# SCFA producing bacteria shape the subtype of ADHD in children

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# Abstract

There is little data on population-based identification of the gut microbiota with ADHD subtypes in children, yet whether the degree ADHD is characterized by short-chain fatty acids (SCFAs) remains unclear. We enrolled 59 ADHD children including 21 inattentive subtypes (ADHD-I), 20 combined subtypes (ADHD-C), 18 hyperactive-Impulsive subtypes (ADHD-H) and 23 healthy controls. The microbiota was characterized by 16S rRNA gene sequencing, and SCFA concentrations were determined by gas chromatographic analysis. Compared to the controls, we observed a decrease of 14 genera belonging to Ruminococcaceae, Lachnospiraceae, Verrucomicrobiaceae and Rikenellaceae family in ADHD-I, while Megamonas, Coprococcus_2 and Paraprevotella were significantly increased in ADHD-C. In addition, a lower abundance of Faecalibacterium, and a higher proportion of Marvinbryantia, Intestinimonas, Prevotella_9 and Eggerthella were detected in the ADHD-H. Analysis of fecal SCFAs showed that elevated levels of acetate and propionate were in ADHD subtypes. Furthermore, most of the bacterium associated with SCFAs overlapped with the differential bacterium in ADHD subtypes. Conclusion: Our data support the clinical distinction among different ADHD subtypes in children may also be reflected in alterations of specific gut microbiota, most of which are SCFA producing bacteria.


# Introduction

Attention-deficit hyperactivity disorder (ADHD) is nowadays the most common behavioral neurodevelopmental disorder estimated to affect approximately 5% of children worldwide. Typically, children with ADHD exhibit age-inappropriate levels of attention, impulsivity and/or hyperactivity, and such symptoms interfere with, or reduce the quality of, social, academic, or occupational functioning [1]. Up to now, the etiology and pathogenesis of ADHD have not been elucidated and its diagnosis and treatment are still challenging. Currently, emerging data suggest that the microbiome, or its disruption, can contribute to the pathology of various neurologic disorders [2]. Using the germ-free (GF) mice, and antibiotic treated specific pathogen free (SPF) mice, it has demonstrated that microbiota deficits alter brain microstructure, gene expression, and neurochemical metabolism across regions of the amygdala, hippocampus, frontal cortex, and hypothalamus, resulting in the occurrence of neurodevelopmental diseases [3–4].

The gut microbiota plays a key role in brain development and function and in the etiology of neurodevelopmental disorders via the gut-brain axis, a bidirectional communication between the gut and the brain relying on immune, neuroendocrine and neural pathways [5–6]. The microbiota is increasingly recognized for its ability to promote enteric and circulating serotonin production, control the differentiation and function of immune cells, and regulate expression of the 5-hydroxytryptamine receptor (5-HT1A), brain-derived neurotropic factor (BDNF), and NMDA receptor subunit 2 (NR2A), in turn, impact the brain [7–9]. Dysbiosis (alterations in microbiota composition and function) of the human microbiome has been reported in subjects diagnosed with several neurodevelopmental diseases, such as schizophrenia, autism and ADHD [10]. Furthermore, microbiota shift in early adolescence with neurodevelopmental disorders has been proved to ameliorate some, but not all, of the behavioral dysfunctions [11].

In addition to neural, endocrine, and immune pathways, gut microbial metabolites may be an additional mechanism in signaling with the potential to affect host physiology [12]. A growing body of evidence shows that microbial metabolites have a major influence on neurodevelopmental disorders. The best known bacterial fermentation products are short-chain fatty acids (SCFAs) such as butyrate, acetate and propionate, which exert several effects, including maintenance of gut barrier function, providing a source of energy for colonocytes and bacterial communities, and possessing neuroactive properties [13]. Previous studies have shown that administration of a high dose of propionate in rats induced behavioral alterations (increased levels of locomotor activity) related with neurodevelopmental disorders [14]. Acetate and propionate, considered as the common preservatives in food products, has been demonstrated to aggravate hyperactivity in ADHD children, but when artificial food colourings and benzoate preservatives diminished, these symptoms may be ameliorated [15]. Moreover, butyrate, well known for its capacity to inhibit histone deacetylase, exerts beneficial behavioral effects via an epigenetic mechanism [16].

Although some published studies have initially observed gut microbiome profile changes in ADHD children, it is worth further investigation whether there are differences in the composition of gut microbiome between different clinical manifestations of ADHD children. Moreover, no population-based identification of children with ADHD has been performed to assess the association between microbiota and a spectrum of SCFAs [17–18]. By subtyping our subjects based on their specific type of ADHD, the purpose of this study was therefore to detect the gut microbiota profiling and the levels of microbial metabolite SCFAs, and gave analysis on the relation between these two indices.

## Materials And Methods

## Study Participants

## ADHD children

All ADHD children (age 6–12 years) were recruited from Department of Child Health Care at Beijing Children's Hospital, Capital Medical University, from June 2017 to May 2018, and the ADHD children were first coming to the child health care and had not previously used psychiatric medication. Using DSM-IV criteria, there are three defined subtypes of ADHD in children: predominantly hyperactive-impulsive (ADHD-H), predominantly inattentive (ADHD-I), and combined (ADHD-C). Finally, a total of 59 participants were classified into three groups which were ADHD-I (n = 21), ADHD-C (n = 20), ADHD-H (n = 18). ADHD children met the DSM-IV diagnosis and scored above clinical threshold for ADHD symptoms on both the Strength and Difficulty Questionnaire (SDQ) and the Conners' Parent Rating Scale-Revised and had no comorbid condition. Wechsler Intelligence Scale for Children (WISC)-Chinese Revision was used to determine participants' current level intellectual ability. Exclusion criteria included history of epileptic seizures, mental retardation and an intelligence quotient of <85.

