Original Article

Investigation of the association between imbalance of the intestinal flora and infantile spasms: a pilot case-control study

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Background: The intestinal flora (IF) regulates brain function *via* the neuroendocrine and neuroimmune systems and influences the development of several neuropsychiatric diseases, including epilepsy. Here, we investigated the specific relationship between the IF and infantile spasms (IS), a specific form of epilepsy.

Methods: Twenty-three children suffering from IS were recruited from the Chinese PLA General Hospital. According to patient response to adrenocorticotropic hormone (ACTH) treatment, the cohort was subdivided into 2 groups: an ACTH-response group and an ACTH-no response (NR) group. A total of 21 healthy children were recruited as a control group (healthy controls: HCs) during the same time period. Fecal samples were collected from infants in the IS and HC groups, and the population of fecal microorganisms was analyzed by 16s ribosomal DNA sequencing. The α and β diversity of the fecal microflora was determined, and the relative abundance of each species was classified. Tax4Fun2 was used to analyze the metabolic pathways utilized by the microflora, and the Kyoto Encyclopedia of Genes and Genomes database was used to analyze differentially expressed genes and pathways.

Results: No significant differences existed in α or β diversity when compared between the IS and HC groups, nor between the ACTH-response and ACTH-NR groups which were separated before and after ACTH treatment. Although there was no significant difference between the ACTH-response and ACTH-NR groups with respect to α diversity, there was a significant difference in β diversity. Compared with that of the HCs, the IF of the IS group featured lower proportions of *Lactobacillus*, *Roseburia*, and *Lachnospira*, and a higher proportion of *Clostridium*. In the IS group, the proportion of *Staphylococcus* in the IF was higher before treatment than after treatment. Compared with the ACTH-NR group, the ACTH-response group had reduced populations of *Odoribacter*, *Phascolarctobacterium*, *Anaerotruncus*, *Mitsuakella*, and *Robinsoniella*. However, an increase was observed in the population of *Bifidobacterium*. A significant difference was also identified between the IS and HC groups with regard to the expression levels of genes associated with lipoic acid synthesis.

Conclusions: Our analysis demonstrated that imbalance of the IF may be involved in the pathogenesis of IS and is related to response to ACTH. Regulating the composition of the IF may pave the way to developing a potential adjuvant therapy for patients with IS.

Keywords: Infantile spasm (IS); gastrointestinal microbiome; adrenocorticotropic hormone; treatment

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Introduction

Infantile spasm (IS) is a rare and specific form of epilepsy that constitutes a serious threat to the health of infants. IS affects approximately 0.31 in 1,000 live births (1), and typically manifests as a cluster of spasms, hypsarrhythmia on electroencephalograms, and developmental delay (2). More than 200 known pathogenic causes of IS have been identified; however, the specific pathogenic mechanisms underlying IS have yet to be illuminated (3).

At present, adrenocorticotropic hormone (ACTH) is the first-line treatment for IS. However, the effects of ACTH are unsatisfactory, with the treatment attaining success in only 60% of cases. Furthermore, the mechanisms underlying the effects of ACTH on IS are not fully understood. Studies have suggested that ACTH may exert its effects on IS by creating negative feedback on the hypothalamus-pituitary-adrenal (HPA) axis and by inhibiting the release of corticotropin-releasing hormone (CRH) (4). Previous research has revealed IS to differ from other types of epilepsy and that dysfunction of the HPA axis is involved in its onset.

Several studies (5-7) have shown that the intestinal flora (IF) may affect brain function *via* mechanisms involving the brain-gut axis, and that imbalance of the IF may be associated with the occurrence of epilepsy. It is possible that the IF may participate in the epileptogenic process by mediating the pro-excitatory effects of peripheral inflammation via immune system activation. This process could occur through a series of mechanisms, including proinflammatory cytokine and chemokine release, modulation of neural networks via the production of neurotransmitters (particularly serotonin, γ-aminobutyric acid, and glutamate), and activity involving the balance between excitation and inhibition (E/I balance). The IF can also induce its effects by dysregulating the endocannabinoid system, adjusting the permeability of the gut barrier (for instance, by increasing the levels of lipopolysaccharide), and adjusting both neuroendocrine pathways (e.g., the HPA axis) and neural pathways (e.g., vagus afferents and the enteric nervous system) (5). However, no previous study has investigated the link between the IF and IS.

Here, we attempted to investigate the specific association between the IF and IS. The present study focused on children with IS who were being treated with ACTH in the Pediatrics Department of the First Medical Center of PLA General Hospital and used a group of healthy controls (HCs) for comparison.

We present the following case in accordance with the STROBE reporting checklist (available at http://dx.doi.org/10.21037/tp-20-384).

Methods

Study subjects

This study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The Ethics Committee of PLA General Hospital (reference no. 20190156) approved the study protocol. For each participant, written informed consent to participate in the study was obtained from the parents or a legal guardian.

From March to September 2019, 23 infants with IS were recruited from the Pediatrics Department of the First Medical Center of PLA General Hospital. All of the included patients met the specific diagnostic criteria for IS, as described by Hrachovy in 2013 (1). The age at onset for all cases was <1 year. The form of onset, the frequency of seizures, electroencephalographic manifestations, and the use of oral antiepileptic drugs (AEDs) prior to admission were recorded for each participant. These data were recorded again for each patient after 14 days of receiving treatment with ACTH (2.5 U/kg, ≤25 U; Biochemical and Pharmaceutical Co., Ltd., Shanghai, China) and magnesium sulfate (0.25 g/kg, ≤2.5 g). Twenty-one healthy infants, aged 6 to 15 months, were also recruited as HCs. None of the participants experienced any obvious problems, such as diarrhea, or respiratory or gastrointestinal infection, in the 4 weeks preceding the study initiation. It was also ensured that none of the subjects had taken any antibiotics or probiotics during the 4 weeks prior to the study commencing.

Collection of fecal samples

Fecal samples were collected from all infants in the study group on the day of admission and after the completion of ACTH treatment. For the collection of samples from the HCs, the infants defecated into containers in a dedicated room in the outpatient department. After collection, all samples were then frozen at –80 ℃ for analyses.

Response evaluation

At the end of the course of treatment, we evaluated short-term efficacy as a specific response. A “complete response”

(CR) denoted no spasms, a "partial response" (PR) denoted a reduction in seizure frequency by >50%, and "no response" (NR) denoted that the frequency of seizures had been reduced by <50% or had not been reduced at all.

