Characteristics of Intestinal Microbiota and Mother's Reproductive Tract Flora in Children With Down Syndrome

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Abstract

Background: Patients with Down's syndrome (DS) often have an increased rate of infections, hypertension, objectivity and gastrointestinal disorders, which are the most common abnormalities and have a significant impact on their daily life. The gut microbiota plays an important role in maintaining gut homeostasis and improving immunity and has been linked to the development of obesity, hypertension, and colon cancer. However, there are few studies on the intestinal flora and the mother's reproductive tract flora of children with DS in childhood. Therefore, 16S sequencing technology was used to analyze and explore the intestinal flora of children with DS and CHD patients in DS and the microbial abundance and diversity composition in the mother's reproductive tract.

Results: We found that the gut microbiota in children with DS was mainly composed of *Escherichia*, *Bifidobacterium*, *Clostridium* and *Bacteroides*, which have significant differences in the abundance and diversity of intestinal flora compared with healthy children, and the abundance of *Enterococcus* and *Erysipelatoclostridium* in the intestine of children with CHD was significantly higher than that of children without CHD, and the relative abundance of *Lactobacillus* in the reproductive tract of mothers with DS was significantly higher than that of mothers with healthy children, which may suggest potential ways of using microbiome composition for prognosis and diagnosis. Through functional analysis, it was found that Down's syndrome patients significantly downregulated immune system and their cell growth and nucleotide metabolism were lower than those of healthy children.

Conclusion: We performed 16S rRNA gene sequencing on fecal samples from 60 children and vaginal swab samples from 63 mothers to identify a number of potentially important taxonomic, functional and microbiomes associated with congenital heart disease and Down's syndrome. Structural changes and its correlation with the mother's vaginal flora. Our analysis shows that the ecosystem associated with childhood congenital heart disease affects the selection of bacterial communities in the native microbiota, and we focus on specific bacteria and their relevance to disease.

Background

Down syndrome, also known as trisomy 21 syndrome, is an autosomal dominant genetic disease in children caused by the absence of separation of egg cells or sperm during meiosis of chromosome 21[1, 2]. It is the most common chromosomal disease that causes developmental disorders and intellectual defects in children[3]. The incidence of DS in the world is approximately 1/500 ~ 1/1000 of live births[4]. In the United States, the incidence rate is approximately 1.26%[5]. DS children have many clinical phenotypes, such as mental retardation, growth retardation, and special facial features, accompanied by multiple system malformations, which affect their health[6]. The risk of developing deformities in each group is significantly higher than that in normal children. The most common complication of DS is congenital heart disease (CHD). It has been reported that approximately 40% – 60% of children with DS have CHD[7]. The main reason may be related to chromosome abnormalities in DS patients, and the differences in morbidity and mortality in DS are related to the type of congenital heart defect. Some

studies have shown that microRNAs on chromosome 21 (mir-nov21) are involved in the regulation of cardiac development by regulating the expression of GATA4 and other genes, while the inactivation of the GATA4 gene in the early stage of cardiac development can lead to cardiac hypoplasia and endocardial cushion defects, which may be one of the reasons for the frequent combination of CHD in patients with DS[8, 9]. In addition, aberrations on chromosome 21 have also been confirmed to affect the fusion of endocardial cushions, leading to CHD[10]. Studies have shown that AVSD, ASD and VSD are the most common CHD types in children with DS[11]. The main causes of death in children with DS and CHD are pulmonary hypertension, severe pneumonia and heart failure. In addition, some studies have found that the risk of DS has a stronger relationship with mothers[12]. For example, the risk of DS increases with the age of pregnant women. Women over 35 years old have the highest risk of giving birth to children with Down's syndrome. If the childbearing age of the older mother is 40 years old, the risk will increase to 1/110, while if the childbearing age is 45 years old, the risk will be higher[13]. The risk of DS will increase for fetuses with abnormal pregnancy histories and more pregnancies[14].

At present, the prevention of DS mainly occurs through prenatal screening during pregnancy to effectively reduce and prevent the birth of children with Down's syndrome[15]. Testing mainly includes serological screening, fetal malformation ultrasound screening, and cell-free DNA testing technology. When screening high-risk pregnant women, it is necessary to perform chorionic villus biopsy in early pregnancy or amniocentesis in middle pregnancy to avoid the birth of DS babies after the diagnosis of chromosome karyotype analysis. Serological screening is a more common screening method because it is less invasive for pregnant women, detection is convenient and quick, and the cost of the test is low. Lattice is easy to accept and is generally carried out in the clinic at home and abroad, but there are problems with this test, such as a low detection rate and high screening error rate. Radioimmunoassay (RIA), chemiluminescence immunoassay (CLIA), enzyme-linked immunosorbent assay (ELISA), colloidal gold immunochromatography (GICA), time-resolved fluorescence immunoassay (TRFIA) and other detection methods have a longer detection time, more complex operation, increased sample demand, and higher cost[16–18], which limits the application and development to a certain extent. As a result, due to many economic, educational and cultural level reasons, many pregnant women do not participate in the pregnancy screening on time, so the birth rate of DS is still high[2, 19, 20]. After birth, children with DS will face the risk of repeated lung infection, heart failure, severe malnutrition and even death. At present, it is relevant to correct the heart malformation and reduce the death risk of children by surgical treatment in the early stage[21], but the vulnerability, immune function, intelligence level and cognitive development level of children with DS are far lower than those of normal children, and the overall increase in life expectancy in DS comes with a parallel increase in risk for age-related diseases, such as Alzheimer’s disease, increased rate of infections, hypertension and obesity[22] and gastrointestinal disorders, which are the most common anomalies in people with DS and have a significant impact on their daily life[23]. Ottaviano et al. found that patients with DS are characterized by increased susceptibility to autoimmunity and respiratory tract infections that are suggestive of humoral immunity impairment[24].