## Control

Participants in the control group were matched on age and gender with ADHD participants (n = 23). Controls were also screened using DSM-IV to exclude oppositional defiant disorder, conduct disorder, Tourette disorder or any other Axis I psychiatric comorbid disorders and use of any psychotropic medications. Controls were required to have no family history of psychiatric illness in first-degree relatives. Other exclusion criteria were the same as those for the ADHD group.

In addition, all participants of this study were under a normal diet, and no antibiotics, probiotics, or prebiotics have been taken in the 3 months prior to the sample collection. None of the subjects were on anti-inflammatory or antioxidant drugs. The study protocol was designed in accordance with the guidelines outlined in the Declaration of Helsinki and was approved by ethics committee of Beijing Children's Hospital. Written informed consent was obtained from the parents or guardians of all subjects, prior to the study. The characteristics of the participants are presented in *Table 1.*

# Sample preparation, sequencing, and data processing

Stool samples used were obtained in a sterile container, bought to the laboratory and immediately stored at −80 °C. Microbial DNA was extracted from frozen fecal samples using a QIAamp DNA Stool Mini Kit (Cat. No. 51504, Qiagen, Germany) according to the manufacture's protocol. DNA extraction was assessed by agarose gel electrophoresis. The V3-V4 region of the bacterial 16S ribosomal RNA gene was amplified by PCR using primers 338F (5'ACTCCTACGGGAGGCAGCA3') and 806R (5'GGACTACHVGGGTWTCTAAT3'). The PCR conditions were as follows: 95 °C for 5 min, followed by 25 cycles at 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 25 s and a final extension at 72 °C for 5 min. PCR amplification was performed in a 20 μL reaction system using TransGen AP221–02: TransStart FastPfu DNA Polymerase (TransGen Biotech, China) with an ABI GeneAmp® 9700 sequence detection system (ABI, Foster City, CA, USA). The PCR products were purified using an AxyPrep DNA Gel Extraction Kit (AXYGEN, USA) and then mixed equally before pyrosequencing. The PCR products of the V3–V4 region of the 16S rRNA gene were sequenced by the Next Generation Sequencing Core using an Illumina MiSeq PE300 as previously described [19].

To obtain high-quality sequences, the raw sequences were filtered and trimmed as previously reported [20–21]. After the sequences were optimized, operational taxonomic units (OTUs) assignment was performed for all sequences with a distance limit of 0.03 (equivalent to 97% similarity) using the U search software (version 7.1). According to the results of the taxonomy analysis, Rarefaction and Shannon-Wiener curves, the Chao and Ace estimator for community richness, the Shannon and Simpson index for community diversity, and the Good's coverage for sequencing depth were assessed for each sample using Mothur software (version v1.30.1). Taxonomical assignments of OTUs were performed using Mothur software in accordance with the SILVA database at an 80% confidence level. Finally, the sequences were phylogenetically assigned to taxonomic classifications using an RDP Classifier (version 2.2) at a 70% confidence level. After phylogenetic allocation of the sequences down to the phylum, class, order, family and genus levels, the relative abundance of a given phylogenetic group was defined as the number of sequences affiliated with that group divided by the total number of sequences per sample.

To clarify the similarities of fecal microbiota between the experimental groups, Venn diagrams and species rank abundance distribution curves (Whittaker plots) were generated using R-project for statistical computing. In addition, non-metric multidimensional scaling (NMDS) and hierarchical cluster analysis were performed to determine whether the OTUs identified using the KW filter discriminate between different groups by examining relationships between ecological communities. Bacterial taxonomic analyses and comparison including bacterial phylum and genus were conducted between any different ADHD subtype and control groups using Wilcoxon rank sum test.

# Analysis of SCFAs concentrations in fecal samples

To measure SCFA concentrations in feces, 150 mg of each fresh stool sample was weighed, suspended in sterile distilled water (1.2 ml) and homogenized for approximately 3 min. Then, the pH of the suspension was adjusted to 2–3 by adding 5 M HCl, and the sample was incubated at room temperature for 10 min with occasional shaking. The suspension was transferred into a polypropylene tube and centrifuged for 10 min at 14,000 rpm, and the centrifugation steps were repeated until the supernatant was clear. The internal standard, 2-ethylbutyric acid solution, was spiked into the supernatant at a final concentration of 1 mM, and the supernatant was injected in an Agilent 6890 N GC system equipped with a flame ionization detector (FID) and an N10149 automatic liquid sampler (Agilent, USA). A high-resolution gaschromatography column (DB–624UI, J&W Scientific, Agilent Technologies Inc., USA) of 30 m × 0.25 mm i.d. coated with 1.40 μ m film thickness was used. Nitrogen was supplied as the carrier gas at a flow rate of 1.1 mL/min. The initial oven temperature was 60 °C, maintained for 1.0 min, raised to 160 °C at 10 °C/min and held for 2.0 min, then increased to 200 °C at 5 °C/min, and finally held at 200 °C for 3 min. The temperatures of the FID and the injection port were 220. The flow rates of hydrogen, air and nitrogen as makeup gas were 30, 350 and 45 mL/min, respectively. The injected sample volume for GC analysis was 1 μ L, and the run time for each analysis was 23.5 min. Data handling was carried out with HP ChemStation Plus software (B.04.03; Agilent Technologies).