Sequencing of 16S rRNA genes

For the analysis of fecal samples from the IS and HC groups, at least 200 mg of fecal material was collected into an Ex-DNA/RNA Extraction Kit (Tianlong Technology Co., LTD., Xi'an, China). Then, a polymerase chain reaction (PCR) was performed to amplify the V3-V4 hypervariable regions of the bacterial 16S rRNA gene using universal primers (319 forward 5´-ACTCCTACGGGAGGCAGCAG-3´; 806 reverse 5´-GGACTACHVGGGTWTCTAAT-3´) incorporating FLX Titanium adaptors and a barcode sequence. Subsequently, we pooled the purified amplicons in equimolar amounts and performed paired-end sequencing on a MiSeq platform (Illumina, San Diego, CA, USA) in accordance with standard protocols described by Majorbio Bio-Pharm Technology (Shanghai, China). Raw FastQ files were demultiplexed and quality-filtered using QIIME (q2; Version 1.50; https://qiime2.org). Then, trimmed sequences were clustered into operational taxonomic units (OTUs) with a 97% similarity cut-off using q2-deblur (https://qiime2.org). Finally, the taxonomic assignment of OTUs was carried out using naïve Bayes classifiers against a model provided by QIIME (gg-13-8-99-515-806-nb-classifier.qza).

Bioinformatics analysis and functional gene annotations

QIIME was also used for the calculation of diversity for the 16S rRNA gene sequencing analysis. Differences in α diversity were calculated using diversity indices (Chao1, Shannon, and Simpson). In contrast, β diversity was determined using weighted UniFrac phylogenetic distance matrices and visualized in principal component analysis (PCA) plots. Statistically significant differences in the relative abundance of genera were identified using linear discriminant analysis (LDA) effect size (LEfSe). Only LDA values >2 were considered to be significantly enriched.

OTUs, as well as the representative sequence obtained by QIIME2, were annotated using Tax4Fun2 (R version 3.5.3; R Institute for Statistical Computing, Vienna, Austria), with reference to the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. PCA and permutational multivariate analyses of variance (Adonis) were also carried out for the comparison of overall differences.

Sample size

Prior to performing the experiments, we determined that a sample size of 21 individuals was needed in each group in order to determine significant differences with a statistical power of 80% with a probability of a type I error of 5%.

Statistical analyses

All statistical analyses were performed in SPSS 21.0 (IBM, Armonk, NY, USA), with $P<0.05$ considered as the criterion for significance. Descriptive data were presented as the mean ± standard deviation if the data were normally distributed (as determined by the Kolmogorov-Smirnov test) and as the median (with 25^{th} and 75^{th} percentiles) if the data were not normally distributed. The independent-samples *t*-test was used to identify significant differences in normally distributed data, while the Mann-Whitney rank-sum test was used for data with non-normal distribution. Frequency data were compared using the Chi-squared test and the Fisher's exact probability method. Differences in metabolism between the 2 groups were identified through PCA and Adonis analyses.

Results

Comparison of clinical data

A total of 23 patients with IS were enrolled in the study. Following 14 days of treatment with ACTH, 18 patients who had a CR or PR to ACTH were identified as the ACTH-response group (ACTH-response group), and the remaining 5 patients, who had no response to ACTH, were identified as the no response group (ACTH-NR group). No significant differences were found between the ACTH-response group and the ACTH-NR group, or between the IS and HC groups, with respect to sex, age, birth mode, gestational age, feeding mode, body mass index (BMI), the number of AEDs, or the provision of supplementary food ($P>0.05$, *Tables 1* and *2*).

IF diversity and abundance of the fecal bacterial community

There were no significant differences between the IS and HC groups in terms of the α and β diversity of fecal

Table 1 A comparison of clinical data between the infantile spasms (IS) and healthy control (HC) groups

	Infantile spasms group (n=23)	Health control group (n=21)	χ^2/t/F	P
Sex				
Male (n, %)	9 (39.1)	9 (42.9)	0.063	0.802[a]
Female (n, %)	14 (60.9)	12 (57.1)		
Age (months, mean ± SD)	8.7±4.2	8.0±3.2	0.617	0.54[b]
Feeding patterns				
Exclusive breastfeeding (n, %)	10 (43.5)	10 (47.6)		0.427[c]
Formula milk (n, %)	7 (30.4)	3 (14.3)		
Partial breastfeeding (n, %)	6 (26.1)	8 (38.1)		
Body mass index (BMI) (month, mean ± SD)	17.6±2.3	17.1±1.2	0.75	0.391[b]
Mode of delivery				
Normal childbirth (n, %)	9 (39.1)	13 (61.9)	2.277	0.131[a]
Cesarean delivery (n, %)	14 (60.9)	8 (38.1)		
Gestational age				
<36 weeks (n, %)	4 (17.4)	1 (4.8)		0.666[c]
36–42 weeks (n, %)	18 (78.3)	19 (90.5)		
>42 weeks (n, %)	1 (4.3)	1 (4.8)		
Birth weight				
<2,500 g (n, %)	3 (13)	1 (4.8)		0.455[c]
2,500–4,000 g (n, %)	19 (82.7)	17 (81)		
>4,000 g (n, %)	1 (4.3)	3 (14.3)		
Solid food introduced	15 (65.2)	13 (61.9)	0.052	0.82[a]
Efficacy of ACTH				
ACTH-response	18 (78.3)			
ACTH-NR	5 (21.7)			

[a]Two-sided Chi-squared test; [b]Independent-samples t-test; [c]Fisher's exact test.

microbiota (P>0.05). The Mann-Whitney rank-sum test failed to identify any significant differences between the IS and HC groups at the genus level (P>0.05). However, LEfSe revealed that the IS group had reduced populations of *Lactobacillus*, *Roseburia*, and *Lachnospira*, along with an increased population of *Clostridium* (LDA>2), compared with the control group (*Figure 1*).

The ACTH-response and ACTH-NR groups showed no significant differences in terms of the α diversity of fecal microbiota (P>0.05); however, there was a significant difference in the β diversity of the 2 groups (P<0.05). The Mann-Whitney rank-sum test failed to identify any significant differences between the IS and HC groups at the genus level (P>0.05). However, in the ACTH-response group, LEfSe demonstrated reductions in the populations of *Odoribacter*, *Phascolarctobacterium*, *Anaerotruncus*, *Mitsuokella*, and *Robinsoniella*, as well as an increase in the population of *Bifidobacterium* (LDA>2), in comparison to the ACTH-NR group (*Figure 2*).

In the IS group, no significant differences were observed in the pre- and post-treatment α and β diversity of fecal microbiota (P>0.05). Also, the Mann-Whitney rank-sum test failed to identify any significant differences between the IS and HC groups at the genus level (P>0.05). LEfSe revealed a reduction in the population of *Staphylococcus* following ACTH treatment (LDA >2) (*Figure 3*).

