Gut Microbiota: Effect of Pubertal Status

Xin Yuan
Fuzhou Children's Hospital of Fujian Medical University
Ruimin Chen (chenrm321@163.com)
Fuzhou Children's Hospital of Fujian Medical University https://orcid.org/0000-0003-4114-7706
Ying Zhang
Fuzhou Children's Hospital of Fujian Medical University
Xiangquan Lin
Fuzhou Children's Hospital of Fujian Medical University
Xiaohong Yang
Fuzhou Children's Hospital of Fujian Medical University

Research

Keywords: puberty, children, adolescent, 16s rDNA

Posted Date: August 2nd, 2020

DOI: https://doi.org/10.21203/rs.3.rs-49000/v1

Version of Record: A version of this preprint was published at BMC Microbiology on November 3rd, 2020.
See the published version at https://doi.org/10.1186/s12866-020-02021-0.

Abstract

Background: The make-up of gut microbiota at different puberty stages has not been reported. This cross-sectional study analyzed the bio-diversity of gut microbiota at different puberty stages.

Methods: The fecal microbiome was determined in 89 Chinese subjects aged 5-15 years. Subjects were grouped as non-pubertal (n=42) or pubertal (n=47) according to Tanner stages. Gut colonization patterns were determined by 16S rDNA microbiome profiling.

Results: The subjects were divided into non-pubertal (n=42, male%: 66.7%) or pubertal groups (n=47, male%:44.68); in both groups, *Firmicutes*, *Bacteroidetes* and *Proteobacteria* were the dominant phylum. There was no difference of alpha- and beta-diversity among disparate puberty stages. Non-pubertal subjects had significantly higher members of the genus *Turicibacter* and lower members of genus *Sutterella* than pubertal subjects. Of note, the proportion of genus *Sutterella* increased gradually with the pubertal status and independent of BMI-Z. In the pubertal subjects, the abundance of genus *Adlercreutzia*, *Dorea, Clostridium* and *Parabacteroides* was associated with the level of testosterone.

Conclusion: This is the first report of the diversity of gut microbiota at different puberty stages. The various species of gut microbiota changed gradually associated with puberty stages. Differences in gut microflora at different pubertal status may be related to androgen levels.

Introduction

Puberty constitutes a phase of life associated with profound physiological changes related to sexual maturation during the transition toward adulthood. These somatic developmental changes are predominantly driven by hormones and are accompanied by psychological adjustment. Therefore, this dynamic period represents an unparalleled opportunity to assess potential hormonal impacts on gut microbiota (1). Previous studies concluded that human gut microbiota were relatively stable and were adult-like after the first 1 to 3 years of life (2, 3). However, although healthy pre-adolescent children (ages 7-12 years) and adults harbored similar numbers of taxa and functional genes, their relative composition differed significantly (4). Nonetheless, a large scale study by Enck et al. using conventional colony plating to assess numbers of several bacterial genera, found no noticeable changes in children between 2-18 years old, including stable levels of *Bifidobacterium* and *Lactobacillus* (5). Recently, with the expansion and availability of bacterial DNA sequencing technology, a study revealed that comparison of distal intestinal microbiota composition between adolescents (11-18 years of age) and adults, a statistically significant higher abundance of the genera *Bifidobacterium* and *Clostridium* was found in adolescent samples. Also, the number of detected species was similar between sample groups, indicating that it was the relative abundances of the genera, and not the presence or absence of a specific genus that differentiated adolescent and adult samples (6).

Every organ is affected by the extensive changes in circulating hormone during puberty (7, 8). We postulated that these marked changes in hormone levels would alter the intestinal flora. No previous

microbiota study has explored the full span of growth from pre-puberty to late puberty. Such information is instructive given the association between gut microbiota during growth and adult disease risk (9). To this end, we utilized 16s rDNA gene sequencing to compare fecal microbiota profiles from pre-puberty to adolescence, ranging in age from 5-15 years.

Methods

Study population

This study was approved by the Ethics Committee of the Fuzhou Children's Hospital of Fujian Province, and informed consent was obtained.

The cross-sectional study consisted of healthy children managed by Fuzhou Children's Hospital of Fujian Province from September 2017 to March 2018. This study was limited to subjects who met the following criteria: (a) ages between 5 to 15 years old, and (b) residence of Fujian province.

Additionally, children with any of the exclusion criteria below were not eligible: Patients with any endocrine disease, a history of antibiotic therapy, probiotics, excessive vitamin intake, hospitalization (>24 h) any time point during 6 months prior to the study, any gastrointestinal, chronic illness, or diarrheal disease (World Health Organization definition) during one month prior to the study or gastro-intestinal-related medication (antibiotics prescription).

Clinical assessment

Height and weight were measured by trained endocrine nurses. BMI-Z scores were calculated based on Li Hui et al's reference values, and the diagnostic criteria for obesity or normal weight of Chinese children were as published (10). Tanner stage of pubertal development was assessed in all subjects by the professionally trained pediatric endocrinologists. Subjects were divided into non-pubertal and pubertal groups. The former group was further subdivided into younger children (5-8 years old) and pre-puberty (Tanner stage 1, >8 years old) group, and the puberty group was sub-divided into early (Tanner 2), middle (Tanner 3) and late (Tanner 4 and 5) stages for multi-point analysis. All participants maintained their usual dietary pattern at least 3 days before blood sampling. After 12 h of fasting, 5 ml venous blood was drawn from the left arm of the participants by registered nurses. All blood samples were stored at −80℃ and analyzed within two weeks of sampling. Levels of estradiol (E2) and testosterone (T) were measured by chemiluminescent immunoassays (IMMULITE 2000, Siemens Healthcare Diagnostics Products Limited, Germany) using specific reagents.

Brief medical history

A brief medical history was obtained by questionnaire completed by parents. No participant was taking medication that would affect their gut microbiota. No subjects had a history of constitutional delay in growth or maturation. A standardized survey was completed including demographic data (birth, sex, body size and weight, mode of birth, feeding patterns, dietary habits (high-carbohydrate diet or high-protein

diet), and pre-existing illnesses (including fever in the last 7 days). No subjects smoked. As mentioned previously, a recent gastrointestinal illness was exclusionary.

Fecal sample collection and processing

Subjects collected fecal samples at home in standard stool collection tubes. The samples were shipped immediately (within 2 hours) at room temperature and were stored at −80°C until processing.

Genomic DNA extraction

The microbial community DNA was extracted using MagPure Stool DNA KF kit B (Magen, China) following the manufacturer's instructions. DNA was quantified with a Qubit Fluorometer by using Qubit® dsDNA BR Assay kit (Invitrogen, USA) and the quality was checked by 1% agarose gel.

