The Studies to correlation between gut microbiota diversity and functional erectile dysfunction

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Abstract

Objective: To analyze the distribution of gut microbiota in the ED patients, and explore the relationship between the diversity of gut microbiota and psychogenic erectile dysfunction.

Methods: 30 cases of patients with erectile dysfunction (ED) and 30 healthy persons (healthy donor, HD) stool specimen were collected, using Illumina's Miseq platform samples V3-V4 region sequences bacterial 16SrRNA gene Paired end (PE) 300 sequencing, sequencing results were analyzed differences in species composition and diversity. Analysis contains five modules: sequencing data quality control, OTU species clustering and annotation, alpha diversity, beta diversity and the use of T-test and the analysis of the LEfSe differences.

Results: 1. The flora diversity in the group of ED than HD significantly different ($P<0.01$), ED group has a low bacterial diversity. 2. Between ED group and HD group, abundant bacteria (TOPlO) and core flora (90%) had no significant difference in the genus level; all bacteria flora (>1%) display, Alloprevotella groups genus presents differences, Alloprevotella only be identified in the HD group. 3. ED and HD groups present in well separated PCoA analysis, having a significant difference in the two kinds of microflora. 4.T-test shows six species were significantly different, in the ED group, Streptococcus and Subdoligranulum were increasing, and Prevotella, Prevotella sp.9, Blautia, Lachnospiraceae NK4A136 groups and Roseburia were decreasing. 5.LEfSe analysis revealed 24 species were significantly different between ED and HD groups.

Conclusion: Gene sequencing was performed on the two groups of specimens and finding that microbial community structure and diversity had significant difference, suggesting that ED have low gut microbiota diversity.

Introduction

There are a large number of microorganisms living in the human intestinal tract. The normal gut microbiota is the natural barrier of the human body and plays an important part in maintaining human health (1).The normal gut microbiota in the human body is mainly composed of firmicutes, Bacteroidetes, proteobacteria and actinomycetes. According to the impact on human health, it can be divided into three categories: symbiotic bacteria, opportunistic bacteria and pathogenic bacteria, which are dynamically balanced to maintain the homeostasis of the intestinal environment (2). Intestinal beneficial bacteria (i.e., probiotics) include bifidobacteria and lactobacillus, etc., harmful bacteria include Escherichia coli and enterococcus (3).Changes in the internal and external environment of the body could affect the structure of gut microbiota, resulting in gut microbiota imbalance (4), it could lead to inflammatory bowel disease (5), irritable bowel syndrome(6), non-alcoholic liver disease(7), viral hepatitis(8), metabolic syndrome (9) and other diseases aggravation and rapid progression. Sexual function is a complex phenomenon affected by sex hormones, psychology, nerves and hemodynamics, such as hormone disorder, obesity, stress, anxiety, hypertension, diabetes, etc.Therefore, it is speculated that there is a correlation between

erectile dysfunction and gut microbiota distribution. Take into account this inference, this study tested the gut microbiota diversity of patients with erectile dysfunction and the normal control group.

Materials And Methods

1.1 Research Objects

ED group: 30 ED patients with an average age of (29.33±2.73) years were accepted by the First Affiliated Hospital of Tianjin University of Chinese Medicine from October 2018 to October 2019. HD group:30 healthy volunteers aged 20-40 with an average age of (29.17±2.66) years. There was no statistically significant difference in age between the two groups(P□0.05).

1.2 Inclusion Criteria

Married men aged 20-40 with regular sexual partners; Participants know and agree to participate in the study; Normal genital and secondary sexual characteristics; No acute or chronic gastrointestinal diseases; No history of severe hypertension, myocardial infarction or other serious cardiovascular disease, diabetes, blood, kidney or liver disease; No history of infectious diseases such as tuberculosis, viral hepatitis and AIDS; Did not participate in other clinical trials in the last 3 months, did not use antibiotics, steroid drugs, did not eat probiotics and probiotics and other microbiological preparations. In the ED group, the diagnostic criteria of mild to moderate ED were satisfied. The IIEF-5 score was between 8 and 21, and the diagnosis was consistent with two attending physician or above. In the HD group, male physicians with the title of associate chief physician or above were interviewed in IIEF-5 and scored 22-25. The study was approved by the Ethics Committee of the First Teaching Hospital of Tianjin University of Traditional Chinese Medicine, and was strictly followed during the study implementation. The participants gave informed consent and signed the informed consent.