# Data analysis

One-way analysis of variance (ANOVA) was performed to compare means in different groups with normally distributed data using SPSS version 19.0 for Windows; for data with a non-normal distribution, the differences between groups were assessed using the Mann–Whitney U-test and the Wilcoxonsigned-rank test. Significant differences between ADHD subtypes and the controls were assessed using Dunnett's test. Spearman's correlation between fecal SCFAs and associated genera was calculated and scaled by coefficients of each respective linear model. All values are expressed as the mean ± SD, and $P < 0.05$ was considered to be statistically significant.

## Results

# Demographic and clinical comparisons

Demographics and clinical description of the participants with ADHD-I, ADHD-C, ADHD-H and controls are presented in *Table 1.* No significant differences were present between any two subtypes or relative to controls in terms of age, gender distribution, or total IQ. However, the higher score of inattention index and lower scores of hyperactivity/impulsivity symptoms in ADHD-I group than that of ADHD-C and ADHD-H groups according to Conners' Parent Rating Scale and DMS-IV.

# Changes in gut microbiota profiles of different ADHD subtypes

Based on the sequencing data, a total of 2442813 valid reads were obtained, with an average of 56810±7148 reads per sample. The gut microbiotas of all samples were classified into 507 OTUs at a 97% similarity level, representing 12 phyla, 22 classes, 34 orders, 61 families, 188 genus, and 358 species.

The alpha and beta diversity values of the bacterial communities between any ADHD subtypes and control groups were assessed using various indices based on the OTU level. To determine alpha diversity, the bacterial phylotype richness, reflected by the ACE and Chao indexes was calculated. Meanwhile, the bacterial diversity expressed as Shannon and Simpson indexes. Detailed data on the estimators in each group are presented in *Table 2.* Compared to healthy controls, richness indices (ACE and Chao) and diversity index (Shannon) were all significantly lower only in ADHD-I, not in ADHD-C and ADHD-H subjects. However, another diversity index (Simpson) was significantly higher in ADHD-I and ADHD-C groups than that in the control. Rarefaction and Shannon-Wiener curves for each group indicated that the total bacterial diversity was well represented. More than 99% of coverage in all of the samples indicated that the sequencing depth was sufficient to reflect the whole bacterial diversity and the real composition of gut microbiota.

NMDS was performed to identify for dissimilarities in the microbial composition between three subtypes of ADHD patients and the controls (*Fig 1F).*. Analysis using the KW test showed that the microbial clusters from any ADHD subtypes were all located a shift to the left, which indicated compositional differences from healthy children. However, no significant differences in microbial composition were found among three subtypes of ADHD patients.

The gut microbiota composition in three subtypes of ADHD patients and healthy controls from the phylum to the genus is illustrated in *Fig 1A-E.* At the phylum level, the predominant bacterial taxa were *Firmicutes* and *Bacteroidetes,* followed by *Proteobacteria, Actinobacteria* and Verrucomicrobia in all samples. Compared with controls, only children in ADHD-I group have a significant lower Verrucomicrobia. At the genus level, the changes of gut microbial composition in each ADHD subtype were distinguishable. Comparisons of the ADHD-I and control groups revealed that the levels of 14 genera belonging to *Ruminococcaceae, Lachnospiraceae, Verrucomicrobiaceae* and *Rikenellaceae* family were decreased in the ADHD-I group. However, there was a greater abundance of *Megamonas, Coprococcus_2* and *Paraprevotella* in the ADHD-C group relative to the control group. We also found that a lower percentage of

*Faecalibacterium,* and a higher proportion of *Marvinbryantia, Intestinimonas, Prevotella_9* and *Eggerthella* in the ADHD-H group compared to the control group (*Fig 2).*.

# Fecal SCFAs in different ADHD subtypes

As shown in Fig 3, compared to healthy controls, total SCFA propionate concentrations in feces were increased in ADHD subtypes, especially the concentration of acetate and propionate, were increased in ADHD-H subtypes. No significant differences were seen in butyrate, isovalerate and valerate concentrations between any subtype of ADHD and control groups (*Fig 3).*.

# Relationship between gut microbiota composition and fecal SCFA

The relativity of fecal SCFA concentration and the bacterial genera showed that *Veillonella, Klebsiella* were positive correlation with acetate concentration, and *Lachnospiraceae_NK4A136_group, unclassified_f_Lachnospiracese* were negative correlation with acetate concentration. *Alloprevotella, [Ruminococcus]_gnavus_group* were positive correlation with propionate concentration, and *Christensenellaceae_R–7*

*_group, Lachnospira, Lachnospiraceae_NK4A136_group, Lachnospiraceae_UCG004,* were negative correlation with propionate concentration. *Klebsiella* was positive correlation with butyrate concentration, and *Lachnospira, norank_f_ Lachnospiracese* were negative correlation with butyrate concentration. *Phascolarctobacterium, norank_f_Ruminococcaceae, Odoribacter, [Eubacterium]_ruminantium_group, Ruminococcaceae_UCG–005* were negative correlation with valerate concentrations. *Alloprevotella, [Ruminococcus]_gnavus_group* were positive correlation with iso-valerate concentrations, and *Lachnospiraceae_UCG004, Faecalibacterium* were negative correlation with iso-valerate concentrations (*Fig 4).* More than half of the bacterium associated with SCFAs overlapped with the differential bacterium among ADHD and control groups, and this indicated that SCFAs might play the vital role in the occurrence of ADHD.