The gut microbiota plays an important role in maintaining homeostasis and improving immunity[25, 26]. The intestinal microbiota develops during early childhood, and this normal colonization is critical for

immune system development in infants and young children, as gut dysbiosis can lead to long-term disease[25, 27]. In particular, dysbiosis has been linked to the development of obesity[28], hypertension, and colon cancer[29]. In addition, children and adults with DS may experience unexplained neurodevelopmental regression[30]; however, in the current study, it was found that the intestinal flora is also closely related to autism and depression in children.

Based on this information, this study used 16S rRNA sequencing as a technical means to explore the microbial diversity and species distribution of intestinal flora in children with DS and CHD for the first time to analyze the difference in reproductive tract flora between mothers of DS and normal healthy children, determine the key difference in intestinal flora between DS and healthy children, and provide a theoretical basis for disease-related research and treatment methods.

Results

Overall structural changes in microbiota composition

Good's coverage for the six groups was greater than 98.0%, indicating a considerable sequencing depth for the analysis of the microbiota (Fig. 1a-c). For alpha diversity analysis, Chao and ACE estimators and the Shannon and Simpson indexes were used to assess community richness and diversity, respectively. As shown in Fig. 1a, the richness of the bacterial population (Chao1 and ACE indices) in the DSC group was significantly higher than that in the NC group ($p < 0.01$), while there was no significant difference between the species richness of intestinal flora in CHD and NCHD group (Fig. 1b), and there was no significant difference between the species richness of the reproductive tract of DSM and that of NM (Fig. 1c). For the Shannon and Simpson analyses, we can see that the Shannon index of the DSC group is significantly lower than that of normal group ($p < 0.05$), which indicated that the diversity of intestinal flora of children with DS was less than that of healthy children, while the diversity index of children with congenital heart disease was not significantly different from that of children without congenital heart disease, and the reproductive tract microbial species diversity of DS mothers was not significantly different from that of healthy children.

To measure the extent of the similarity between microbial communities, PCoA plots of unweighted and weighted UniFrac distances were generated. From the PCoA analysis, we can see that the composition of intestinal flora in children with DS is significantly different from that in the healthy control group (Fig. 1d). Among these algorithms, the PC1 distribution with the largest contribution rate revealed changes of 16.32%, and the PC2 distribution revealed changes of 6.5%, which showed that there was a significant difference between the two groups. However, the distribution of flora with CHD and NCHD was similar. There was a partially significant difference between the reproductive tract flora of DSM and NM (Fig. 1e). Among these algorithms, the PC1 distribution with the largest contribution rate revealed changes of 57.1%, and the PC2 distribution revealed changes of 18.1%; that is, the total microbial composition was not similar between the two groups. We also used cluster analysis to explore the similarity between samples. From Fig. 1f, we can see that DSC group samples tended to gather together and had similar

microbial composition and diversity distribution, and it can be seen that the composition and diversity of intestinal flora in NC were similar as a whole, with *Bacteroides* as the majority. At the same time, it shows the accuracy of the results of our sample collection. We found that the composition of the diversity of the microbial flora in the group of mothers of children with DS tended to clump together, had a similar microbial composition and had a relatively high abundance of *Lactobacillus* (Fig. 1g), indicating that there is a significant difference in the overall composition compared with the mothers of healthy children.

Taxonomy-based comparisons at the phylum and genus levels

From the phylum-level analysis (Fig. 2a), we could clearly see that the DSC group was mainly composed of 16.67% *Bacteroides*, 37.35% *Firmicutes*, 26.13% *Proteobacteria*, 50% *Verrucomicrobia* and 15.64% *Actinobacteria*. The normal group was mainly composed of 45.30% *Bacteroides*, 22.85% *Firmicutes*, 15.64% *Proteobacteria*, 28% *Verrucomicrobia* and 3.02% *Actinobacteria*. Therefore, we found that *Bacteroides* is the most different intestinal flora between children with DS and healthy children. The DSM group was mainly composed of 9.36% *Bacteroides*, 28.99% *Firmicutes*, 6.55% *Proteobacteria* and 29.96% *Actinobacteria*. The NM group was mainly composed of 13.39% *Bacteroides*, 25.42% *Firmicutes*, 6.51% *Proteobacteria* and 24.14% *Actinobacteria*. The most different intestinal flora were *Firmicutes* and *Bacteroides.*

We further analyzed the composition of intestinal flora at the genus level (Fig. 2b), and we could clearly see that the DSC group was mainly composed of 18.42% *Escherichia*, 14.93% *Bifidobacterium* and 14.66% *Bacteroides*. The normal group was mainly composed of 44.30% *Bacteroides*, 9.94% *Escherichia* and 2.90% *Bifidobacterium*. We found that the relative abundance of *Escherichia* in the intestines of children with DS was significantly higher than that of healthy children ($p < 0.01$) (Fig. 2c), followed by that of *Bifidobacterium* (Fig. 2d), and the relative abundance of *Bacteroides* in the intestines of children with DS was significantly lower than that of healthy children ($p < 0.01$) (Fig. 2e). Therefore, we speculated that the greater abundance of *Escherichia* and the lower abundance of *Bacteroides* in the intestines of children with DS might be related to the susceptibility of children with DS to intestinal diseases.