1.3 Exclusion Criteria

Having obvious primary diseases and/or complications of the heart, liver, kidney and nervous system; With other sexual dysfunction, such as decreased libido, orgasm suppression or no orgasm, ejaculation suppression or no ejaculation; Female partners have significant sexual dysfunction, such as decreased libido, painful sexual intercourse, etc. drug abuse within 2 years; Acute and chronic inflammation of the reproductive system; Penis deformities, genital deformities that obviously impair erectile function; Patients with serious mental disorders.

1.4 Collection samples

According to the instructions, the two groups were instructed to take about 50g of fresh stool specimens before eating food in the morning, taking them into a special specimen bottle respectively, and quickly put them in an ice box for preservation at -80°C. All samples are kept in dry ice and sent to Confidante Future for gene sequencing and data analysis.

1.5 Gene sequencing and data analysis

1.DNA extraction: According to different sample types, the corresponding kit (with kit) is used to extract the sample DNA and remove other non-DNA substances from the sample; 2.DNA quality control: Using 0.8-1% agarose gel to detect the integrity, degradation and degradation degree of DNA; Q-bit instruments and matching DNA-specific dyes were used to detect the DNA concentration.The A260/A280 and A260/A230 of DNA were detected by namedrop to determine the purity of DNA.3. Library construction: V3 and V4 regions was amplified using the universal primers of 16S rRNA gene; The PCR amplification products were purified and the redundant primers and various components in the PCR reaction system were removed. The purified product was supplemented by PCR, and the terminals of the purified product were added to the connector for sequencing and the index label sequence for distinguishing each sample. The second round of PCR amplification products was purified and the redundant primers and various components in the PCR reaction system were removed. 4. Library quality inspection and pretreatment before sequencing: Q-BIT was used to identify the concentration of each library, and all libraries were mixed according to the equal molality;Fluorescence quantitative PCR was used to detect the molality of the mixed library.The total library of sodium hydroxide treatment was single-stranded DNA.5. Clustering: In alumina's Miseq sequencer, the single-stranded DNA generated was hybridized with the single-stranded sequence of Flowcell, and then clusters of single-stranded DNA were formed on Flowcell by bridging PCR amplification. Finally, the DNA in the cluster was converted into single-stranded DNA and then Paired end(PE) was sequence. 6. Sequencing: On Miseq, the base sequence of each cluster on FlowCell was observed by simultaneous synthesis and sequencing. 7. Disinformation analysis: Raw Data were firstly quality-controlled, filtered, spliced and removed to obtain Clean Data, then the effective Data were analyzed for Operational Taxonomic Units and species classification. According to the OTUs clustering results, species annotation was made on the representative sequence of each OTU to obtain the analogous species information and specie-based abundance distribution. At the same time, OTUs was tested for relative abundance, Alpha diversity calculation, Alpha diversity, etc., so as to obtain species richness and uniformity information in samples, as well as differences between different samples or groups. In order to further explore the differences in community structure between the group samples, t-test, LEfSe and other statistical analysis methods were utilized to test the significance of the differences in species composition and community structure between the ED group and the HD group.The data(P 0.05)was seen as the criterion of statistical significance.

1.6 Ethical approval. All experiments and methods were performed in accordance with relevant guidelines and regulations.

Results And Analysis

2.1 Data preprocessing and quality control: The total DNA of the sequenced samples was tested. The concentration and purity of 49 samples (ED: 26, HD: 23) met the sequencing requirements of Illumina MiSeq platform.