# Discussion

To date, there are limited studies to identify that the fecal microbiota composition exist differences among different ADHD subtypes. In this study, we sequenced the total bacteria DNA of stool samples from 59 ADHD children including 21 ADHD-I, 20 ADHD-C, 18 ADHD-H and 23 control individuals, and examined the gut microbial composition with different ADHD subtypes. Compared to healthy controls, ADHD-I subtypes showed more reduced gut microbial diversity and more differences in microbial composition than the ADHD-C and ADHD-H subtypes.

Further analysis revealed that a significantly lower *Verrucomicrobia* phylum were shown only in ADHD-I children. Moreover, the majority of decreased *Verrucomicrobia* ascribed to the reduced *Akkermansia* genus.

Some research has shown that *Akkermansia* has a beneficial role on the intestinal mucosal layer and enhances the barrier function of the gut epithelium, and may mediate obesity, diabetes, and inflammation [22]. Thus, in conditions of low *Akkermansia* abundance, the maintenance of a healthy gut barrier may be possible destroyed and pathogenic factors of other bacteria, like Lipopolysaccharide (LPS), could consequently harm the host [23].

At the family, *Ruminococcaceae* significantly decreased in ADHD-I and ADHD-H subtypes. *Ruminococcaceae* constitute the major taxonomic group of the human gut microbiota and include many putative anti-inflammatory and thus potentially protective genera. The key members of *Ruminococcaceae* showed a confirmed decrease and were negatively correlated to disease duration in some neurodegenerative diseases, such as Parkinson's disease (PD), multiple system atrophy (MSA), multiple sclerosis (MS), and amyotrophic lateral sclerosis (ALS) [24]. The animal model for autism spectrum disorders also demonstrated that dysregulation of *Ruminococcaceae* are responsible for the anxiety-like phenotype [25]. The modulating social behavior of *Ruminococcaceae* may have been related to degrade complex polysaccharides to short-chain fatty acids, including acetate, butyrate, and propionate, that could induce alterations in T-cell differentiation and microglial function, and affect expression of neuroinflammation and neuroplasticity related genes in the prefrontal cortex or hippocampus [26–27].

Although the family *Ruminococcaceae* was found no difference between ADHD-H subtypes and controls, the genus *Faecalibacterium* which belongs to *Ruminococcaceae* was decreased in ADHD-H subtypes. The decreased *Faecalibacterium* in ADHD-H subtypes were in agreement with other previous ADHD studies [28–29]. Low *Faecalibacterium* levels are associated with the elevated markers of inflammation, which impact on the development of atopic diseases including asthma, eczema, and allergic [30–31]. Inflammatory cytokines released during conditions of low *Faecalibacterium* levels in the gut have the ability to cross the blood–brain barrier, which makes them likely candidates to play a role in the development of ADHD [32]. At present, *Faecalibacterium* is suggested as a potential future probiotic due to some strain-dependent anti-inflammatory features, like butyrate production [33].

However, the LEfSe approach in the present study also showed that an increased abundance of the family *Prevotellaceae* wasexhibited in ADHD-C and ADHD-H subtypes. *Prevotellaceae* is a well-known and often discussed genus in the phylum of *Bacteroidetes.* As a 'fermenting' group of bacteria, *Prevotellaceae* produce butyrate, which has been identified as a specific inducer of Treg cell differentiation [34]. The relative abundance of *Prevotellaceae* was also significantly higher in autoimmune disease, such as Graves' disease (GD), irritable bowel syndrome (IBS) and Crohn's disease [35–36]. Apparently, the high abundance of *Prevotellaceae* contributed to the inflammatory processes, with leading to a breakdown of the mucosal barrier [37].

In addition to its role in the inflammation, we identified the majority of changed gut microbiota in ADHD children also participated in SCFA metabolism. Previous studies indicate that *Ruminococcaceae* and *Lachnospiraceae* are robust butyrate-producers, and expansion of gut-residing *Lachnospiraceae* may result in increased butyrate production [38]. Species such as *Akkermansia* have been identified as key propionate producing mucin degrading organisms [39]. SCFAs play a pivotal role in host gut, metabolic and immune

function with regulation of various elements of gut–brain axis. Moreover, circulating SCFAs produced by gut microbiota can directly influence the integrity of the blood–brain barrier (BBB) by increasing production of the tight junction proteins claudin–5 and occluding which limits entry of undesirable metabolites into brain [40–41].