Through the analysis of the intestinal flora of the DSC and NC groups at the level of Metastats (Fig. 2f), we found that the main differences between the two groups were *Bacteroides*, *Bifidobacterium*, *Escherichia*, *Clostridioides*, *Erysipelatoclostridium*, *Faecilibacterium*, *Haemophilus*, *Klebsiella*, *Parabacteroides* and *Ruminococcus*. The results show that the distribution and differential flora of DS patients in the intestinal flora are different from those of healthy children and provide a theoretical basis for revealing the related disease mechanisms of DS. Through analysis of the intestinal flora of groups CHD and NCHD through Metastats (Fig. 2g), we found that the main differences between the two groups were *Enterococcus* and *Erysipelatoclostridium*, indicating that DS patients with CHD and DS patients without CHD have significant distribution of intestinal flora, which indicated that the relative abundance of these two intestinal bacteria was higher in children with CHD. This related discovery provides a certain theoretical basis for exploring the pathogenesis of CHD; then we analyzed the flora of DSM and NM at the genus level of Metastats (Fig. 2h), and we found the main differences in flora between the two groups

were *Lactobacillus*, *Atopobium* and *Corynebacterium*. The findings provide a basis for exploring the correlation between children with DS and their mothers.

The differences in the dominant members of the microbiota

From 3.2, we determined the different species in the DSC and DSM groups from the relative abundance level of OTUs. To verify and further determine the more significant microorganisms in different groups, we also conducted LEfSe analysis. LEfSe was used to identify the specific phylotypes related to the DSC and DSM groups. As shown in Fig. 3a-c, the main differential microbial species between the control group and the DSC groups were *Bacteroides, Clostridioides, Pseudomonadales, Bifidobacterium, Streptococcus* and *Escherichia*. The main differential microbial species between the CHD group and the NCHD groups were *Enterococcus, Pseudomonadales, Bacteroides, Haemophilus, Romboutsia* and *Paeniclostridium*. The main differential microbial species between the DSM group and the NM groups were *Lactobacillus, Pseudomonas, Corynebacterium, Peptostreptococcus, Sneathia* and *Gaiella*.

Functional gene prediction

The intestinal flora plays a certain role in the intestine and is closely related to the function of the body. Since we found that the intestinal microbes changed through 16S rRNA sequencing technology, we further analyzed and predicted the functions between different groups.

Through the L1 level KEGG analysis of the gene pathway (Fig. 4a), we found that the expression of the functional pathway of the group NC was mainly concentrated in Metabolism, Cellular Processes and Organic Systems, and the DSC and CHD groups were significantly lower than the normal group in terms of the Genetic Information Processing pathway, indicating that DS patients were weak in the transmission and expression of genetic information. The expression of group DSM functional pathways was mainly concentrated in Genetic Information Processing and Environmental Information Processing, and the functional pathways of group NM were significantly lower in Metabolism, Cellular Processes and Organic Systems. Among other groups, the difference in flora between mothers of children with DS and healthy mothers is also revealed here, which provides a reference basis for exploring the relevant mechanism of mothers' influence on children with DS.

We further analyzed the L2 KEGG of the gene pathway (Fig. 4b) and found that group DSC and CHD significantly downregulated Immune System, Cell Growth and Death, Replication and Repair, Nucleotide Metabolism and Folding, Sorting and Degradation compared with the other groups. The expression of signaling molecules and interacting genes was significantly upregulated, indicating that the immune function of DS patients was weak, and their cell growth and nucleotide metabolism were lower than those of healthy children, which was consistent with the content reported in the literature. The poor immunity of DS children easily causes infection and various complications. Children with DS with poor immunity easily develop infections and various complications. Normal group NC had significantly increased Cell Motility, Lipid Metabolism, Carbohydrate, Metabolism, Amino Acid Metabolism, Metabolism of Terpenoids and Polyketides, Endocrine System, Digestive System, Biosynthesis of Other Secondary

Metabolites, Energy Metabolism, Glycan Biosynthesis and Metabolism, which were expressed more in lipid and amino acid metabolism than group DSC. Group NM significantly upregulates Excretory System, Neurodegenerative Diseases and Sensory System and significantly downregulates Signal Transduction, Xenobiotics Biodegradation and Metabolism, Transport and Catabolism, Metabolism of Cofactors and Vitamins, Cell Motility, Lipid Metabolism, Carbohydrate Metabolism, Cellular Community -prokaryotes, Metabolism of Other Amino Acids and Amino Acid Metabolism Pathways. Group DSM significantly downregulated Metabolism of Terpenoids and Polyketides and Endocrine System. The analysis of the L2 level metabolic pathway can provide a basis for exploring the pathogenesis and prevention of DS.