Table 2 A comparison of clinical data between the response and no response (NR) groups to ACTH

	ACTH-response group (n=18)	ACTH-NR group (n=5)	P
Sex			
Male (n, %)	6 (33.3)	3 (60.0)	0.343[a]
Female (n, %)	12 (66.7)	2 (40.0)	
Age [months, M (P_{25}, P_{75})]	7 (6, 12)	9 (4, 13)	0.857[b]
Feeding patterns			
Exclusive breastfeeding (n, %)	9 (50.0)	1 (20.0)	0.166[a]
Formula milk (n, %)	6 (33.3)	1 (20.0)	
Partial breastfeeding (n, %)	3 (16.7)	3 (60.0)	
Body mass index (BMI) M (P_{25}, P_{75})	17.1 (15.3, 18.9)	18.1 (16.9, 19.75)	0.325[b]
Mode of delivery			
Normal childbirth (n, %)	7 (38.9)	2 (40.0)	0.131[a]
Cesarean delivery (n, %)	11 (61.1)	3 (60.0)	
Gestational age			
<36 weeks (n, %)	4 (22.2)	0	0.166[a]
36–42 weeks (n, %)	14 (77.8)	4 (80.0)	
>42 weeks (n, %)	0	1 (20.0)	
Birth weight			
<2,500 g (n, %)	3 (16.7)	0	0.309[a]
2,500–4,000 g (n, %)	15 (83.3)	4 (80.0)	
>4,000 g (n, %)	0	1 (20.0)	
Solid food introduced	12 (66.7)	3 (60.0)	1[a]
No. of oral AEDs			
0	9	3	1[a]
1	7	2	
2	2	0	

[a]Fisher's exact test; [b]Mann-Whitney rank-sum test.

In the IS groups, no significant differences were observed in the α and β diversity of fecal microbiota before and after treatment (P>0.05). Furthermore, neither the Mann-Whitney rank-sum test nor LEfSe identified any significant differences (P>0.05; LDA >2) (*Figures 4* and *5*).

KEGG-based comparison of metabolic pathways between the IS group and HC group based on IF testing

PCA and Adonis were used to determine global metabolic differences in 1st-, 2nd-, and 3rd-level KEGG pathways. Mann-Whitney rank-sum tests were also carried out on 6 1st-level pathways, 45 2nd-level pathways, and 326 3rd-level pathways. The pathway associated with lipoic acid metabolism showed significant differences between the IS group and the HC group. Patients in the IS group showed upregulation of certain genes, including *lipB* and *lipA* (P<0.05, *Figure 6*).

Discussion

In recent years, an increasing number of studies have shown

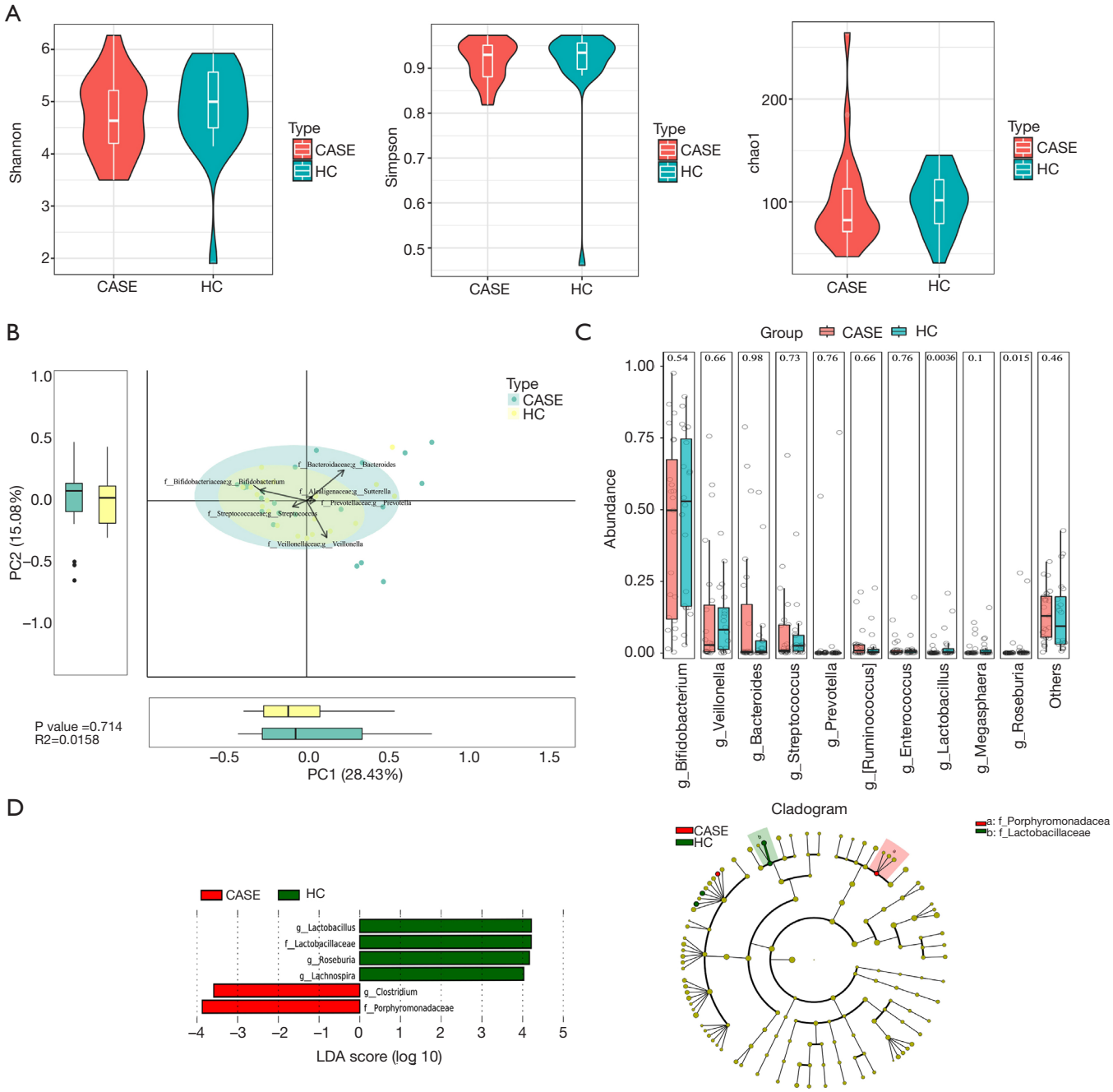


Figure 1 Diversity and relative abundance of intestinal flora between the infantile spasms (IS) group and the HC group. (A) Comparison of the α diversity indices between the IS and HC groups. (B) Comparison of the β diversity indices between the IS and HC groups. (C) Mann-Whitney rank-sum tests showing the top 10 genera by abundance. (D) Linear discriminant analysis effect size of the top 10 genera with regard to abundance [case: IS group; HC: healthy control group; before: prior to ACTH treatment; after: following ACTH treatment; invalid: ACTH-no response group; valid: ACTH-response group; red and green: significant differences (LDA >2)].