Recent clinical studies suggested that the changed concentrations of SCFAs, including acetate, propionate and butyrate, were likely to be an important risk factor in PD, autism spectrum disorders (ASD), epilepsy and some inheritable metabolic disorders [42–43]. Exogenous sodium propionate supplementation lead to rapid intracellular acidification, and subsequently caused transient and variable decreases in excitatory postsynaptic current [44]. Furthermore, intraventricular infusions of PPA produced reversible repetitive dystonic behaviours, hyperactivity, turning behaviour, retropulsion, caudate spiking, and the progressive development of limbic kindled seizures [45]. In addition, Butyrate altered the metabolic behavior of macrophages to increase oxidative phosphorylation and also promoted alternative macrophage activation. Oral antibiotics disrupt this process to promote sustained T cell-mediated dysfunction and increased susceptibility to infections, highlighting important implications of repeated broad-spectrum antibiotic use [46]. In this study, we detected the increased acetate and propionate concentrations in ADHD subtypes, however, there were no differences among ADHD subtypes.

These SCFAs have been shown to affect the host through multiple mechanisms including the regulation of histone acetylation and methylation, G-protein coupled receptors (GPCRs), facilitating the secretion of various hormones (e.g. GLP-1 and PYY) and neurochemicals (e.g. serotonin), and the induction of vagus nerve signaling [49]. Since HDACs regulate gene expression, inhibition of HDACs has a vast array of downstream consequences [47]. Another intriguing function of SCFAs was able to affect the activity of epigenetic enzymes or act as the substrates necessary for epigenetic modifications which lead to changes of DNA methylation status in inflammatory bowel disease (IBD), type 1 diabetes mellitus (T1D), and obesity [48–49]. Recently, aberrant DNA methylation and histone acetylation were indicated in ADHD patients [50]. It is needed to confirm whether associations between SCFAs and DNA methylation levels might exist.

In conclusion, the present study identified preliminary significant differences in microbial composition between different ADHD subtypes and controls, and these gut microbiota changes are associated with SCFA producing process, which indicated that SCFA alteration may involve in the pathological symptoms of ADHD. Future studies will be needed to validate the present findings and elucidate the possible link of SCFAs and ADHD in children.

## Declarations

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# Declaration of Interest

None.

# Ethical Standards

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008

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# Tables

**Table 1. Demographic and clinical description for three subtypes of ADHD children**

| | ADHD-I | ADHD-C | ADHD-H | Control | P |
|---|---|---|---|---|---|
| Gender (male/female) | 17/4 | 14/2 | 11/1 | 21/3 | 0.84 |
| Age (m), mean±SD (range) | 105.7±18.2 | 100.7±25.0 | 99.4±13.7 | 106.5±14.9 | 0.66 |
| Total IQ, mean±SD (range) | 101.2±10.2 | 100.8±13.9 | 99.1±7.9 | 106.9±12.6 | 0.56 |
| Conners' Parent Rating Scale | | | | | |
| Conduct problem | 12.5±8.1[a] | 10.1±5.9 [a] | 14.3±6.8 [a] | 5.3±4.3[b] | 0.001 |
| Study problem | 9.2±3.8 [a] | 9.6±5.3 [a] | 10.4±6.2 [a] | 3.0±2.7 [b] | 0.001 |
| Psychosomatic | 1.6±1.2 [a] | 1.8±1.5 [a] | 1.0±0.8 [a] | 0.3±0.4 [b] | 0.001 |
| Hyperactivity-impulsivity | 2.7±1.0 [a] | 5.5±2.0 [a] | 6.2±3.3 [a] | 0.6±0.4 [b] | 0.001 |
| Anxiety | 0.7±1.0 | 1.1±1.0 | 0.9±1.4 | 0.6±0.7 | 1.38 |
| ADHD Index | | | | | |
| Total score | 14.0±3.5 [a] | 23.8±2.6 [a] | 16.5±5.6 [a] | - | 0.001 |
| Inattention | 6.6±1.5 [a] | 7.9±0.6 [a] | 4.4±0.8[b] | - | 0.001 |
| Hyperactivity/Impulsivity | 2.2±1.7 [a] | 7.0±0.8[b] | 6.9±1.1[b] | - | 0.001 |

Note: Post hoc tests were calculated using the Tukey test (for continuous variables) or the Fisher exact test (for categorical variables). Columns with different superscripts differ at P 0.05.

ADHD = attention-deficit hyperactivity disorder

ADHD-I = predominantly inattentive ADHD

ADHD-C = combined ADHD

ADHD-H = predominantly hyperactive-impulsive ADHD

**Table 2. Characteristics of sequences in different ADHD subtypes**

| group | Valid Sequence | Coverage(%) | Chao | Ace | Simpson | Shannon |
|---|---|---|---|---|---|---|
| C | 57524 | 99.95±0.01 | 220.48±46.32 | 215.91±43.47 | 0.07±0.03 | 3.39±0.36 |
| ADHD-I | 56944 | 99.95±0.02 | 176.49±49.87* | 176.34±49.72* | 0.14±0.07* | 2.83±0.38* |
| ADHD-C | 54456 | 99.94±0.01 | 231.80±49.98 | 229.94±51.23 | 0.19±0.05* | 2.93±0.96 |
| ADHD-H | 57295 | 99.95±0.00 | 211.75±36.20 | 214.25±36.23 | 0.07±0.03 | 3.44±0.04 |