Discussion

Human intestinal microbes are closely related to health, as intestinal microbes help regulate host metabolism and immune system development[31]. Numerous studies have shown that intestinal microbes influence and are significantly associated with many diseases of the human body, such as intestinal inflammation, obesity, diabetes, and tumors[32, 33]. An important stage of intestinal microecology colonization occurs during infancy. The colonization pattern of intestinal microbiota in infants directly affects the diversity of the microbiota, physiological development and the development of the immune system, thus exhibiting a long-term impact on the risk of long-term disease. The human intestinal microbiota is established from the time of the baby's birth, and the intestinal microbiota becomes increasingly complex as the baby grows[34, 35]. As the baby grows and develops, the intestinal microbiota continues to evolve into an adult state, and the abundance of *Bacteroides* gradually increases[36–38]. Kamng'ona et al. found that the dominant intestinal microorganisms are *Bacteroides*, which is consistent with our results in this research[39]. We found that the relative abundance of *Bacteroides* in the intestinal tract of healthy children was the highest, accounting for 50% of the entire intestinal microbiota, thereby making it the dominant component of the microbiota. We also found that the intestinal flora of children with DS was significantly different from that of healthy children. The relative abundance of *Escherichia* in the intestine of children with DS was significantly higher than that of healthy children, while the relative abundance of *Bacteroides* was significantly lower than that of healthy children. In the sampling results of healthy children, the relative abundance of *Bacteroides* accounted for approximately half (44%) of the whole intestinal flora, while *Escherichia* accounted for 9.94%, which was significantly lower than the 18.42% of children with DS. The relative abundance of *Bifidobacterium* and *Clostridioides* in children with DS was significantly higher than that in healthy children, which was consistent with the results of Biagi et al. [22]. They found that the gut microbiota of persons with DS was well known to provide the host with short-chain fatty acids (SCFAs), butyrate, propionate and acetate, from fermentation of indigestible polysaccharides in the gut, and their results showed that the DS microbiota was largely dominated by the butyrate producers *Clostridium* cluster, propionate producing *Bacteroidetes*, and the acetate producer *Bifidobacterium*. However, they also found that persons with DS were enriched in *Sutterella* and reduced in *Veillonelaceae*, which was inconsistent with our results. The reason may be because the population they studied was adult patients with DS (19–35 years), and the

human intestinal flora changed more with age. The distribution of bacteria in adults is quite different from that in childhood.

The distribution of the intestinal microbiota in infants at 6 months and 12 months of age is unstable[40]. In the later stages of childhood, chronic inflammatory diseases are caused by a balance of intestinal microbiota damage[41]. By constructing a mouse model, it was shown that after a reasonable change in the intestinal microbiota of mice, the progeny of the offspring had a reduced incidence of allergic diseases and inflammatory diseases[42]. During this period, the construction of the intestinal microbiota in infants and young children is easily affected by various factors, such as dietary intake, probiotics and dietary supplements. In a study of the intestinal microbiota composition in 12-month-old infants, infants were given different diets[43]. Compared with the infants in the other two groups, the infants in the iron-fortified cereal group had prominent differences in the bacterial composition of the gut microbiota, and the abundance of the *Lactobacilli* populations was significantly reduced. Therefore, we speculate that we can improve the diversity of the intestinal flora of children with DS to make it consistent with the composition of the intestinal flora of healthy children to reduce or slightly alleviate the internal inflammation or other disease reactions of children with DS to a certain extent and increase intestinal immunity. Zhang et al. carried out metagenomic shotgun sequencing and a metagenome-wide association study (MGWAS) of fecal, dental and salivary samples from a cohort of individuals with rheumatoid arthritis (RA) and healthy controls[44]. They found that dysbiosis was detected in the gut and oral microbiomes of RA patients, but it was partially resolved after RA treatment, and alterations in the gut, dental or saliva microbiome distinguished individuals with RA from healthy controls, were correlated with clinical measures and could be used to stratify individuals on the basis of their response to therapy. Their results establish specific alterations in the gut and oral microbiomes in individuals with RA and suggest potential ways of using microbiome composition for prognosis and diagnosis. Khocht et al. used checkerboard DNA-DNA hybridization to explore the subgingival microbiota in DS and non-DS adults[□□!□□ □□□□□]. They found that most microbial species were present in DS subjects at levels similar to those in non-DS subjects, except for higher proportions of *Selenomonas noxia*, *Propionibacterium acnes*, *Streptococcus gordonii*, *Streptococcus mitis* and *Streptococcus oralis* in DS subjects than in non-DS study subjects, higher proportions of *Treponema socranskii* in DS subjects than in non-DS mentally retarded subjects, and higher proportions of *Streptococcus constellatus* in DS subjects than in mentally normal subjects. DS adults with periodontitis had higher subgingival levels of *T. socranskii* than DS subjects without periodontitis. Higher subgingival proportions of *S. constellatus*, *Fusobacterium nucleatum ssp*. *nucleatum*, *S. noxia* and *Prevotella nigrescens* showed significant positive correlations, and higher proportions of *Actinomyces naeslundii* and *Actinomyces odontolyticus* showed negative correlations, with increasing mean subject attachment loss in DS adults.

In general, the microbiota of children with DS was mainly composed of *Escherichia*, *Bifidobacterium*, *Clostridium* and *Bacteroides*, which have significant differences in the abundance and diversity of intestinal flora compared with healthy children. *Enterococcus* and *Erysipelatocolostridium* were the main differentiating microorganisms in children with and without CHD, and the relative abundance of *Lactobacillus* in the reproductive tract of mothers with DS was significantly higher than that of mothers

with healthy children, which may suggest potential ways of using microbiome composition for prognosis and diagnosis.

Materials And Methods

Study design

The experiment was divided into six groups: 30 fecal samples of children with DS (DSC), 30 fecal samples of healthy children (NC), 16 fecal samples of DS with congenital heart disease (CHD), 8 fecal samples of DS without congenital heart disease (NCHD), 28 reproductive tract samples of mothers of children with DS (DSM) and 35 reproductive tract samples of mothers of healthy children (NM). The collected fresh fecal samples and genital tract samples were put into aseptic containers, then quickly put into the refrigerator, transferred to the laboratory and placed in the refrigerator at − 80 ℃ for the next experiment.