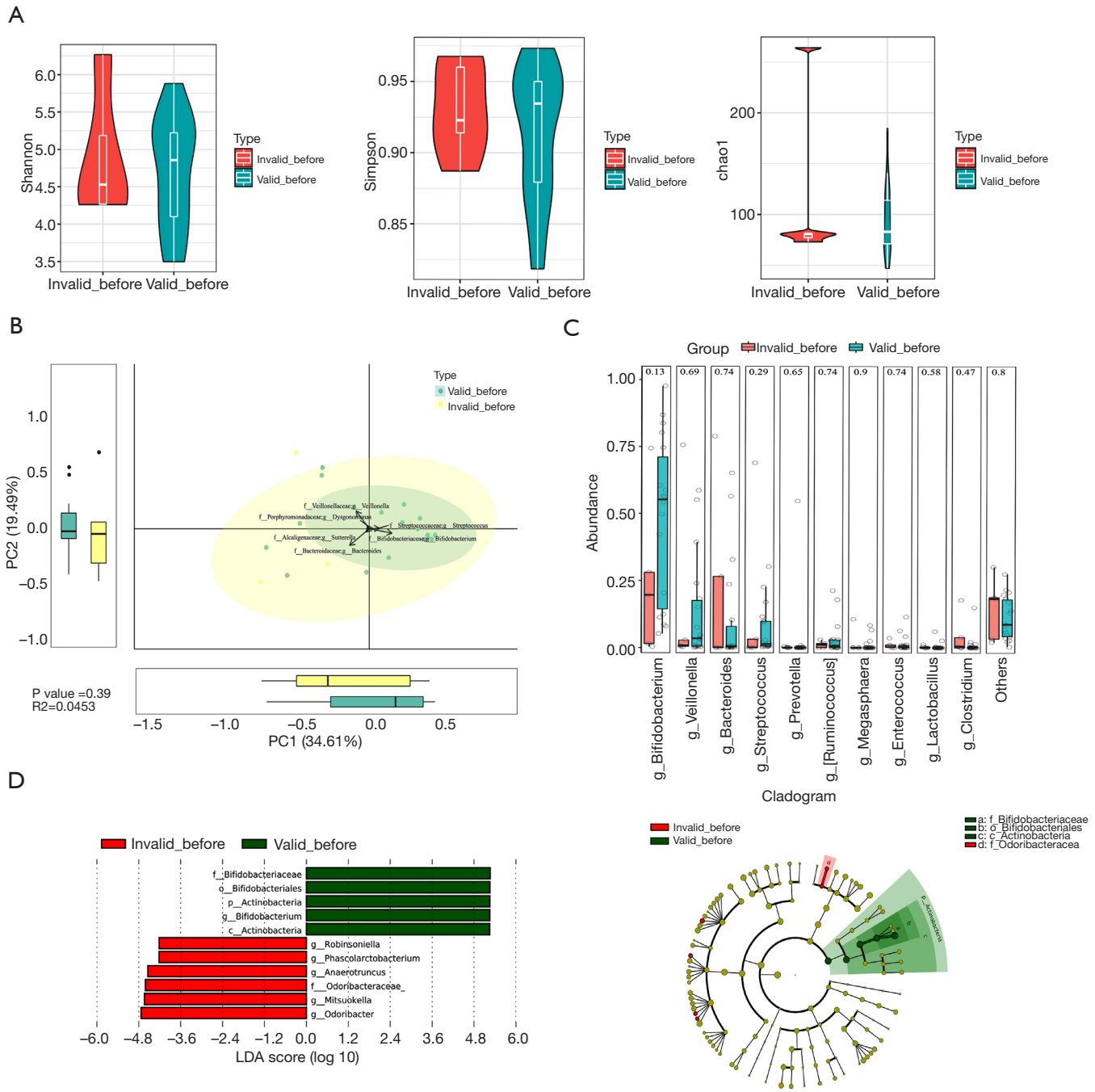


Figure 2 Diversity and relative abundance of intestinal flora between the ACTH-response group and the ACTH-NR group. (A) Comparison of the indices of α diversity between the ACTH-response and ACTH-NR groups prior to treatment. (B) Comparison of the indices of β diversity between the ACTH-response and ACTH-NR groups prior to treatment. (C) Mann-Whitney rank-sum tests of the top 10 genera in terms of abundance. (D) Linear discriminant analysis effect size of the top 10 genera by abundance [case: infantile spasms group; HC: healthy control group; before: prior to ACTH treatment; after: following ACTH treatment; invalid: ACTH-NR group; valid: ACTH-response group; red and green: significant differences (LDA >2)].