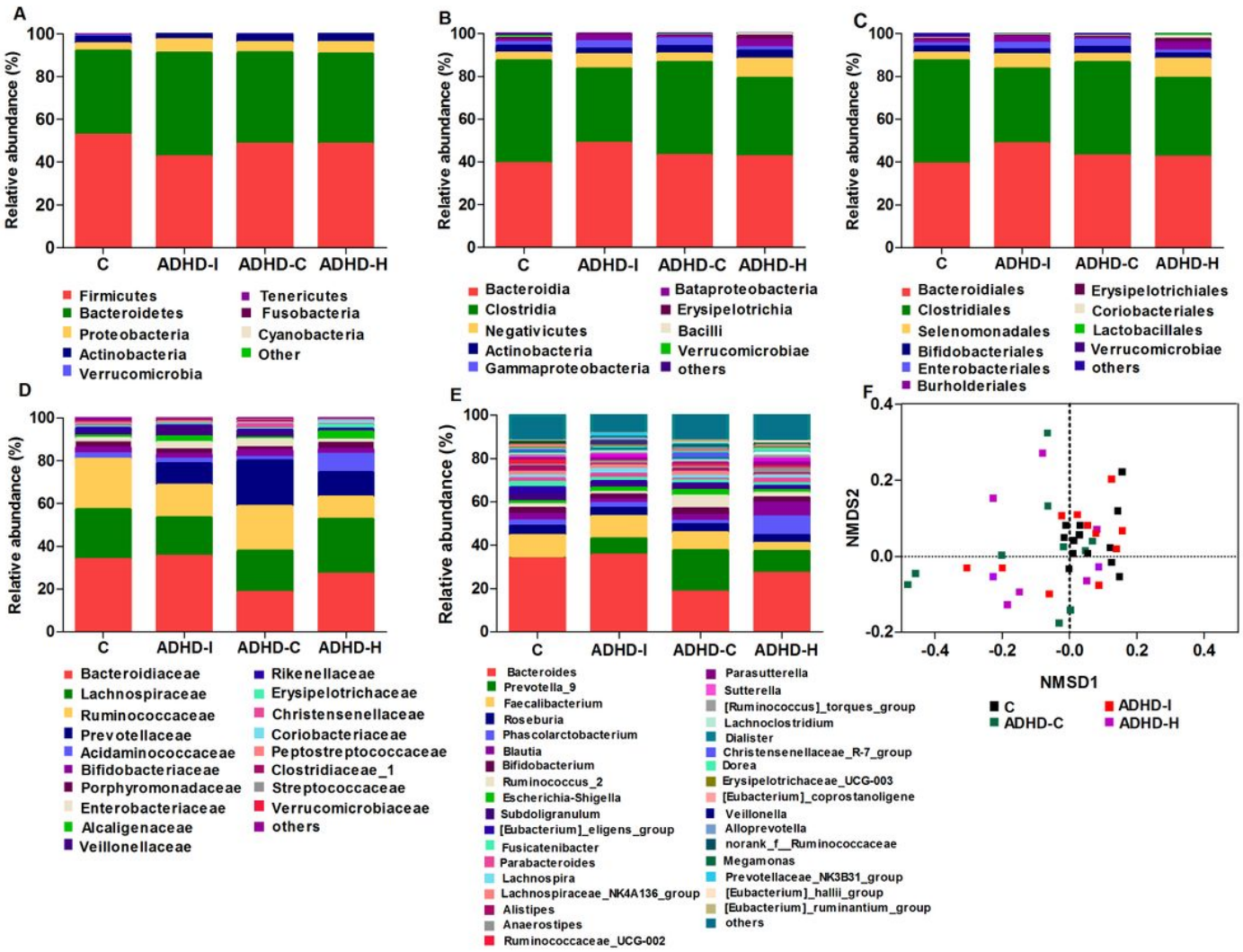
Note: the number of valid sequence, coverage percentages, richness estimators (ACE and Chao), and diversity indices

(Shannon and Simpson) were calculated at 3% distance. Data are means ± SD. *Compared to the control (C), $P < 0.05$.

ADHD = attention-deficit hyperactivity disorder, ADHD-I = predominantly inattentive ADHD,