DNA extraction and 16S rRNA gene sequencing

DNA was extracted from feces using the Qiagen QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany). The primers used were 338F 5'-ACTCCTACGGGAGGCAGCA-3' and 806R 5'-GGACTACHVGGGTWTCTAAT-3'. The library was sequenced on the Illumina Miseq platform. Quality filtering of the raw reads was performed under specific filtering conditions to obtain high-quality clean reads according to Cutadapt.

Bioinformatics and statistical analysis

Sequences with ≥ 97% similarity were assigned to the same operational taxonomic units (OTUs). QIIME (version 1.7.0) was used for the analysis of alpha diversity, including observed species, Chao1, Shannon, Simpson, and ACE. Beta diversity analysis was used to evaluate differences in samples in species complexity. Beta diversity on both weighted and unweighted UniFrac was calculated by QIIME software (Version 1.7.0). Cluster analysis was preceded by principal component analysis, which was used to reduce the dimension of the original variables using the FactoMineR package and ggplot2 package in R software (Version 2.15.3). Principal Components Analysis (PCoA) was performed to obtain principal coordinates and visualize complex, multidimensional data. A weighted UniFrac distance matrix among samples obtained previously was transformed to a new set of orthogonal axes, by which the maximum variation factor was demonstrated by the first principal coordinate, the second maximum variation factor was demonstrated by the second principal coordinate, and so on. Graphics drawing was calculated by using the ggplot2 package in R software (Version 2.15.3).

Data shown are the mean ± SD. Data between 2 groups were analyzed by the unpaired t-test (Prism 6.0; GraphPad Software) if the data were in a Gaussian distribution and had equal variance, by unpaired t-test with Welch's correction (Prism 6.0; GraphPad Software) if the data were in Gaussian distribution but with unequal variance, or by nonparametric test (Mann-Whitney U test, Prism 6.0; GraphPad Software) if the data were not normally distributed. Data among more than two groups were analyzed by one-way ANOVA followed by Dunnett's multiple comparisons (Prism 6.0; GraphPad Software) if the data were Gaussian

distributed and had equal variance or analyzed by Kruskal-Wallis followed by Dunn's multiple comparisons (Prism 6.0; GraphPad Software) if the data were not normally distributed. The Gaussian distribution of data was analyzed by using the D'Agostino-Pearson omnibus normality test (Prism 6.0; GraphPad Software) and Kolmogorov-Smirnov test (Prism 6.0; GraphPad Software). Differences with $p < 0.05$ were considered significant.

Conclusions

In this study, we confirmed the microbiomes structural changes in the child fecal and mother vaginal flora related to congenital and Down's syndrome. The results showed that significant differences in the abundance and diversity of intestinal flora compared with healthy children. Especially, the abundance of *Enterococcus* and *Erysipelatoclostridium* in the intestine of children with CHD was significantly higher than that of children without CHD, and the relative abundance of Lactobacillus in the reproductive tract of mothers with DS was significantly higher than that of mothers with healthy children, which may suggest potential ways of using microbiome composition for prognosis and diagnosis. Using functional analysis, it was discovered that Down's syndrome patients' immune systems were significantly downregulated and their cell growth and nucleotide metabolism were lower than healthy children. Our analysis shows that the ecosystem associated with childhood congenital heart disease affects the selection of bacterial communities in the native microbiota, and we focus on specific bacteria and their relevance to disease.

Abbreviations

DS: Down syndrome

CHD: congenital heart disease

DSC: children with Down syndrome

NC: healthy children,

NCHD: DS without congenital heart disease

DSM: mothers of children with DS

NM: mothers of healthy children

Declarations

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Authors' contributions

YNL, DW and WHS conceived and designed the experiments. YNL and DJH performed the sample collection. CFD performed the DNA extraction and assisted with the 16S rRNA gene sequencing data analysis. YNL performed the 16S rRNA gene sequencing and wrote the majority of the article. WHS's contributions included advising the project and revising the manuscript. The authors read and approved the final manuscript.

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Availability of data and materials

The raw data of all samples used in this project have been uploaded to the NCBI SRA database, and the accession number was SRP292977.

Ethics approval and consent to participate

This study was approved by the Institutional Review Board Children's Hospital Chongqing Medical University (Approval NO. 005/2014). All participants and their legal guardians were provided a written informed consent upon enrollment. The authors declared that the experimental research on the protists described in this paper was in compliance with institutional, national and international guidelines.

Consent for publication

Not applicable.

Competing Interests

The authors declare no conflicts of interest.