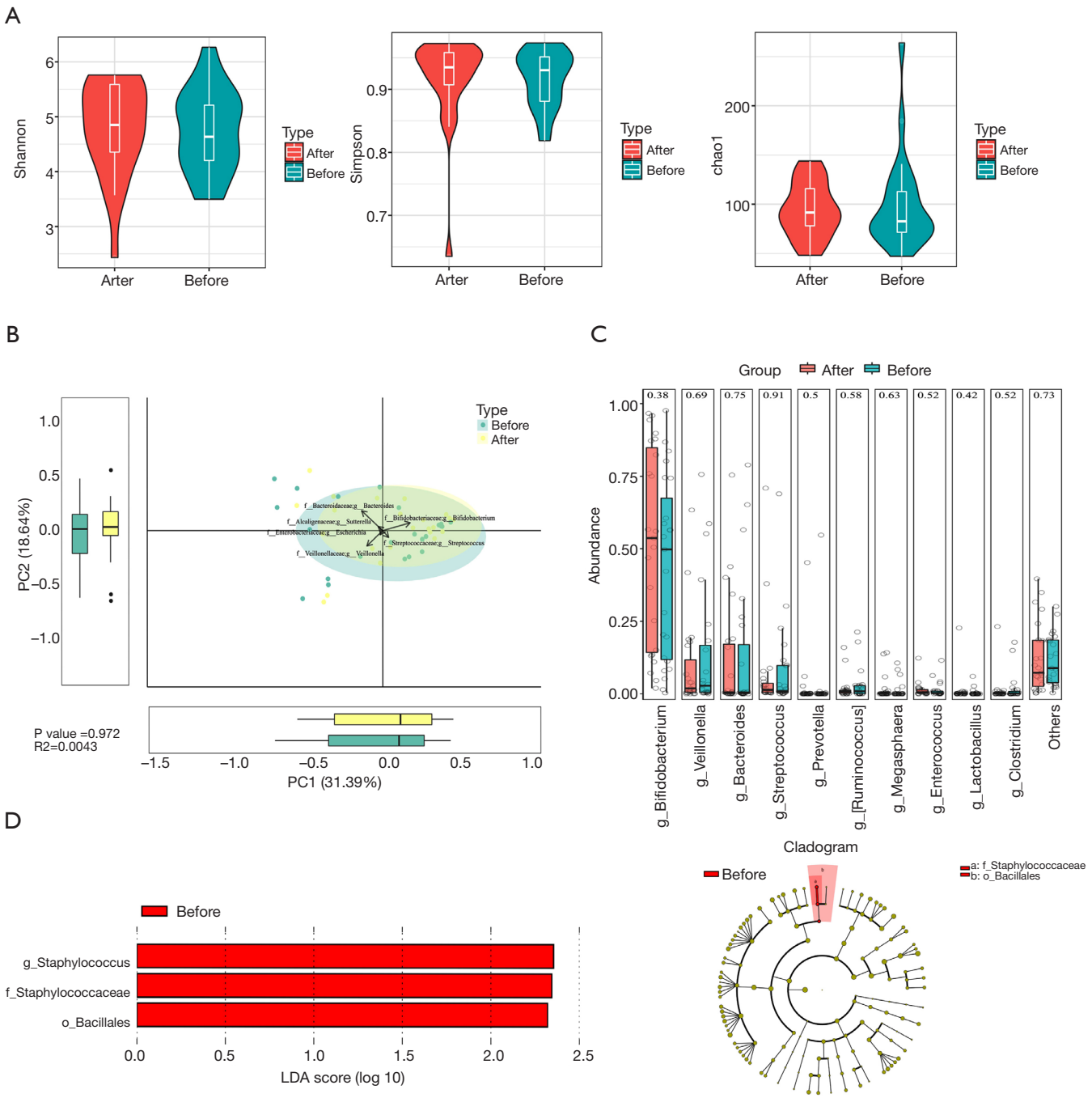


Figure 3 Diversity and relative abundance of intestinal flora before and after treatment in the infantile spasms (IS) group. (A) Comparison of the indices of α diversity before and after treatment in the IS group. (B) Comparison of the indices of β diversity before and after treatment in the IS group. (C) Mann-Whitney rank-sum tests of the top 10 genera in terms of abundance. (D) Linear discriminant analysis effect size of the top 10 genera by abundance [case: infantile spasms group; HC: healthy control group; before: prior to ACTH treatment; after: following ACTH treatment; invalid: ACTH-NR group; valid: ACTH-response group; red and green: significant differences (LDA >2)].

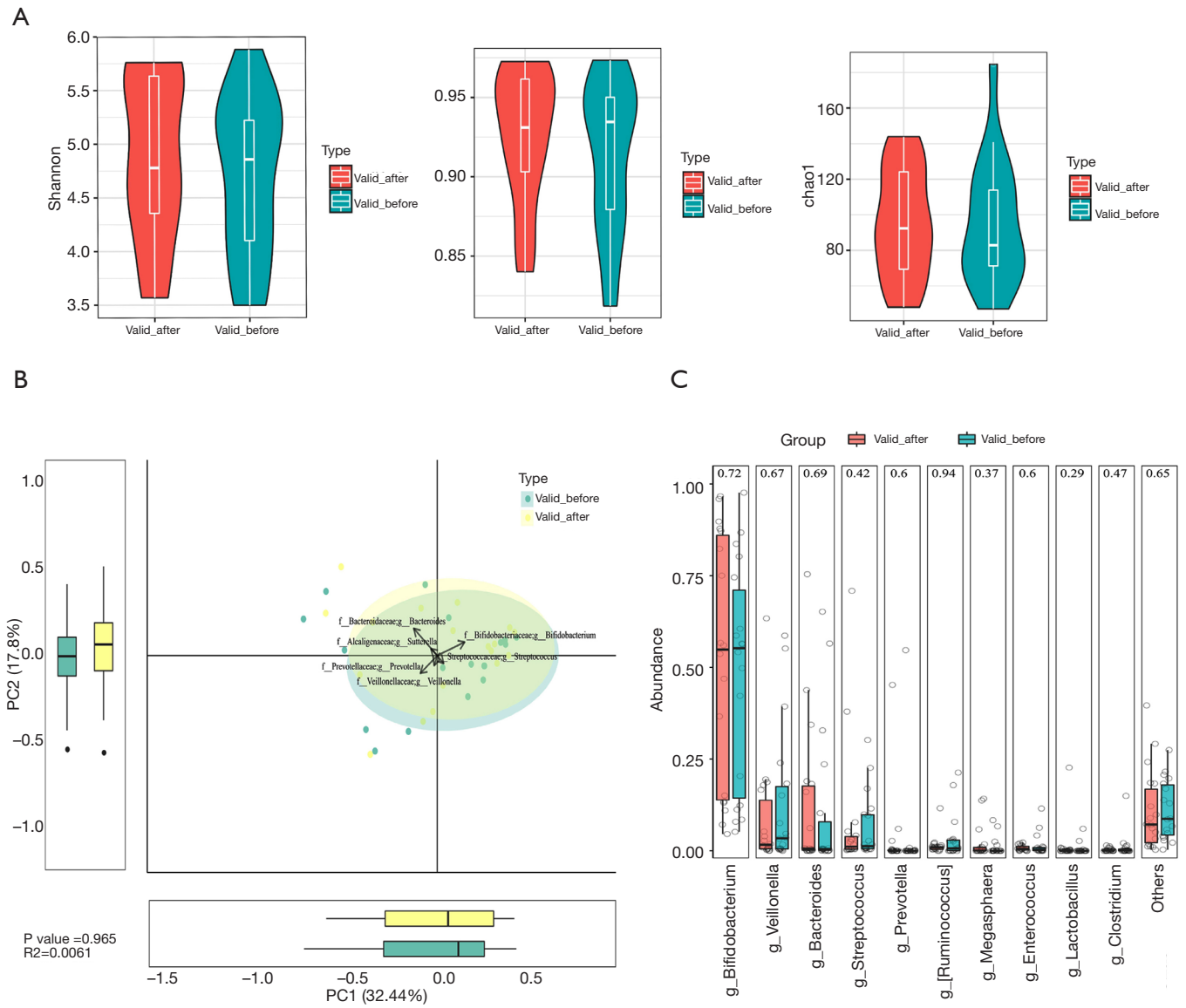


Figure 4 Diversity and relative abundance of intestinal flora before and after treatment in the ACTH-response group. (A) Comparison of the indices of α diversity before and after treatment in the ACTH-response group. (B) Comparison of the indices of β diversity before and after treatment in the ACTH-response group. (C) Mann-Whitney rank-sum tests of the top 10 genera by abundance (case: infantile spasms group; HC: healthy control group; before: prior to ACTH treatment; after: following ACTH treatment; valid: ACTH-response group).