Library Construction

Variable regions V3-V4 of bacterial 16s rDNA gene were amplified with degenerate PCR primers, 341F(5'-ACTCCTACGGGAGGCAGCAG-3') and 806R(5'-GGACTACHVGGGTWTCTAAT-3'). Both forward and reverse primers were tagged with Illumina adapter, pad and linker sequences. PCR enrichment was performed in a 50μL reaction containing 30ng template, fusion PCR primer and a PCR master mix. PCR cycling conditions were as follows: 94℃ for 3 minutes, 30 cycles of 94℃ for 30 seconds, 56℃ for 45 seconds, 72℃ for 45 seconds and final extension for 10 minutes at 72℃ for 10 minutes. The PCR products were purified with AmpureXP beads and eluted in Elution buffer. Libraries were qualified by Agilent 2100 bioanalyzer (Agilent, USA). The validated libraries were used for sequencing on Illumina MiSeq platform (BGI, Shenzhen, China) following the standard pipeline of Illumina, generating 2 × 300bp paired-end reads.

The raw data were filtered to eliminate adapter contamination and low quality reads, then paired-end reads with overlap were merged to tags. And tags were clustered to OTU at 99% sequence similarity. Taxonomic ranks were assigned to OTU representative sequence using Qiime2-feature-Classifier. Alpha diversity, beta diversity and the different species screening were analyzed based on OTU and taxonomic ranks.

Statistical analysis

Statistical analyses of clinical data were performed using the Statistical Package for the Social Sciences software version 23.0 (SPSS Inc. Chicago, IL, USA). The normality of the data was tested using the Kolmogorov-Smirnov test. Data are expressed as mean ± SD depending on the data distribution. Comparisons of the results were assessed using independent samples t test, Mann-Whitney U test and Kruskal-Wallis test. Comparison of rates between two groups used chi-square test. A value of $P<0.05$ was considered statistically significant.

Statistical analyses of 16s rDNA sequencing data were performed on alpha- (reflecting intra-individual bacterial diversity) and beta- (inter-individual dissimilarity) diversity measurements. Alpha-diversity indices contained the Shannon diversity index (calculates richness and diversity using a natural logarithm), observed OTUs, Faith's Phylogenetic Diversity (Measures of biodiversity that incorporates phylogenetic difference between species) and Pielou's evenness (Measure of relative evenness of species richness). Beta-diversity indices contained Jaccard distance, Bray-Curtis distance, unweighted Unifrac and weighted Unifrac using PERMANOVA methods. Kruskal-Wallis Test was used for two groups comparison. Alpha- and Beta- diversity analysis was done by software QIIME2 (v2019.7) (11). Based on the OTU abundance, OTU of each group was listed. Venn diagram was drawn by Venn Diagram of software R(v3.1.1), and the common and specific OTU ID were summarized. Partial least squares discrimination analysis (PLS-DA) completed by package 'mixOmics' of software R. The statistics and graphics of differential analysis were done in STAMP (12). Welch's t-test was used for two groups comparison, and ANOVA methods was used for multiple groups comparison

Results

1. Study subjects

The mean age of the 89 participates was 9.75 ±1.92 years (ranging from 5.5 to 14.3 years) and 55.06% were boys. The majority (73.03%) were obese based on BMI, and 26.97% had normal BMI.

Based on puberty status, the subjects were divided into non-pubertal group (n=42, 66.7% male) and pubertal group (n=47, 44.68% male). The average age was 8.36 ±1.64 years and 10.99 ±1.15 years, respectively. There was no statistical difference in BMI-Z scores or dietary habits between the two groups (p=0.783 and 0.641, respectively). The non-pubertal group was further subdivided into a younger children group (n=18, 66.7% male) and pre-pubertal group (n=24, 66.7% male). And the pubertal group classified as early (n=18, 77.8% male), middle (n=14, 35.7% male), late (n=15, 13.3% male). Of the 40 girls, 21 had E2 measured, 2 were non-pubertal with a level of E2 <5pg/ml, and 19 were pubertal with a level of E2 33.68±35.80 pg/ml; 21 of the 49 boys had T measured, 6 were non-pubertal with a level of T 5.10±4.16 ng/dl, and 15 were pubertal with a level of T 83.20 ±98.55 ng/dl. There was no statistical difference in BMI-Z scores, mode of birth, feeding patterns or dietary habits among the groups (p>0.05). Table 1, 2 and Table S1 describes the characteristics of the subjects.

2. Core microbiota in all the subjects

(1) Core microbiota

With 16S ribosomal RNA gene sequencing, 671 discrete bacterial taxa (OTUs) were identified. Most of these species belonged to the "shared" category of those common to multiple but not all samples. We also identified a "core" of 557 species shared among all fecal samples. The non-puberty group had 49 unique species and the puberty group had 66 unique species (Figure 1). The core microbiota were

dominated by phylum *Firmicutes*, *Bacteroidetes* and *Proteobacteria* in both the non-pubertal and pubertal groups (Figure2 and Table S2).

(2) OTU classification with different puberty status

Using PLS-DA, the non-pubertal and pubertal groups can be distinguished to a certain extent, suggesting that the two groups differed in the classification of the gut microbiota (Figure 3).

3. Microbiota profiles with different puberty status

(1) Alpha- and beta-diversity in subjects with different puberty status

Regarding alpha-diversity, the Shannon diversity index, Observed OTUs, Faith's phylogenetic diversity and Pielou's evenness based on OTU distribution (groups of closely related individuals) there was no significant difference between pre-pubertal and pubertal groups (all $p > 0.05$, Table S3).

Beta-diversity also did not differ significantly between these two aforementioned groups after correction for multiple testing (Table S4).

(2) Bacterial taxa differences in subjects with different puberty status

We used STAMP (Welch's t-test) analysis to identify bacteria where the relative abundance was significantly increased or decreased in each phenotypic category. Non-pubertal subjects had members of the genus *Turicibacter* that were significantly more prevalent than puberty subjects, the proportion of sequence were 0.08±0.14% vs 0.02±0.04%, respectively ($p=0.012$, Figure 4 A). Also, the pubertal subjects had members of genus *Sutterella* that were significantly more prevalent than the non-pubertal subjects, the proportion of sequence were 1.92±3.27% vs 0.77±1.34%, respectively ($p=0.034$, Figure 4 B).

4. Microbiota profiles during puberty transition

(1) Alpha-, beta-diversity and bacterial taxa differences in non-puberty subgroups

As for the alpha-diversity between younger children and pre-pubertal groups, the Shannon diversity index, observed OTUs, Faith's phylogenetic diversity and Pielou's evenness based on OTU distribution, there was no statistical difference (all $p>0.05$, Table S3).

Beta-diversity also did not differ significantly between these two groups. None of the comparisons were significantly different (all $p>0.05$) after correction for multiple testing (Table S4).

STAMP (Welch's t-test) found no differential bacterial taxa between pre-puberty group and non-puberty group ($p>0.05$).