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Figures

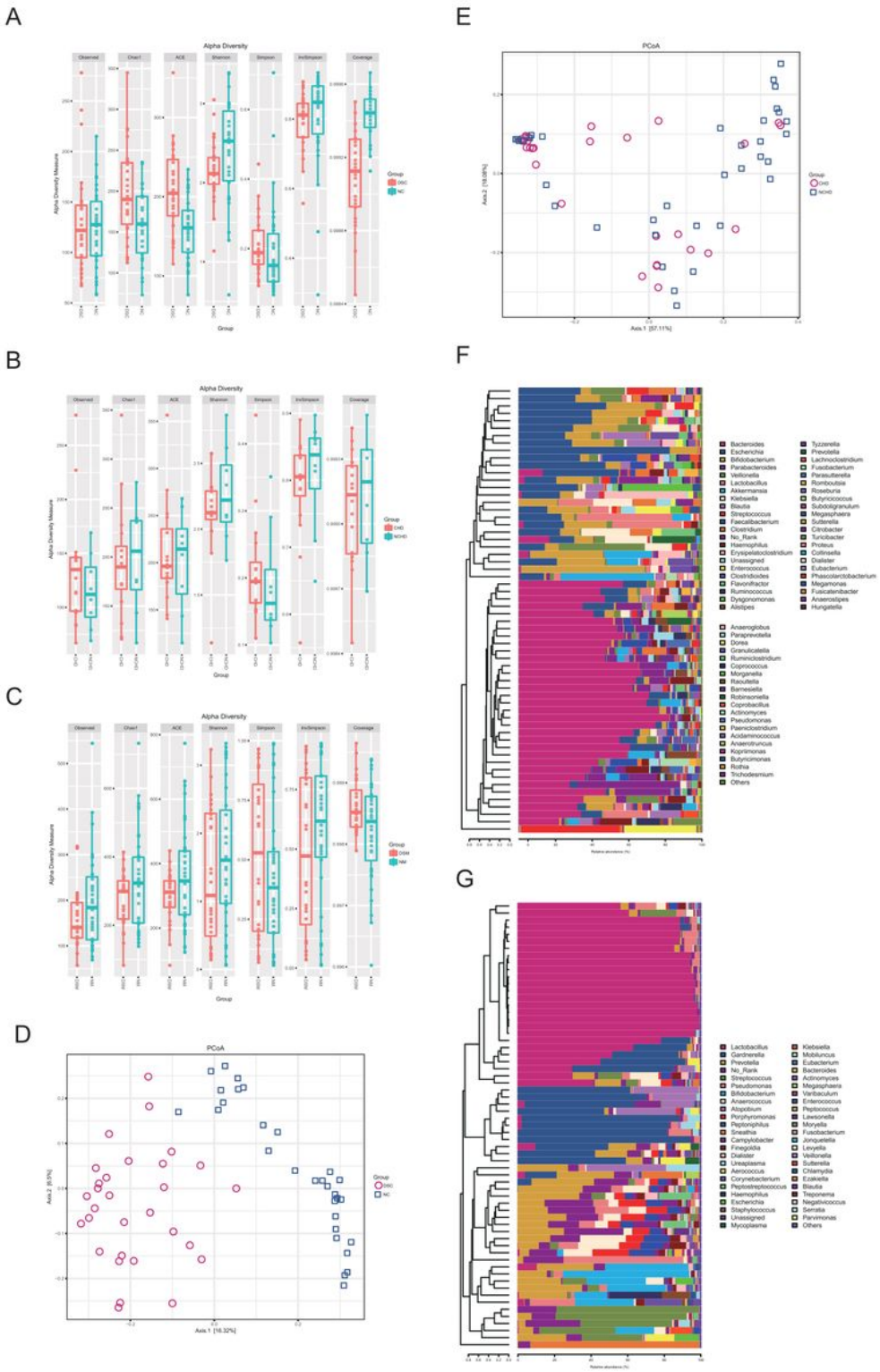

Figure 1

Changes in the diversity of the gut microbiota a-b) The Chao1 index, ACE index, Shannon index and Simpson index of the DS group and CHD group; c) the Chao1 index, ACE index, Shannon index and Simpson index of reproductive tract microorganisms of DSM and NM; d-e) PCoA plots based on Spearman distances are colored by time point. The significant differences between groups were calculated by analysis of similar (ANOSIM) tests; f-g) unweighted UniFrac cluster tree based on the group

method with arithmetic mean (UPGMA) clustering tree to study the similarity between different samples based on Bray-Curtis distance. The data were analyzed by one-way ANOVA (*p <0.05; **p < 0.01). DSC: Down syndrome, NC: normal, CHD: Child with congenital heart disease in DS; NCHD: child without congenital heart disease in DS; DSM: the mother of DS; NM: the mother of healthy child. n=25-40/group.