that several mechanisms work together to form the brain-gut axis, thus creating a link between the IF and the brain. As the largest mammalian ecosystem, the intestinal tract normally contains in excess of 100 trillion microbes. This population of microbes can affect the physiological function of the host (8). The theory underlying the microbial-intestinal-brain axis proposes that the IF and the brain have a bidirectional regulatory mechanism that features a complex neuroendocrine pathway (9). Studies have also reported a close relationship between the occurrence of some diseases in the central nervous system, including epilepsy, and the IF (10,11). The pathogenesis of IS may be related to the excessive release of CRH resulting from dysfunctional activity in the HPA axis (4). Recent research has shown that abnormalities in the IF may lead to dysfunction of both the HPA axis and the immune

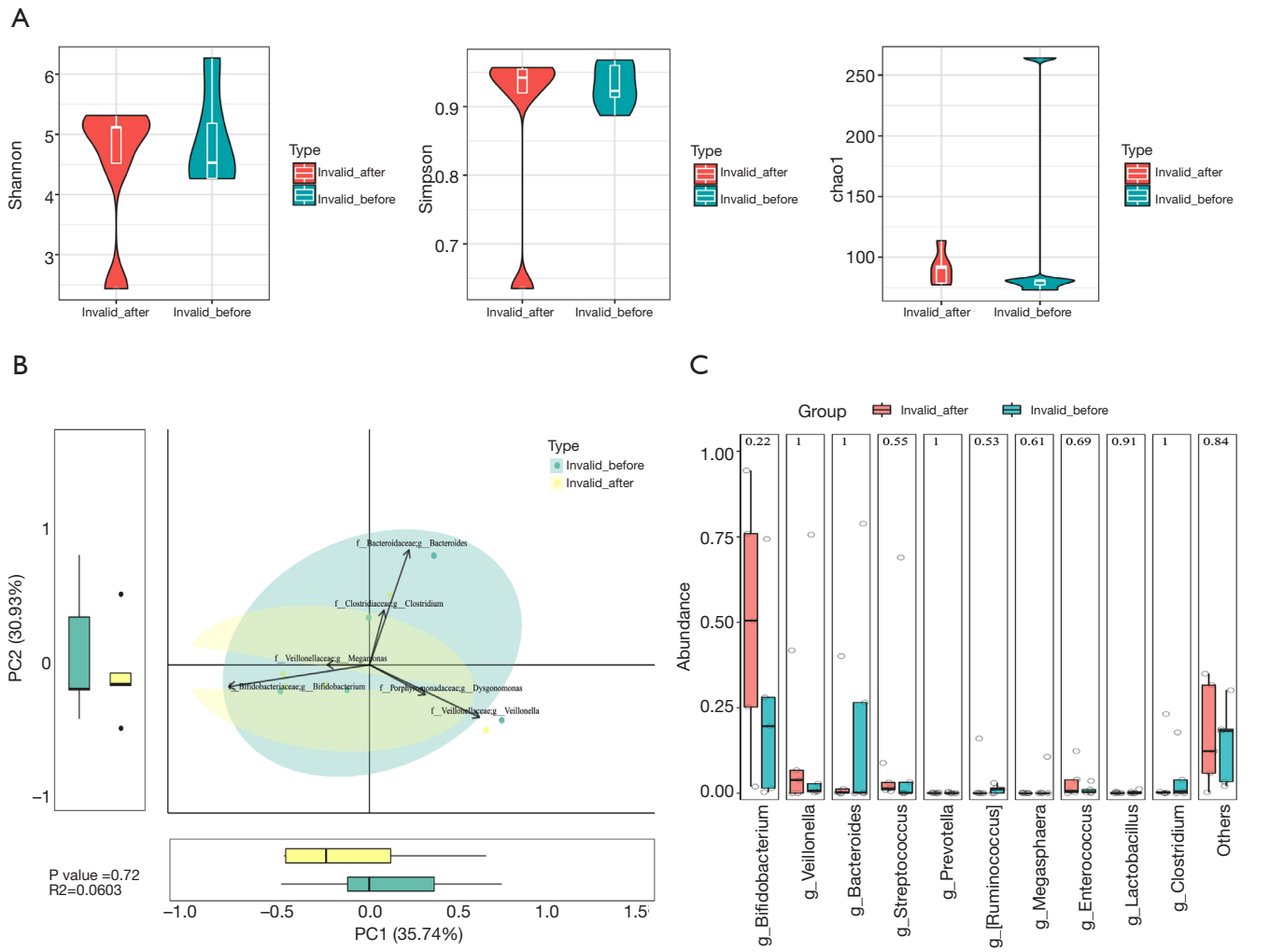
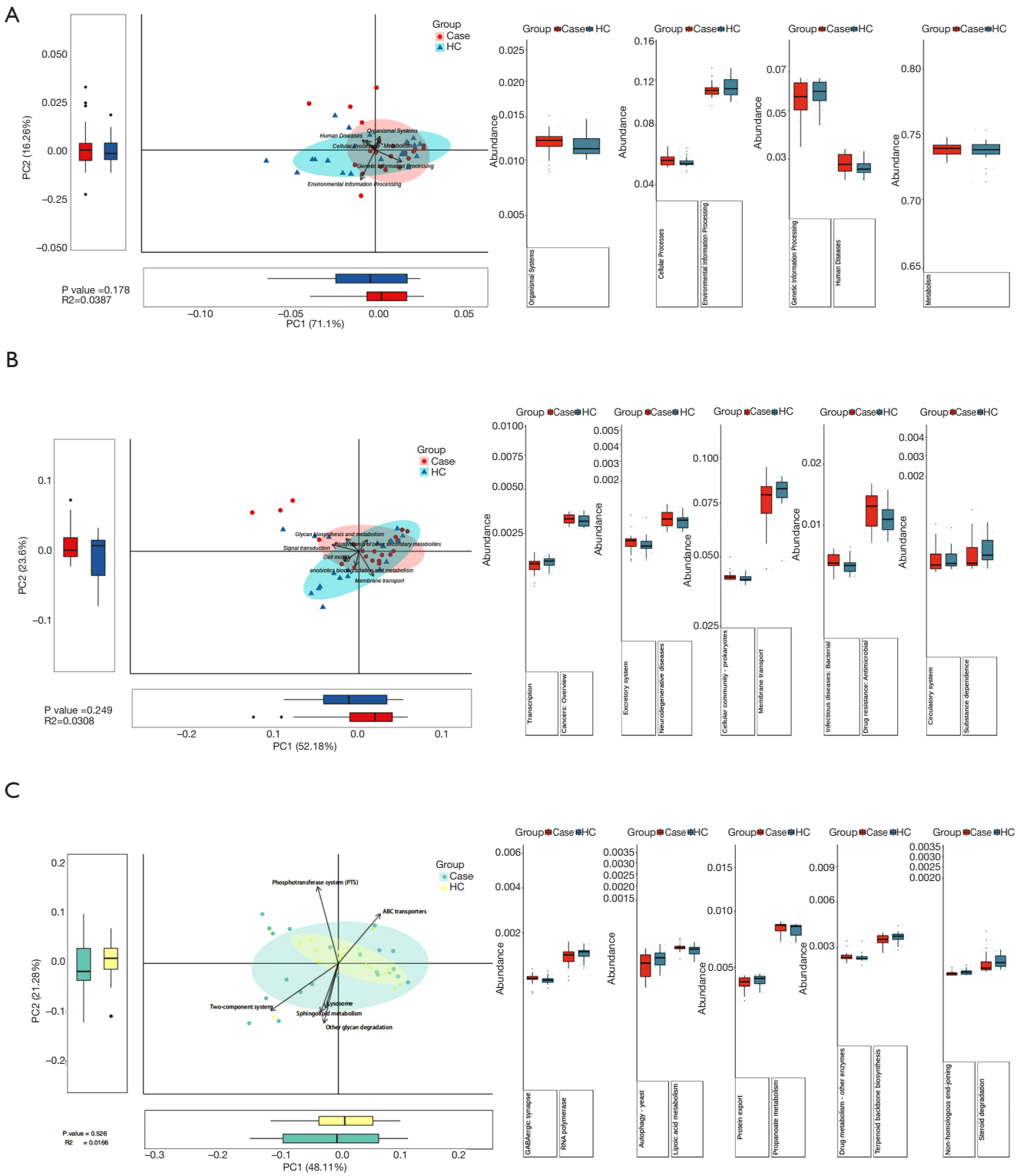