(2) Alpha-, beta-diversity and bacterial taxa differences in puberty subgroups

As for the alpha-diversity between the three subgroups at the different puberty stages, the Shannon diversity index, observed OTUs, Faith's phylogenetic diversity and Pielou's evenness based on OTU distribution, there was no significant differences (all p>0.05, Table S3).

Beta-diversity also did not differ significantly between the three subgroups (all p> 0.05) after correction for multiple testing (Table S4).

STAMP (ANOVA methods) revealed that among early, middle and late puberty groups, the proportion of the genus *Anaerotruncus* increased gradually in association with the puberty stages (0.005±0.008%, 0.010±0.011%, and 0.033±0.049%, respectively). The proportion of the genus *Coprococcus* first increased, and then waned to a similar proportion with early puberty group (0.005±0.008%, 0.010±0.011%, and 0.033±0.049%, respectively). Differences among groups were statistically significant (p=0.025 and 0.025, respectively, Figure 5).

Spearman correlation analysis was used to detect an impact of BMI-Z on genera *Anaerotruncus* and *Coprococcus*: Genus *Coprococcus* did not correlate with BMI-Z (r=-0.025, p=0.865), whereas genus *Anaerotruncus* related to both BMI-Z and Tanner staging (r=-0.326 and 0.327, p=0.025 and 0.025, respectively). After further correcting BMI-Z, the correlation between genus *Anaerotruncus* and Tanner stage was slightly more robust (r=0.350, p=0.017).

(3) Alpha-, beta-diversity and bacterial taxa differences in non-puberty and puberty subgroups

Regarding the Alpha-diversity between the pre-puberty and the early puberty groups, the Shannon diversity index, observed OTUs, Faith's phylogenetic diversity and Pielou's evenness based on OTU distribution did not reveal any significant difference. Comparing the younger children group and the late puberty group, the Alpha- diversity indexes did not differ (all p>0.05, Table S3).

Beta-diversity also did not differ significantly between the three subgroups according to the puberty stages. The results were non-significant (all p>0.05) after correction for multiple testing (Table S4).

STAMP (ANOVA methods) showed that among non-puberty, early, middle and late-puberty groups, the proportion of the genus *Butyricicoccus* progressively increased, and then waned toa similar proportion with non-puberty group (0.27±0.23%, 0.30±0.26%, 0.70±0.85% and 0.28±0.22%, from non-pubertal, early-pubertal, middle-pubertal to late-pubertal, respectively). The proportion of the genus *Sutterella* increased steadily associated with puberty stages (0.77±1.34%, 1.26±1.92%, 1.63±3.96% and 3.00±3.57%, from non-pubertal, early-pubertal, middle-pubertal to late-pubertal, respectively), and the difference among groups were statistically significant (p=0.013 and 0.039, respectively, Figure 6).

Spearman correlation analysis was used to assess the impact of BMI-Z on the genera *Butyricicoccus* and *Sutterella*. Both genus *Butyricicoccus* and *Sutterella* did not correlated with BMI-Z (r=-0.079 and -0.131, p=0.459 and 0.222, respectively).

5. Correlations Between Sex hormone and Bacterial Abundance

To evaluate correlations between bacteria and serum sex hormones (testosterone and estradiol), Spearman's rank analysis was adopted. In the pubertal subjects, the abundance of genera *Adlercreutzia* and *Dorea* was positively associated with the level of testosterone (r=0.293 and 0.545, p=0.046 and 0.002, respectively), and the abundance of genera *Parabacteroides* and *Clostridium* was positively associated with the level of testosterone (r=-0.383 and -0.361, p=0.033 and 0.046, respectively). There was no association between the bacterial abundance and serum estradiol (all p>0.05).

Discussion

Recent studies exploring bacterial 16S rDNA genes have yielded insight into the intricate interactions between age and the gut microbiota (13). With increasing age, there is a gradual and steady reduction in the population of aerobes and facultative anaerobes, in concert with a simultaneous upsurge in the number of anaerobes (5, 14). Previously, the adolescent microbiota was found to differ functionally from that of adults, expressing genes related to development and growth (15), whereas the adult microbiota was associated more with inflammation and obesity (3, 5). However, the make-up of gut microbiota at different puberty stages were not described (16).

In this study, we found that there was no difference in alpha- and beta-diversity between non-pubertal and pubertal subjects. However, non-pubertal subjects had members of genus *Turicibacter* which were significantly more prevalent than pubertal subjects. Yet the latter children had members of genus *Sutterella* that were significantly more prevalent than the former subjects. Few studies have addressed differences in gut microbiota as children age (17). A recent high-throughput analysis of distal gut microbiota found that adolescents harbor a less complex and considerably different microbiota from that of adults, even though they apparently share a core microbiota configuration (5, 6). Furthermore, comparing adults to adolescents, a significantly higher abundance of both *Bifidobacterium* and *Clostridium* genera in the adolescents was found, but the number of species was similar in the two groups (6). Paliy O et al. developed a microbiota microarray based on the Affymetrix GeneChip platform and found that fecal samples from adults harbored more *Clostridia*, and less *Bacteroidetes* and *Proteobacteria*, than those from children. A number of other putative differences were also reported at the genus level (18). In particular, whereas adolescents have significantly higher levels of *Clostridia* and *Bifidobacteria* genera compared to adults, the number of species between the two groups was similar (2, 5).

In addition to the bursts of GnRH with puberty, many other circulating hormones come of age. These profound hormonal changes may be accompanied by changes in the composition of gut microbiota. In a study from the Netherlands, 61 children (2 - 18 years) collected fecal samples weekly for 6 weeks, along with a follow-up sample after 18 months. The microbial composition stability varied per phylum at both short-term and long-term intervals. However, the age span of this study precluded the characterization of the gut microbiota at distinct puberty stages. We found there was no significant difference in alpha-, beta-diversity, or the differential bacterial taxa between the younger children and pre-pubertal groups. A study of Asian school-age children assessed the presence and ratios of *Bifidobacterium / Bacteroides* and

Prevotella enterotype-like clusters, and the prevalence of which depended on geographic regions with varying local diets and living environments (19).

Among the three pubertal subgroups, notwithstanding that there were no significant differences in alpha- and beta-diversity, differential analysis detected significant microbiota differences amongst the groups. Notably, the proportion of the genus *Anaerotruncus* increased gradually with the puberty stages, and the proportion of the genus *Coprococcus* first increased, and then decreased to a similar proportion with the early puberty group, suggesting that the two genera are closely associated with puberty. It has been reported that gut *Coprococcus* was enriched in girls with idiopathic central precocious puberty (20). All microbes in genera *Coprococcus* promote SCFAs production (21-23). Therefore, it is plausible that SCFAs-producing bacteria are increased in pubertal subjects to promote the expression of the leptin gene, which in turn activate the HPG axis, and lead to the onset of puberty (20).