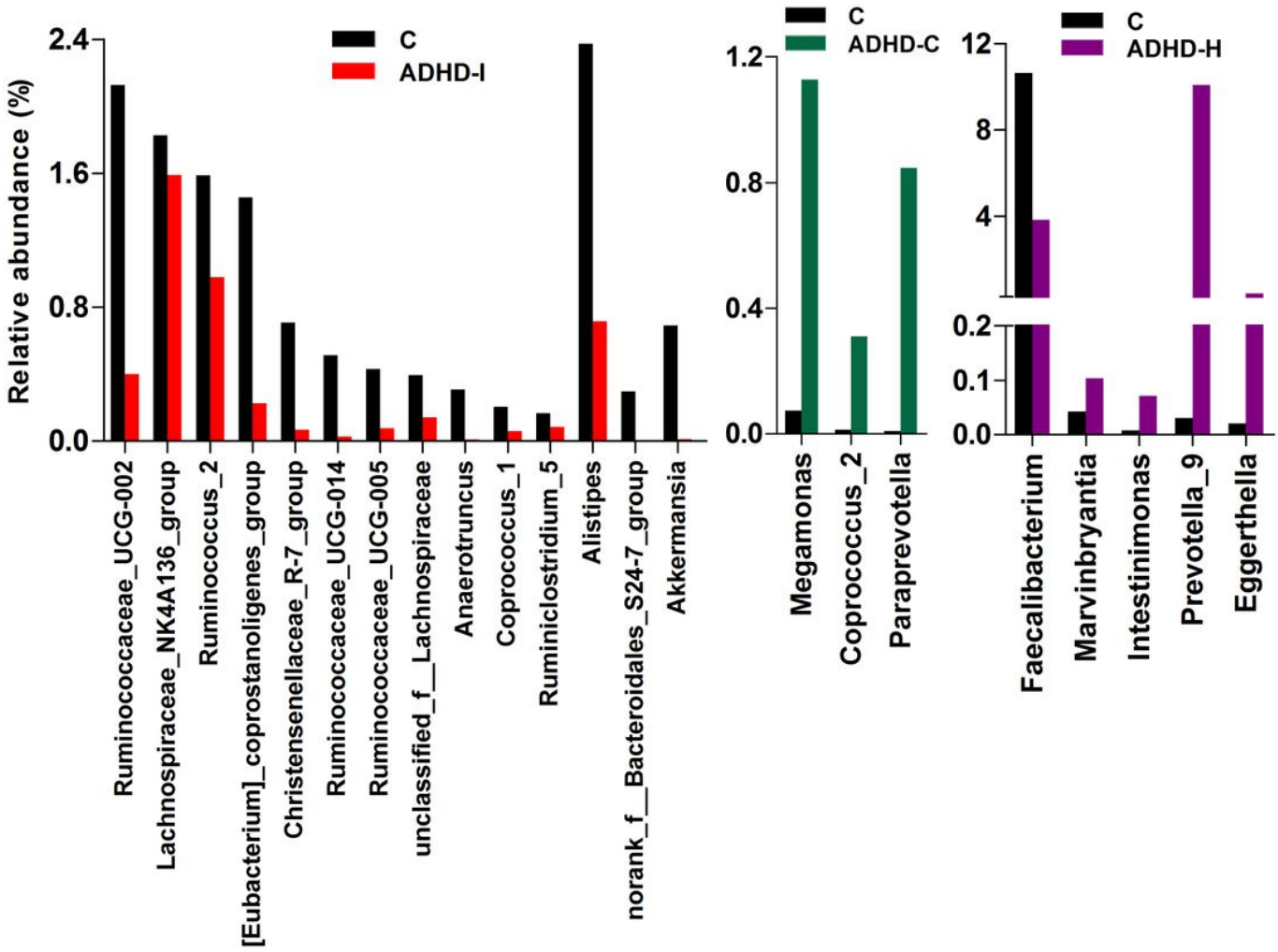
ADHD-C = combined ADHD, ADHD-H = predominantly hyperactive-impulsive ADHD

## Figures




## Figure 1

Gut microbiota profiles in different subtypes of children's ADHD. (A) phylum level, (B) class level, (C) order level, (D) family level and (E) genus level. (F) Non-metric multidimensional scaling (NMDS) of different ADHD subtypes and healthy controls. ADHD = attention-deficit hyperactivity disorder, ADHD-I = predominantly inattentive ADHD, ADHD-C = combined ADHD, ADHD-H = predominantly hyperactive-impulsive ADHD.




## Figure 2

The bacterial abundance of differential microbial genus in ADHD subtypes and controls (P< 0.05). ADHD = attention-deficit hyperactivity disorder, ADHD-I = predominantly inattentive ADHD, ADHD-C = combined ADHD, ADHD-H = predominantly hyperactive-impulsive ADHD.