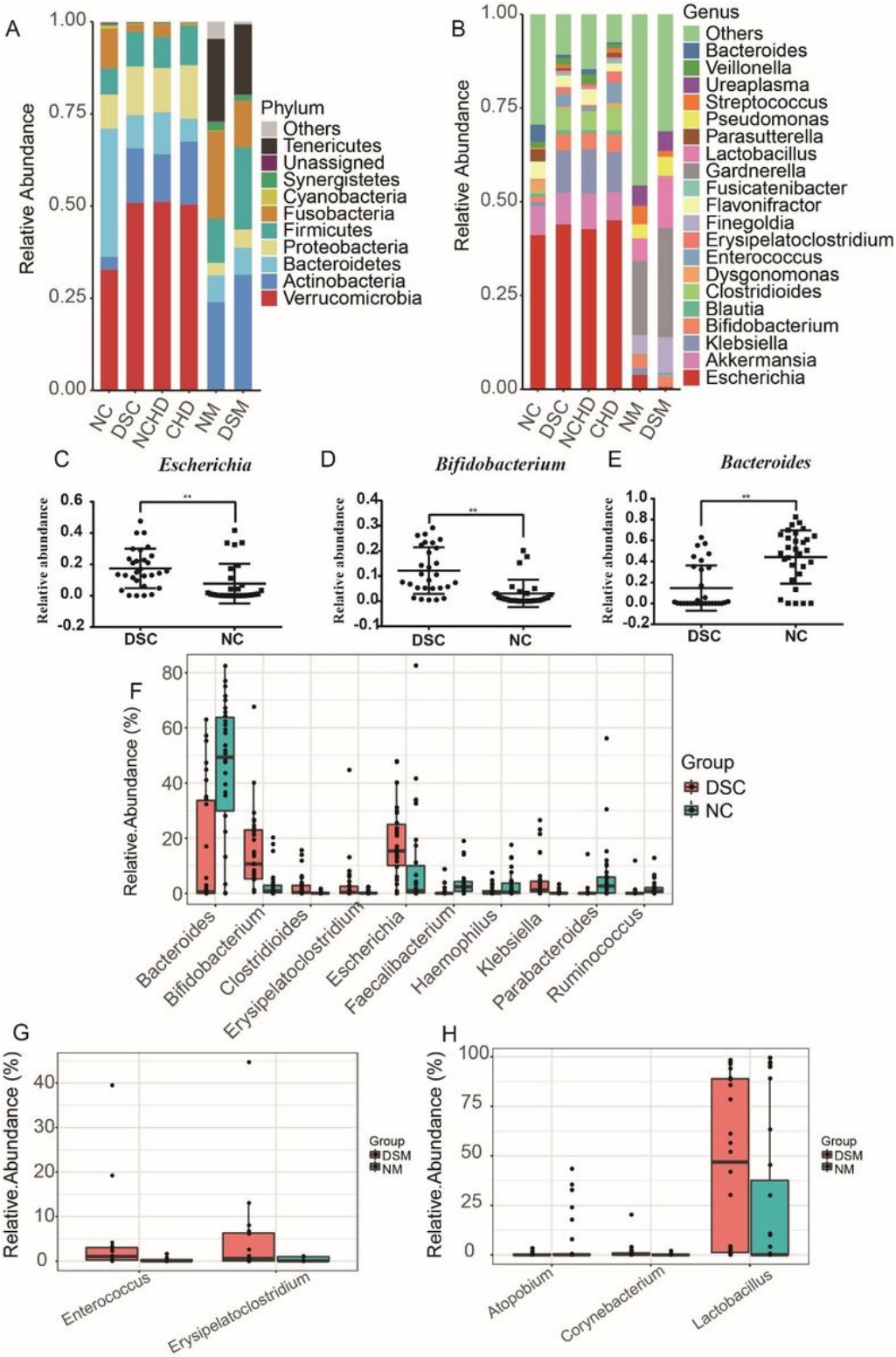

Figure 2

Composition of the gut microbiota a) The relative contribution of the top 30 phyla in each group; b) the relative contribution of the top 30 genera in each group; c) relative abundance of Escherichia in the DSC and NC groups; data were analyzed by the unpaired t-test (*p <0.05; **p < 0.01); d) relative abundance of Bifidobacterium in the DSC and NC groups; data were analyzed by the unpaired t-test (*p <0.05; **p < 0.01); e) relative abundance of Bacteroides in the DSC and NC groups; data were analyzed by the unpaired t-test (*p <0.05; **p < 0.01). f-h) Metastats analysis was performed on the DSC and NC groups, groups CHD and NCHD, DSM and group NM.