Previous research inferred that during childhood there was less diversity in the gut microbial community compared to adults (24). In the present study, upon comparing the non-pubertal group with early-, middle- and late-puberty subjects, there was no statistical difference in alpha- and beta-diversity. Nonetheless, the *Butyricicoccus* and *Sutterella* genera of fecal microbiota varied with puberty stage.

Despite the limitations imposed by heterogeneity of the groups, the present study found a relationship between puberty and gut microbiota. During sexual development and growth through the adolescent period, the gut microbiota undergoes progressive changes, likely due to the hormonal surge or other age-related factors. Accordingly, the association between serum sex hormones and bacterial abundance was further analyzed. In the pubertal subjects, it was found that the abundance of genus *Adlercreutzia*, *Dorea, Clostridium* and *Parabacteroides* associated with the level of testosterone. A mouse model study showed that after inoculating male and female germ-free C57BL/6J mice with fecal bacteria from a man with short-term vegetarian and inulin-supplemented diet, *Clostridium* and *Dorea* were over-represented in females (25). Furthermore, Shin JH (26) reported that the abundance of *Dorea* correlated significantly with testosterone levels in men, a finding consistent with the results of our study. We believe that the abundance of the two bacteria *Parabacteroides*, and *Adlercreutzia* are affected by sex hormones, and are known to metabolize phytoestrogens with generation of secondary molecules such as equol, enterolactone, and secoisolariciresinol (27). The association between these bacteria and androgen warrants further investigated.

As a counter-narrative, could the adaptive intestinal microbiome effect serum hormone concentrations or tissue responsiveness (28)? Little is known about the causal inter-relationships between gut microbiota and pubertal development. As differences in gut microbiota become more pronounced at puberty, the role of sex hormones in shaping the gut microbiota composition is terra incognita (29, 30). Relevant to our study, the transfer of gut microbiota from adult male mice to immature females, which altered the recipient's microbiota, resulted in elevated levels of testosterone comparable to males (31). Interesting to ponder, does a similar phenomenon due to the microbiota exists in humans ? It is plausible that

microbiota-driven hormone effects are in play and potentially influenced by genetic, metabolic, or psychosocial factors.

Conclusion

This study is the first report of the characteristics of fecal microbiota during the transitional stages of puberty. Non-pubertal subjects harbored significantly more members of the genus *Turicibacter* and lower members of genus *Sutterella* than puberty subjects. Moreover, the proportion of the genus *Anaerotruncus* escalated in the puberty subgroups. Likewise, the genus *Sutterella* increased in association with the pubertal stage. In the pubertal subjects, it was found that the abundance of genera *Adlercreutzia*, *Dorea, Clostridium* and *Parabacteroides* was associated with the level of testosterone. The explanation for the differences in these gut microbiota, and their potential metabolic and hormonal impact, requires additional study.

Declarations

Ethics approval and consent to participate

This study was reviewed and approved by the Ethics Committee of Fuzhou Children's Hospital of Fujian Medical University, and was conducted in agreement with the Declaration of Helsinki Principles. Informed consent was obtained from all individual participants included in the study.

Consent for publication

Informed consent for publication was obtained from all individual participants included in the study.

Availability of data and materials

The datasets supporting the conclusions of this article are included within the article and its additional files.

Competing interests

The authors declare that they have no competing interests.

Funding

This study was supported by Technology Innovation Team Train Project of Fuzhou Health Committee in China (2016-S-wp1), and sponsored by key Clinical Specialty Discipline Construction Program of Fuzhou, Fujian, P.R.C (201610191).

Author Contributions

XY drafted the initial manuscript; RMC conceptualized and designed the study, and reviewed and revised the manuscript; YZ and XHY collected cases; XQL did the laboratory testing.

Acknowledgements

The authors are grateful to all the participants.

Contribution to the Field Statement

There were no differences in alpha- and beta-diversity of gut microbiota associated with puberty stages, and the proportion of genus *Sutterella* increased gradually with puberty stages. In the pubertal subjects, the abundance of genus *Adlercreutzia*, *Dorea, Clostridium* and *Parabacteroides* was associated with the level of testosterone. The make-up of gut microbiota at different puberty stages. Changes in gut microbiota as adolescence progresses. This is the first report of the diversity of gut microbiota at different puberty stages. The various species of gut microbiota changed gradually associated with puberty stages. Differences in gut microflora at different pubertal status may be related to androgen levels.

References

1. Kundu P, Blacher E, Elinav E, Pettersson S. Our Gut Microbiome: The Evolving Inner Self. *Cell*. **171**, 1481-1493 (2017).
2. Yatsunenko T, et al. Human gut microbiome viewed across age and geography. *Nature*. **486**, 222-227 (2012).
3. Stewart CJ, et al. Temporal development of the gut microbiome in early childhood from the TEDDY study. *Nature*. **562**, 583-588 (2018).
4. Hollister EB, et al. Structure and function of the healthy pre-adolescent pediatric gut microbiome. *Microbiome*. **3**, 36 (2015).
5. Enck P, et al. The effects of maturation on the colonic microflora in infancy and childhood. *Gastroenterol. Res. Pract*. **2009**, 752401 (2009).
6. Agans R, et al. Distal gut microbiota of adolescent children is different from that of adults. *FEMS Microbiol. Ecol*.**77**, 404-412 (2011).
7. Yahfoufi N, Matar C, Ismail N. Adolescence and aging: impact of adolescence inflammatory stress and microbiota alterations on brain development, aging and neurodegeneration. *J. Gerontol. A Biol. Sci. Med. Sci.* Jan 10 (2020).
8. Robert GH, et al. Association of gray matter and personality development with increased drunkenness frequency during adolescence. *JAMA Psychiatry.* Dec 18 (2019).
9. Integrative HMP (iHMP) Research Network Consortium. The Integrative Human Microbiome Project. *Nature*. **569**, 641-648 (2019).

