2021). The dominant microbiota of the cesarean birth group was \textit{g-Staphylococcus} on day 5, similar to findings of previous studies (Li et al., 2018; Wampach et al., 2018; Coelho et al., 2021). However, \textit{Streptococcus} and \textit{Clostridium} found in the cesarean birth group did not increase significantly, in contrast to previous findings (Coelho et al., 2021). Therefore, SGA and delivery mode may have different effects on the development of gut microbiota in term neonates.

Antibiotic therapy can greatly alter the diversity and composition of neonatal gut microbiota (Nobel et al., 2015; Gasparini et al., 2016). Associations between antibiotic therapy and decreased microbial diversity, increased abundance of \textit{Firmicutes}, and decreased abundance of \textit{Bacteroides} and \textit{Bifidobacterium} have been reported (Gasparini et al., 2016; Ficara et al., 2020). In our study, despite no statistical difference between the alpha diversity of the group with antibiotics and the group without antibiotics, the alpha diversity of SGA neonates treated with antibiotics was lower in the first week of life, similar to previous findings (Gasparini et al., 2016; Ficara et al., 2020). Hence, we assumed that the therapeutic duration of antibiotics was insufficient to significantly disrupt gut microbial diversity. We also found that the composition of neonatal gut microbiota was altered by antibiotic therapy. However, there was no difference in
the relative abundance of *Firmicutes*, *Bifidobacterium*, and *Bacteroides* between the two groups, which differed from previous findings (Ficara et al., 2020).

This study is the first to analyze the characteristics and evolution of the gut microbiota of term SGA infants in the first week of life, and also the first to explore the potential association between specific microbiota and neural development in SGA infants. Alongside the novelty of this study, it is worth mentioning the following limitations. (1) This was a single-center study; there may be differences in the gut microbiota of SGA infants from other hospitals. The results need to be further confirmed by multicenter and large-exponent investigations. (2) We only studied the gut microbiota of term SGA infants within 1 week after birth and the neurological prognosis at 6 months of age; we will follow up with SGA infants and discuss the relationship between neonatal gut microbiota and neurological development by comprehensively considering various factors, such as education mode and society. (3) The ASQ-3 is a screening scale, and because of the prevalence of the novel coronavirus, it was difficult to use diagnostic scales such as the Bayley scale to evaluate the prognosis of neurodevelopment.

**Conclusion**

Compared to AGA infants, the gut microbial diversity of term SGA infants was significantly lower in the first week of life. Certain pathogenic and conditional pathogenic bacteria increased or formed the dominant microbiota in SGA infants, such as *Escherichia/Shigella*, *Ralstonia*, and *Clostridium*. This study suggests that there may be associations between alpha diversity, certain gut microbiota, and neurodevelopmental outcomes in SGA infants. The results showed that higher alpha diversity on day 3 was associated with poor communication performance in SGA infants at 6 months of age, and the gut microbiota factors affecting the prognosis of SGA infants included *Bacteroidota*, *Bacteroides*, *Bacteroides_fragilis*, and *Clostridium_saccharobutylicum*. SGA infants are at a higher risk for adverse neurodevelopmental outcomes; however, current methods for the clinical treatment of neurodevelopmental delay in SGA infants are limited. With further studies of the gut microbiota of SGA infants, we hope to provide further insights into the early treatment of SGA infants.

**Data availability statement**

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary material.

**Ethics statement**

The studies involving human participants were reviewed and approved by the Ethics Committee of Peking University First Hospital. Written informed consent to participate in this study was provided by the participants’ legal guardian/next of kin.

**Author contributions**

All authors listed have made great contributions to this work and have read and agreed to the published version of the manuscript.

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**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.

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**Supplementary material**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2022.912968/full#supplementary-material
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