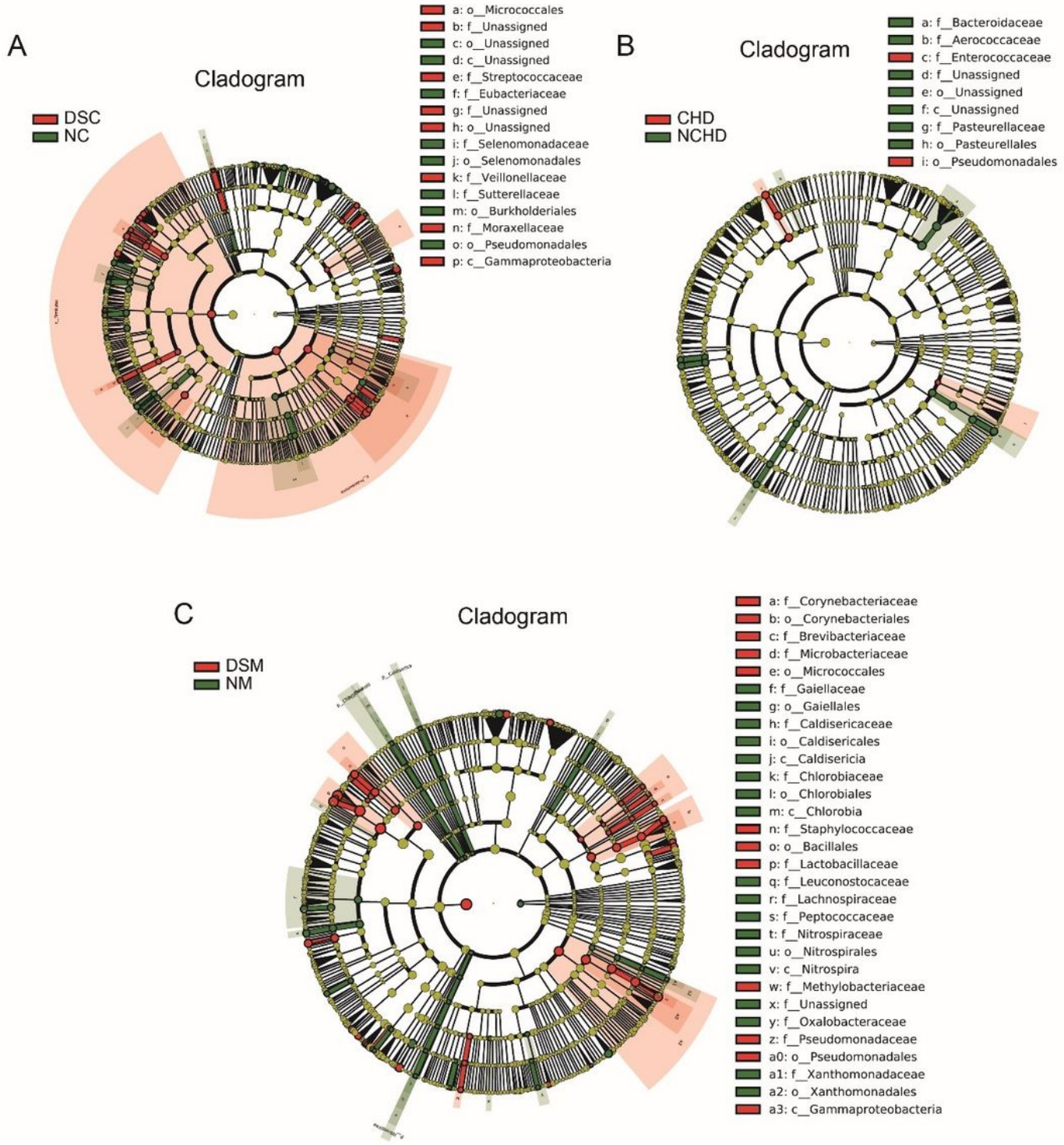

Figure 3

Major differential microbial species a-c) Taxonomic cladogram obtained from LEfSe in the DSC and NC, CHD and NCHD, DSM and NM groups. Biomarker taxa are highlighted with colored circles and shaded areas. Each circle's diameter reflects the abundance of those taxa in the community.

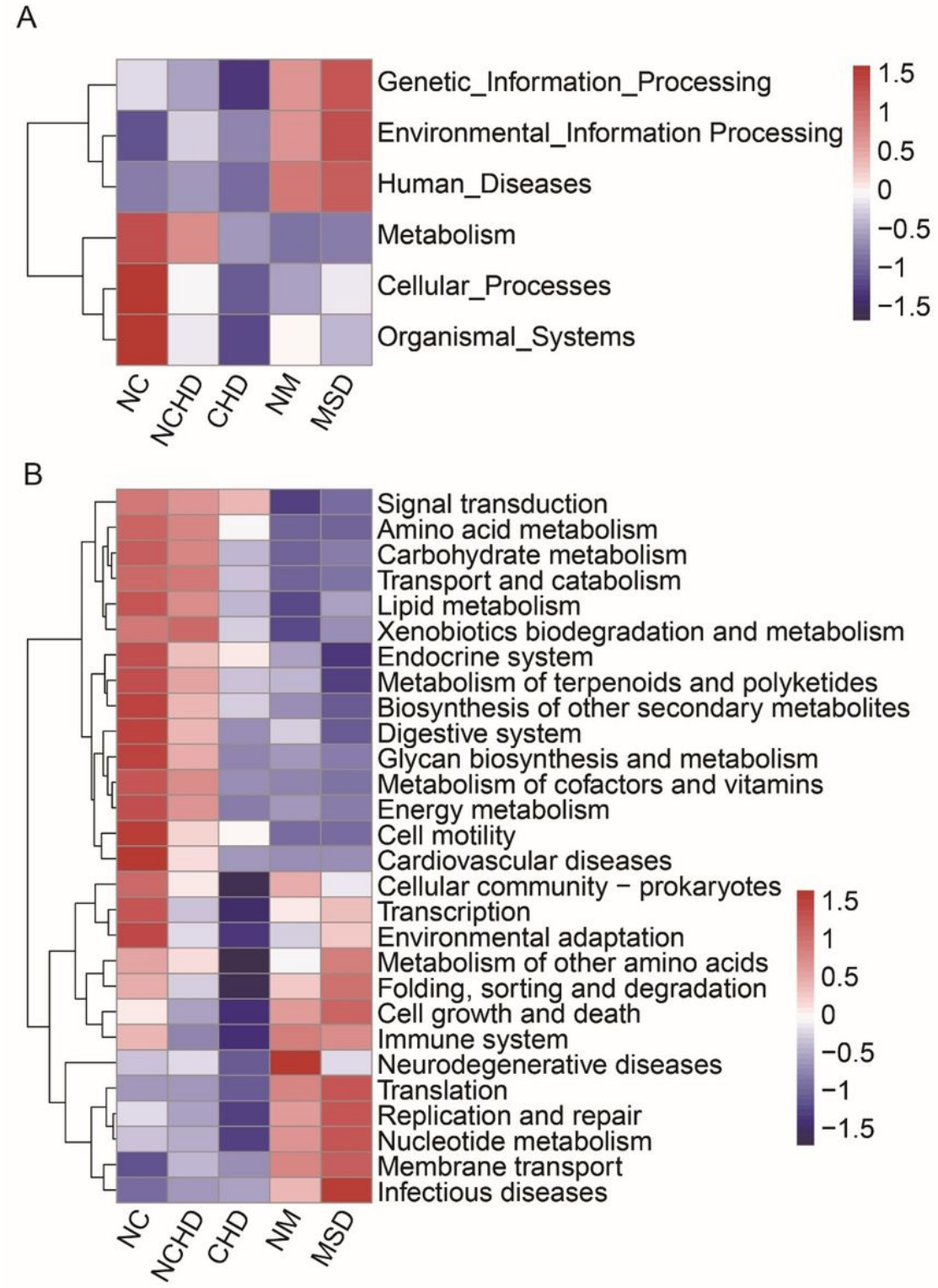

Figure 4

Functional gene prediction a-b) Prediction heatmap of functional genes in the L1 and L2 pathways in different treatment groups