10. Li H, Zong XN, Ji CY, Mi J. Body mass index cut-offs for overweight and obesity in Chinese children and adolescents aged 2 - 18 years. *Zhonghua Liu Xing Bing Xue Za Zhi*. **31**, 616-620 (2010).
11. Bolyen E, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology*, **37**, 852-857 (2019).
12. Parks DH, Tyson GW, Hugenholtz P, Beiko RG. STAMP: statistical analysis of taxonomic and functional profiles. *Bioinformatics*. **30**, 3123-3124 (2014).
13. Xu C, Zhu H, Qiu P. Aging progression of human gut microbiota. *BMC Microbiol.***19**, 236 (2019).
14. Strati F, et al. Age and Gender Affect the Composition of Fungal Population of the Human Gastrointestinal Tract. *Front Microbiol.***7**, 1227 (2016).
15. Yahfoufi N, Matar C, Ismail N. Adolescence and aging: impact of adolescence inflammatory stress and microbiota alterations on brain development, aging and neurodegeneration. *J. Gerontol. A Biol. Sci. Med. Sci.* Jan 10 (2020).
16. de Meij TG, et al. Composition and stability of intestinal microbiota of healthy children within a Dutch population. *FASEB J*. **30**, 1512-1522 (2016).
17. Flannery J, Callaghan B, Sharpton T, Fisher P, Pfeifer J. Is adolescence the missing developmental link in Microbiome-Gut-Brain axis communication? *Dev. Psychobiol.* **61**, 783-795 (2019).
18. Paliy O, Kenche H, Abernathy F, Michail S. High-throughput quantitative analysis of the human intestinal microbiota with a phylogenetic microarray. *Appl. Environ. Microbiol.***75**, 3572-3579 (2009).
19. Nakayama J, et al. Diversity in gut bacterial community of school-age children in Asia. *Sci Rep*. **5**, 8397 (2015).
20. Dong G, et al. The association of gut microbiota with idiopathic central precocious puberty in girls. *Front Endocrinol (Lausanne)*.**10**:941 (2020).
21. Duncan SH, et al. Reduced dietary intake of carbohydrates by obese subjects results in decreased concentrations of butyrate and butyrate-producing bacteria in feces. *Appl Environ Microbiol.* **73**:1073-1078 (2007).
22. Pryde SE, et al. The microbiology of butyrate formation in the human colon. *FEMS Microbiol Lett.***217**:133-139 (2002).
23. Lin H, et al. Correlations of fecal metabonomic and microbiomic changes induced by high-fat diet in the pre-obesity state. *Sci Rep*.**6**:21618 (2016).
24. Ringel-Kulka T, et al. Intestinal microbiota in healthy U.S. young children and adults-a high throughput microarray analysis. *PLoS One*. **8**, e64315 (2013).
25. Wang JJ, et al. Sex differences in colonization of gut microbiota from a man with short-term vegetarian and inulin-supplemented diet in germ-free mice. *Sci Rep*. **6**:36137 (2016).
26. Shin JH, et al. Serum level of sex steroid hormone is associated with diversity and profiles of human gut microbiome. *Res Microbiol.***170**:192-201 (2019).
27. Cady N, et al. Beyond Metabolism: The Complex Interplay Between Dietary Phytoestrogens, Gut Bacteria, and Cells of Nervous and Immune Systems. *Front Neurol.* 11:150 (2020).

28. Hopkins MJ, Sharp R, Macfarlane GT. Variation in human intestinal microbiota with age. *Dig. Liver Dis*. **34**, S12-18 (2002).
29. Yurkovetskiy L, et al. Gender bias in autoimmunity is influenced by microbiota. *Immunity*. **39**, 400-412 (2013).
30. Markle JG, et al. Sex differences in the gut microbiome drive hormone-dependent regulation of autoimmunity. *Science*.**339**, 1084-1088 (2013).
31. Kundu P, Blacher E, Elinav E, Pettersson S. Our Gut Microbiome: The Evolving Inner Self. *Cell*. **171**, 1481-1493 (2017).

Tables

Table 1 Clinical characteristics of the study population divided by puberty status

	non-puberty (n=42)	Puberty (n=47	P value
Age (years)	8.36±1.64	10.99±1.15	<0.001
Gender (male%)	66.7%	44.68%	0.054
Height (cm)	132.94 ±10.62	148.96±8.03	<0.001
Weight (kg)	39.72±15.53	54.09±13.57	<0.001
BMI (kg/cm^2)	21.70±5.56	24.07±4.40	0.028
BMI-Z	1.92±1.79	2.01±1.13	0.783

Table 2 Clinical characteristics of the study population divided by puberty stages

	Younger children (5-8years-old)	Pre-puberty	Early puberty	Middle puberty	Late puberty	P value
N (n)	18	24	18	14	15	
Age (years)	6.81±0.74	9.53±1.05	10.76±0.95	10.85±1.41	11.40±1.05	<0.001
Gender (male%)	66.7%	66.7%	77.8%	35.7%	13.3%	0.001
Height (cm)	124.64±7.55	139.16 ±8.05	148.35±8.06	149.64±9.84	149.06±6.50	<0.001
Weight (kg)	30.02±9.17	47.00±15.46	56.83±12.53	55.55±15.77	49.45±12.18	<0.001
BMI (kg/cm^2)	18.98±4.27	23.74±5.61	25.53±4.21	24.28±4.31	22.12±4.26	0.001
BMI-Z	1.47±1.96	2.26±1.61	2.26±1.08	2.19±1.02	1.55±1.21	0.256

Figures

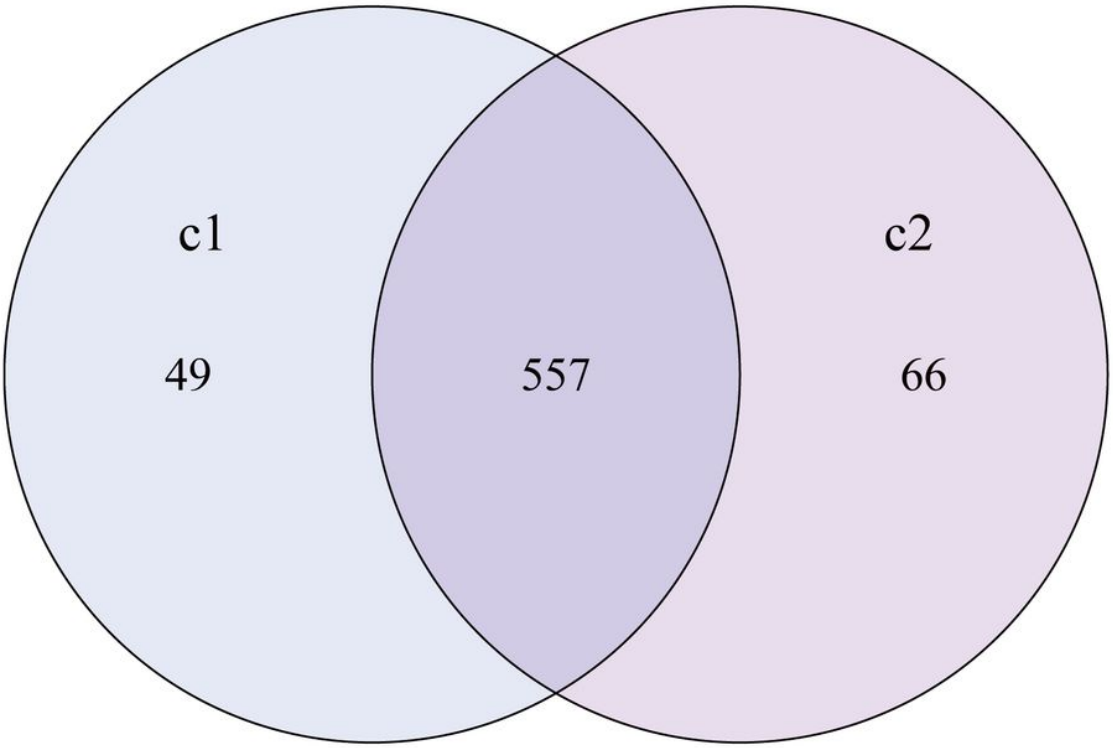

Figure 1

Shared OTU across the non-puberty and puberty groups Different colors represent different groups, the interior of each circle represents the number of observed OTUs in the certain group. The overlapping area or intersection represents the set of OTU commonly present in the counterpart groups. Likewise, the single-layer zone represents the number of OTUs uniquely found in the certain group. C1: puberty group; C2: non-puberty group.