Figure 5 Diversity and relative abundance of intestinal flora before and after treatment in the ACTH-NR group. (A) Comparison of the indices of α diversity before and after treatment in the ACTH-NR group. (B) Comparison of the indices of β diversity before and after treatment in the ACTH-NR group. (C) Mann-Whitney rank-sum tests of the top 10 genera by abundance (case: infantile spasms group; HC: healthy control group; before: prior to ACTH treatment; after: following ACTH treatment; invalid: ACTH- NR group).

system (12). Consequently, it appears that dysregulation of the IF may also be involved in the occurrence of IS.

The theory underlying the microbial-intestinal-brain axis may help to explain an association of IF with epilepsy. Medel-Matus and colleagues previously showed that the transplantation of gut microbiota from stressed rats to non-stressed rats resulted in a marked reduction in the epilepsy threshold of non-stressed rats and the prolongation of their epilepsy (13). Several clinical studies have also demonstrated significant differences in the IF between patients with treatment-resistant epilepsy and healthy populations. However, several authors have presented different proposals with regard to diversity and the specific classification of these differences. In their study of patients with epilepsy, Peng *et al.* found that the IF of patients with drug-refractory epilepsy showed elevated levels of α diversity. In particular, the ratio of *Bifidobacteria* to *Lactobacillus* was significantly lower in the IF of patients with drug-refractory epilepsy than in HCs, while patients with drug-sensitive epilepsy were more likely to be healthier than those with drug-refractory epilepsy (14). Xie *et al.* reported that the α diversity of the intestinal microbiota in infants with refractory epilepsy did not differ significantly from that in healthy subjects; however, they did note a significant