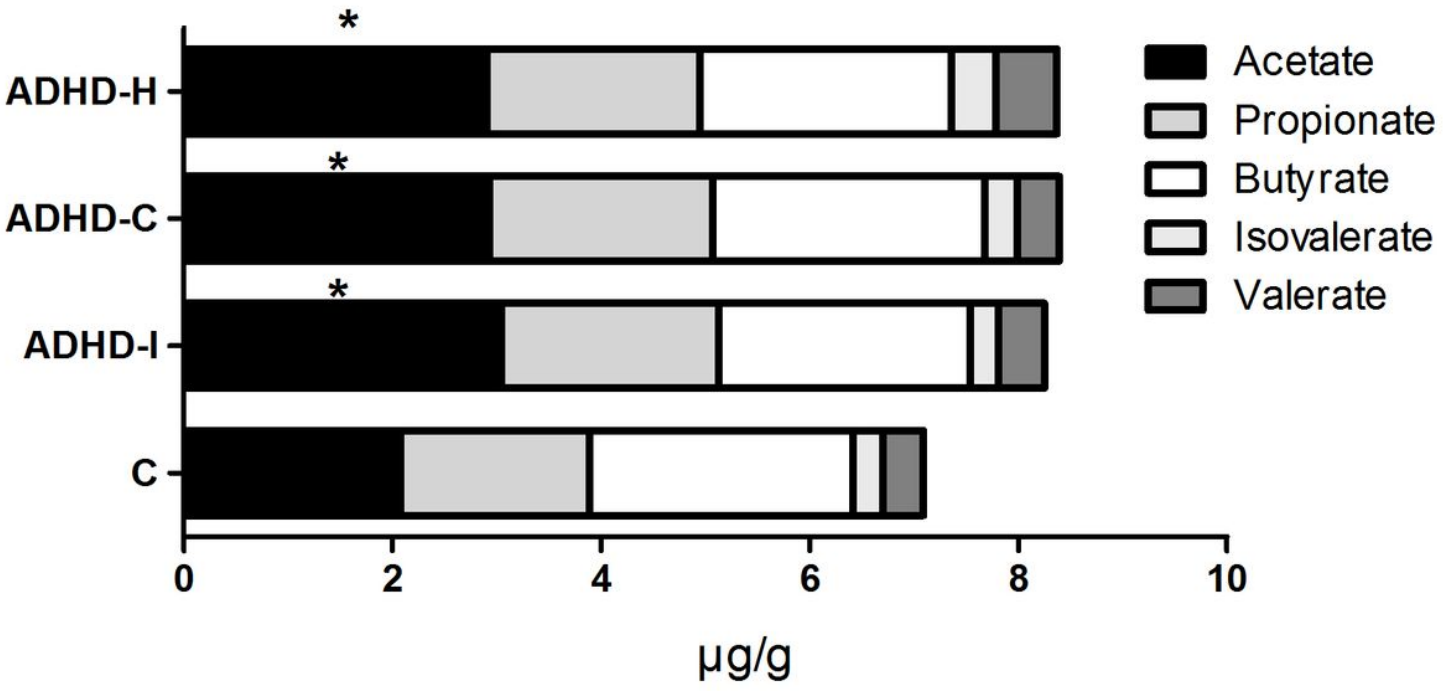
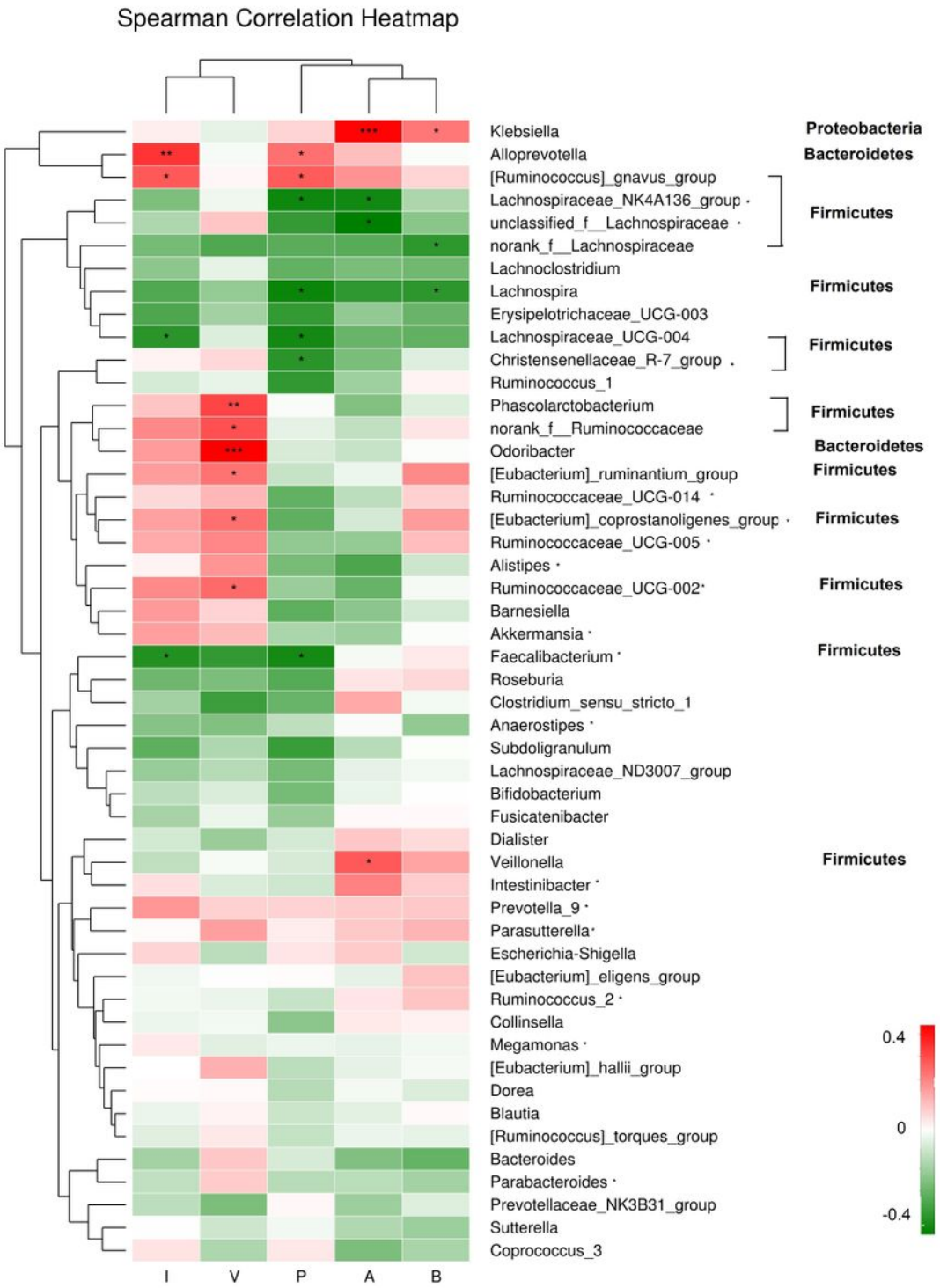



**Figure 3**

The fecal concentrations of SCFAs in different ADHD subtypes and the controls. Significance was established at $P<0.05$.




## Figure 4

Spearman correlations of the relative abundance of microbial genus and the concentrations of SCFAs among ADHD subtypes from controls. The r values are represented by gradient colors, where red and green cells indicate positive and negative correlations, respectively, * P<0.05.