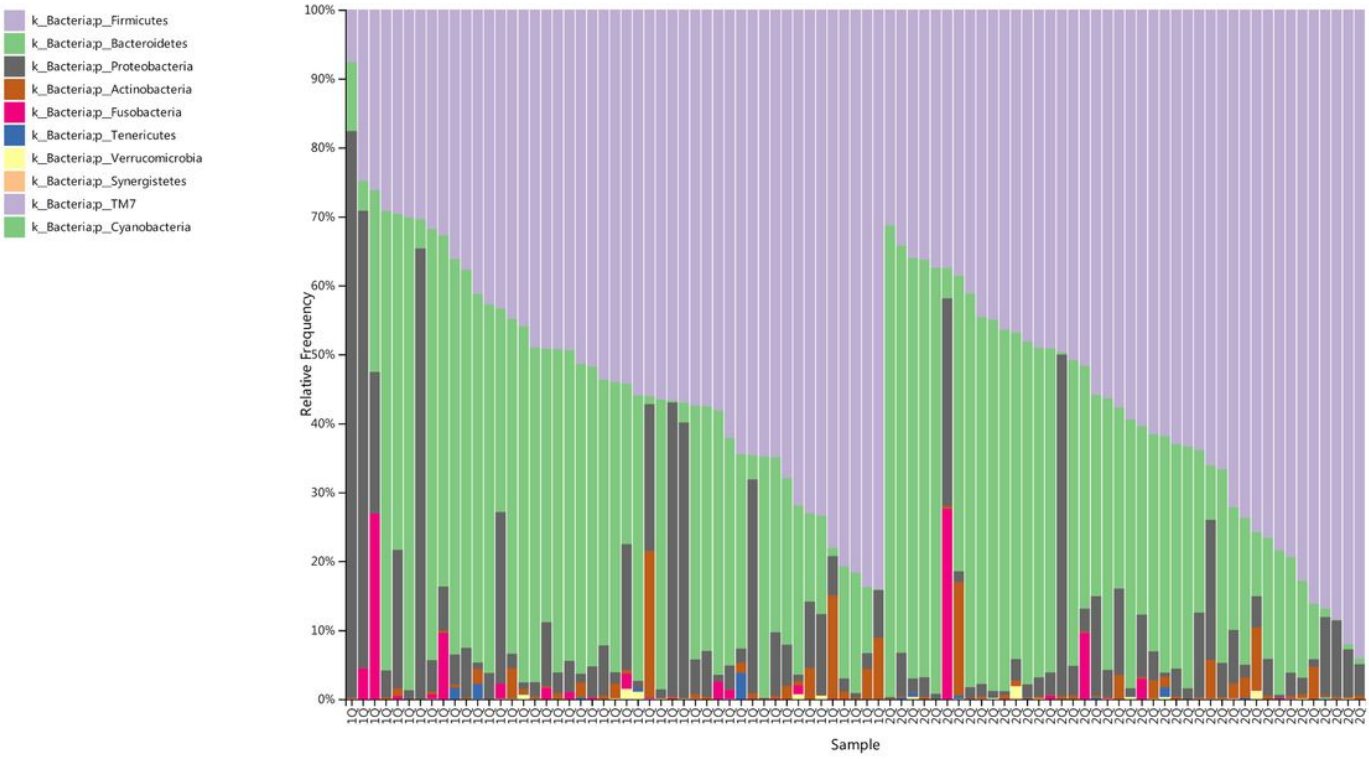

Figure 2

The taxa-bar of gut microbiota in non-pubertal and pubertal groups at phylum level