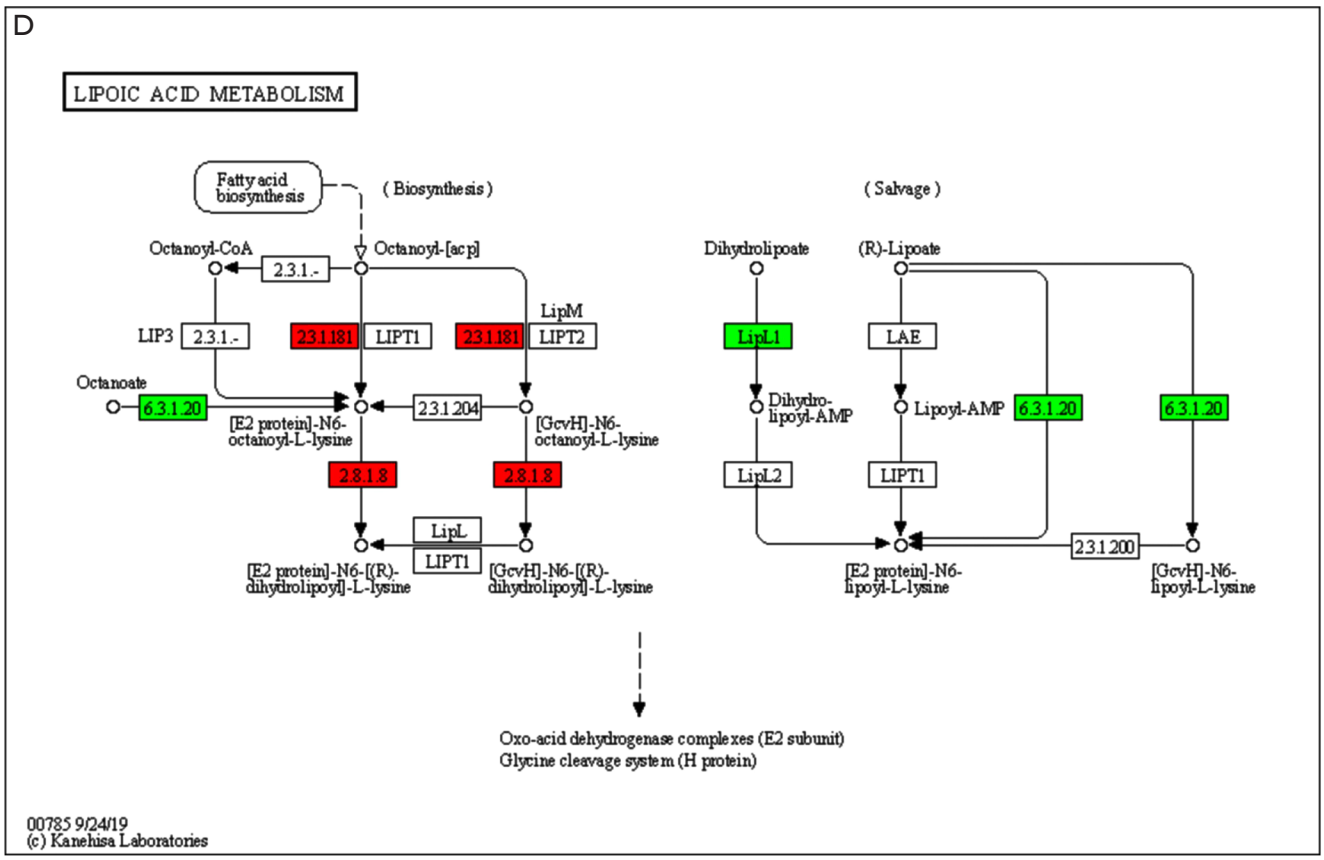


Figure 6 Metabolic pathway analysis of the infantile spasms (IS) group and the HC group. (A) Comparison of metabolic differences at level 1 by PCA and Adonis tests. (B) Comparison of metabolic differences at level 2 by PCA and Adonis tests. (C) Comparison of metabolic differences at level 3 by PCA and Adonis tests. (D) Differences in lipoic acid metabolic pathways, as determined by Mann-Whitney rank-sum tests [case: IS group; HC: healthy control group; red: significant difference ($P<0.05$); green: non-significant difference ($P\geq0.05$)].

difference in β diversity (15). Compared with HCs, infants with refractory epilepsy had significantly higher proportions of *Bacteroidetes*, *Prevotella*, and *Bifidobacterium*, and significantly lower proportions of *Proteobacteria* and *Cronobacter* (15). Also, in another study, Huang *et al.* reported a higher proportion of *Clostridium* in the IF of children with epilepsy (16).

In the present study, we found no significant differences in the α and β diversity of IF between patients with IS and HCs. Hence, our current findings are not entirely consistent with those of previous reports. We also found that the population of *Lactobacillus* was significantly reduced in children with IS, while the population of *Clostridium* was significantly higher. Furthermore, we also observed reductions in the populations of *Roseburia* and *Lachnospira*. Previously, we reported that mice experiencing prenatal stress undergo excessive activation of the HPA axis (17). This action increases the rate of CRH release and results in a marked reduction in the population of *Lactobacillus* in the IF (18), and an abundance of the *Lactobacillus* genus is significantly correlated with the responsiveness of the HPA axis to stress (19). In another study, Peng *et al.* reported that the addition of *Lactobacillus* could reduce the excessive activation of the HPA axis (20). Previously, we also reported that IS may result from abnormalities in the HPA axis (17). We therefore speculate that differences in the IF may constitute 1 of the causes of IS and that the mechanisms involved may have an association with abnormalities in the HPA axis. Our current analysis of metabolic pathways highlighted the upregulated expression of genes associated with lipoic acid synthesis in children with IS, a phenomenon which will lead to the production of excessive levels of lipoic

acid. Tolunay *et al.* reported that overproduction of lipoic acid resulted in status epilepticus and that this condition could not be alleviated by a large dose of levetiracetam, midazolam, or dilantin (21). Therefore, we suspect that metabolic changes arising from changes in the IF may be a key trigger of IS.

Additionally, we discovered a significant difference in the β diversity of the IF between children who responded to ACTH treatment and those who did not. Compared with the ACTH-NR group, the ACTH-response group had reduced populations of *Odoribacter*, *Phascolarctobacterium*, *Anaerotruncus*, *Mitsuakella*, and *Robinsoniella*. In a previous study, Golubeva *et al.* showed that stressed prenatal mice exhibited excessive activation of the HPA axis, accompanied by a significant increase in the population of *Anaerotruncus* (18). We also found a significantly lower proportion of *Bifidobacterium* in the ACTH-NR group. Some studies have indicated that bacteria from this genus can result the excessive activation of the HPA axis (22). We also recorded and analyzed the number of AEDs but found no significant differences between the 2 groups. Subgroup N03A of the Anatomical Therapeutic Chemical classification system of drugs created by the World Health Organization lists a series of AEDs. The 16 representative medicines in subgroup N03A were tested and none exhibited a clear antimicrobial effect. Therefore, we speculate that differences in the IF might lead to an imbalance in the HPA axis, which is involved in the pathogenic process leading to IS. This imbalance may be more obvious between the ACTH-response and ACTH-NR groups , as the administration of ACTH did not appear to alleviate this issue, resulting in treatment failure in some patients.

Two approaches can be used to regulate the composition of the IF in the treatment of epilepsy: a ketogenic diet (KD) and probiotics (23). A KD is a low-carbohydrate diet which has been demonstrated to have anti-epileptic effects. A range of ketones can be produced during treatment, and these products serve as potent energy sources for gut microbiota (24). A previous study of children with epilepsy showed that after 1 week on a KD, 21% of the children were seizure-free and 43% had >50% seizures (15). The authors also analyzed the IF of the children and reported that treatment with a KD led to a reduction in the proportion of bacteria from the *Bacteroidetes* and *Actinobacteria* phyla, and a reduction in bacteria from the *Proteobacteria* phylum (15). These changes in the IF were believed to be the predominant factor underlying seizure control. Zhang *et al.* showed that the proportion of bacteria from the beneficial *Bacteroidetes* phylum was increased in pediatric patients who showed drug-resistance to AEDs, and KD treatment led to a significant reduction in seizure activity within 6 months (25). In another study, Olson *et al.* observed the seizure-protective effects of a KD by transplanting the fecal microbiota of KD-fed mice into germ-free mice; they noted that the antiepileptic effects disappeared upon the administration of antibiotics (26). In a study of 45 patients with drug-resistant symptomatic epilepsy, a "probiotic cocktail" of beneficial bacteria led to a reduction in seizures of >50% in 29% of patients (27). These findings suggest that regulating the composition of the IF with exogenous interventions could reduce or prevent epilepsy, although further research on this subject is needed.

The main limitation of this study was that it involved a small cohort of patients. This limitation was exacerbated by the patients being divided into groups according to their response to treatment with ACTH. Further research should involve the use of animal models of IF transplantation. Combining such work with metabolomics analysis could also help to provide a far deeper understanding of the pathological mechanisms underlying IS than that we have currently.

Conclusions

Our work suggests that an imbalance in the IF and a change in metabolic pathways may be involved in the pathogenesis of IS. Differences in the composition of the IF appear to affect the efficacy of ACTH treatment. Consequently, the regulation of the IF composition may be beneficial for the treatment of IS and represents a potential strategy for adjuvant therapy.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study involved human participants, and the study protocol was approved by the Ethics Committee of PLA General Hospital (Reference no. 20190156). The parents or legal guardians of each participant provided written informed consent for participation in this study.

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