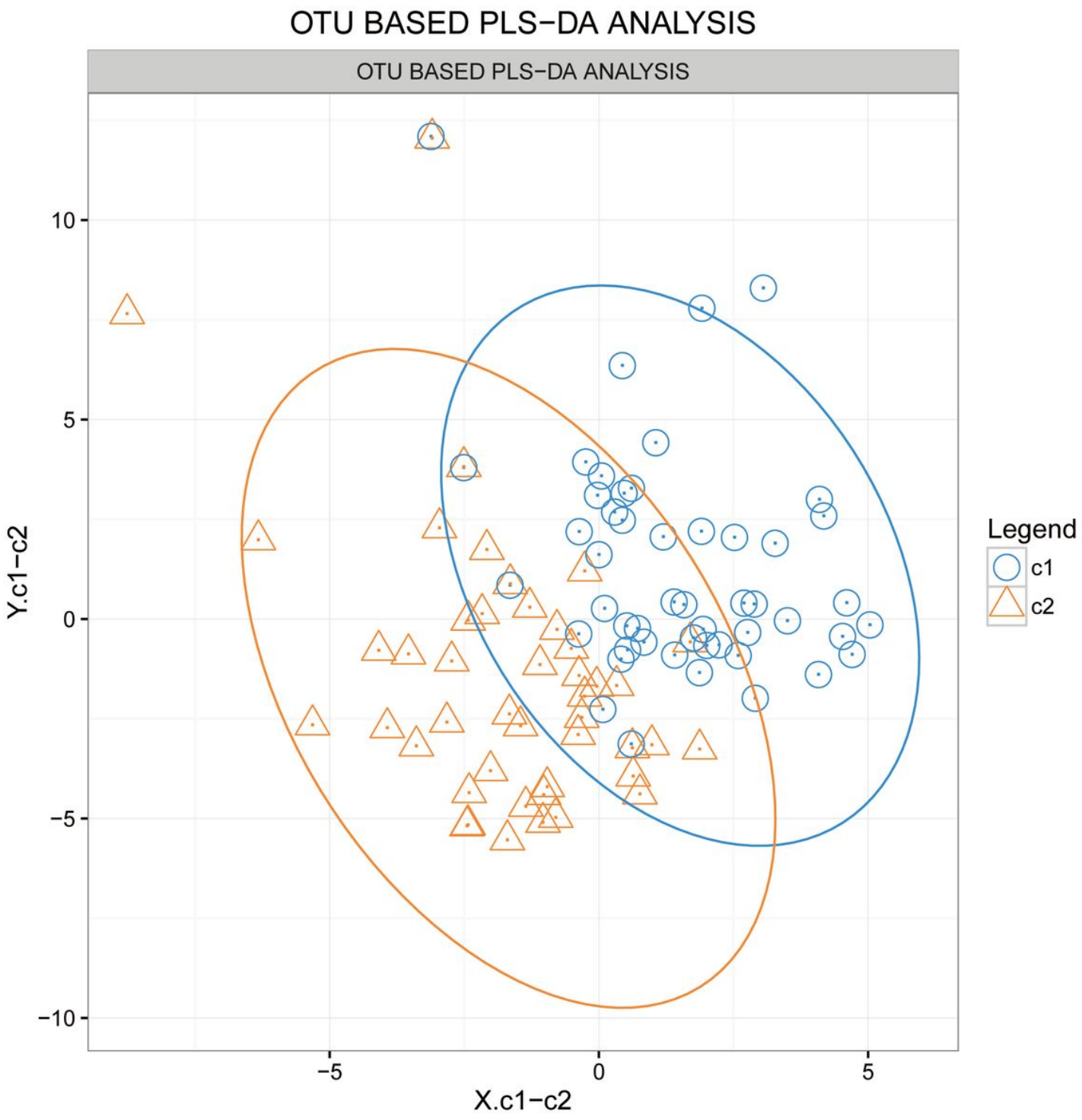

Figure 3

PLS-DA based on OTU abundance The horizontal axis and the vertical axis indicate the top 2 components. Each dot denotes one sample. Samples are colored and grouped by elipse according to their group information. C1: puberty group; C2: non-puberty group.

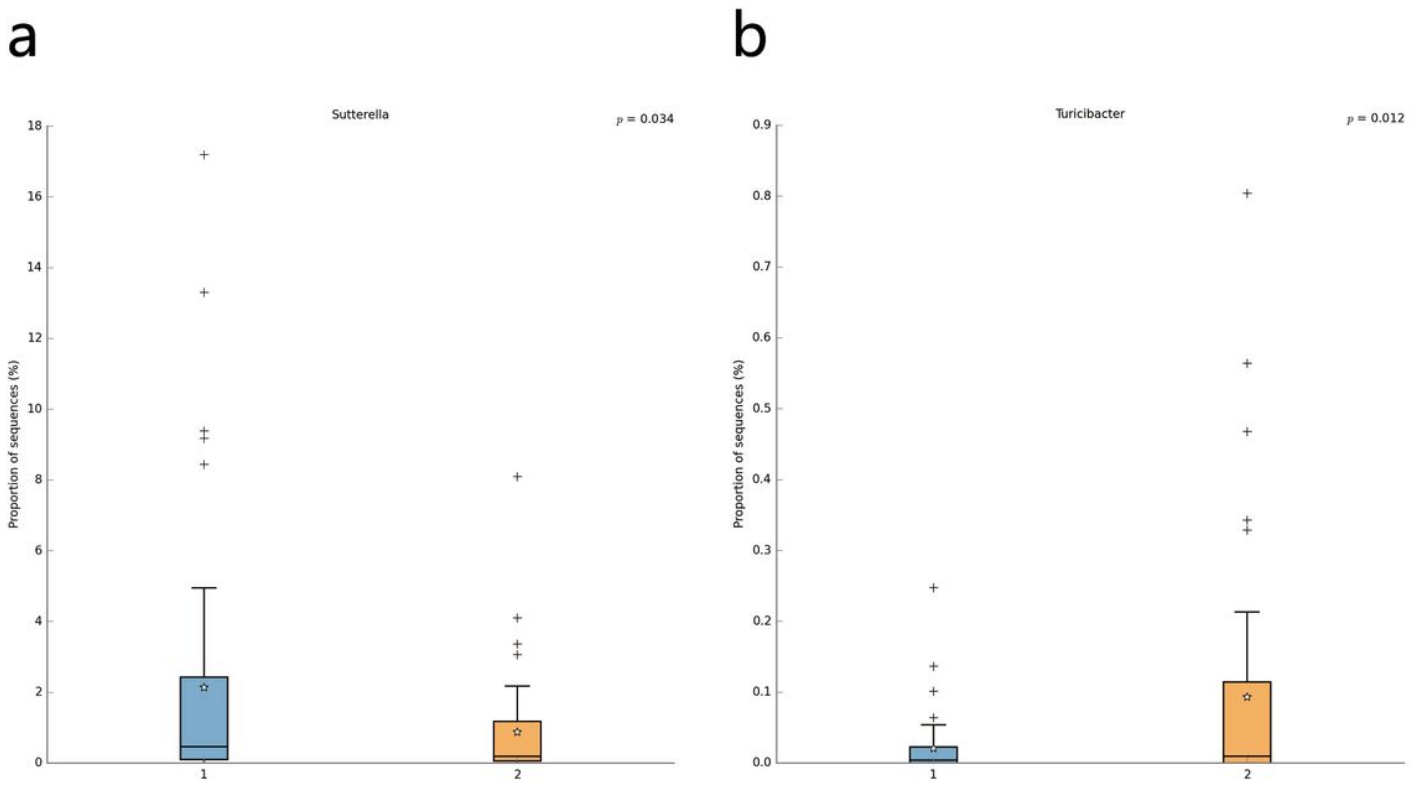

Figure 4

Bacterial taxa differentially represented in the non-pubertal and pubertal groups A: Proportion of genus Sutterella in the non-pubertal and pubertal groups; B: Proportion of genus Turcbacter in the non-pubertal and pubertal groups; 1: pubertal group, 2: non-pubertal group.

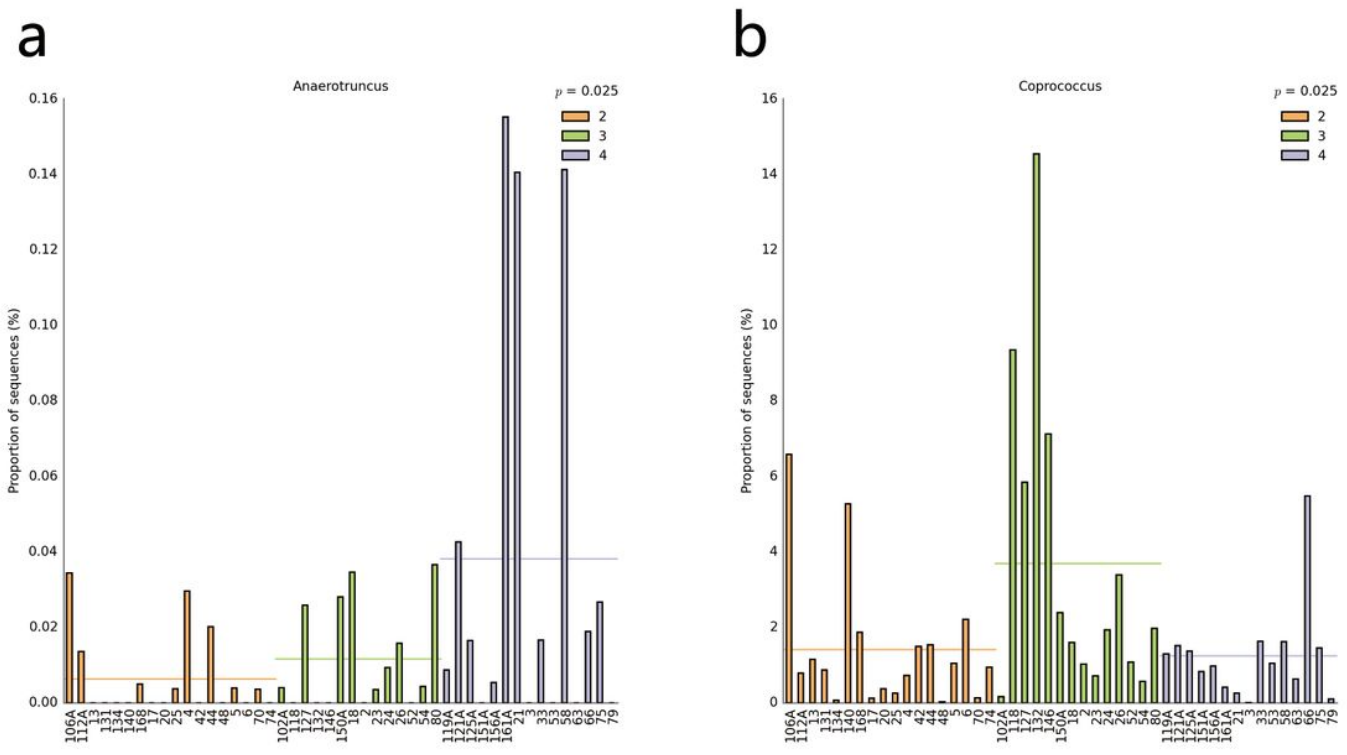

Figure 5

Bacterial taxa differentially represented among the puberty subgroups A: Proportion of genus Anaerotruncus among the early, middle and late-puberty groups; B: Proportion of genus Coprococcus among the early, middle and late-puberty groups; 2: early-puberty group, 3: middle-puberty group, 4: late-puberty group.

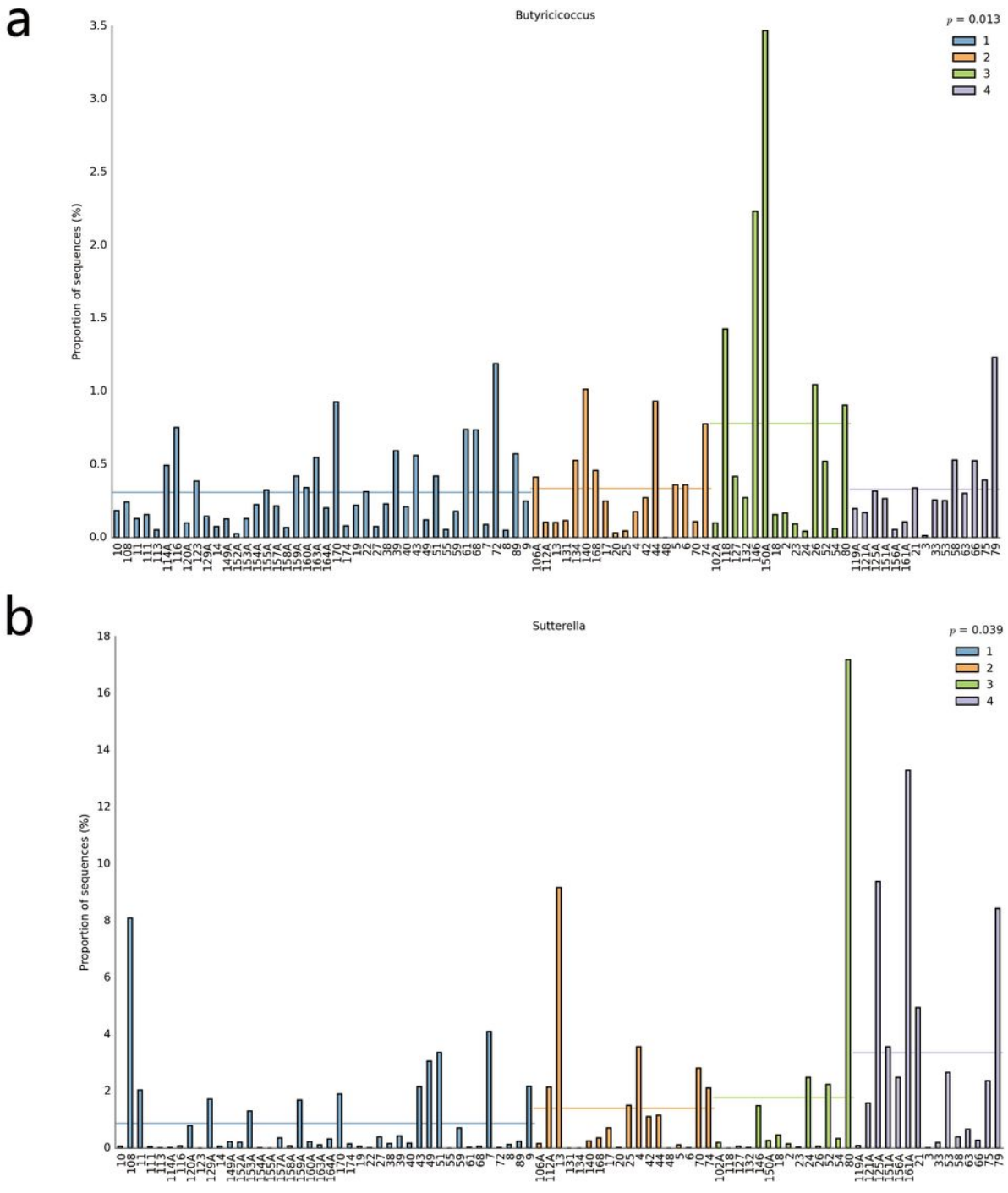

Figure 6

Bacterial taxa differentially represented among the three different puberty stages subgroups A: Proportion of genus Butyricicoccus among the non-pubertal, early, middle and late-puberty groups; B: Proportion of genus Sutterella among the non-puberty, early, middle and late-pubertal groups; 1: non-pubertal group, 2: early-pubertal group, 3: middle-pubertal group, 4: late-pubertal group.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryMaterial.docx