2.2 Analysis of bacterial diversity in fecal samples

2.2.2 Analysis of bacterial flora composition of samples

According to OTU annotation results, histogram of relative abundance of species was made for each sample at different classification levels, which can visually display the bacterial flora of each sample at different classification levels. Figure 1-2 shows the stacking histogram of bacteria with relative abundance greater than 1% in each sample at the classification level of genera. The abscess is the sample name, and the ordinate is the relative abundance ratio of corresponding bacteria. "F_ *" means that it cannot be annotated to genus in biological classification, but can be annotated to family or other classification level.

There was no difference between the genera of the ED-HD group, indicating that there was no statistically significant difference between the high abundance bacteria (top10) and the core bacteria (90%) of the ED-HD group. However, the total intergroup flora(□1%) between groups, Alloprevotella was statistically significant(Alloprevotella was identified only in the HD group.)

2.2.3 Alpha diversity analysis

Alpha Diversity refers to the microbial community Diversity in a specific region or ecological environment, which is a comprehensive index reflecting the richness and uniformity. The Alpha Diversity analysis of a single sample can reflect the richness and diversity of the microbial community in the sample.Figure 3 shows that the ED group has a relatively low Shannon diversity coefficient.(HD group =5.741,ED group □ 4.982), indicating that ED group had lower bacterial diversity (P=0.00074X□0.01).

2.2.4 PCoA analysis based on Bate diversity

Principal component Analysis (PCoA, Principal Co-ordinates Analysis) is used to study the similarity or heterogeneity of sample community composition. The closer the distance the sample, showed that the higher the similarity community structure, otherwise the structure indicates that the larger the difference of the community. Figure 4 is the PCoA analysis conducted by calculating the Unweighted Unifrac distance using the relationship between OTUs systems. The x-coordinate represents one principal component, the y-coordinate represents another principal component, and the percentage represents the contribution value of the principal component to the difference of samples. Each point in the figure represents a sample, and samples of the same group are represented by the same color. The results showed that the ED group (blue) and the HD group (red) were clustered respectively, and the two groups were obviously separated, indicating that the structure of the ED-HD group was different.

2.3 Comparison of relative abundance between groups of all strains

Through statistical analysis, the genera with significant difference in the abundance change between groups can be specifically identified, and the enrichment of the genera with significant difference between groups can be obtained. Meanwhile, the size of the difference between groups and the difference

between groups can be compared to determine whether the community structure difference between groups is statistically significant.

2.3.1 t - test

T-test was used to detect significant differences between groups at the level of genera (P□0.05). Results (Table 1 and FIG. 5-6) show six species were significantly different between groups, Streptococcus and Subdoligranulum were increased in the ED group than HD group, while in platts bacteria genera, Blautia slaughter's species, Lachnospiraceae NK4A136 group and Roseburia were decreased than the HD group.

Table 1 The species were significantly different between ED and HD groups

Class	P-value	显著性
g__Prevotella 9	0.02558353	*
g__Streptococcus	0.043744221	*
g__Blautia	0.048518626	*
g__Lachnospiraceae NK4A136 group	0.006742402	**
g__Roseburia	0.017978557	*
g__Subdoligranulum	0.036087622	*

Note□*□P□0.05□ **□ P□0.01

2.3.2 LEfSe

In order to find the specific bacterial genera of ED, LEfSe analysis was used to compare the different bacterial genera between the two groups. The results (Figure 7) showed that the absolute value of LDA Score of 24 bacterial genera was above 2. It is known that LachnospiraceaeNK4A136 group and Prevotella_9 have the greatest influence on the difference between groups.

Comments:

1) LDA is a supervised dimension reduction method, while PCA is an unsupervised dimension reduction method

2) LDA dimension reduction can be reduced to the dimension of category number K-1 at most, while PCA does not.

3) In addition to dimension reduction, LDA can also be used for classification.

4) LDA selects the projection direction with the best classification performance, while PCA selects the direction with the greatest variance for the projection of sample points.

The main advantages of LDA algorithm are as follows:

1) Prior knowledge of categories can be used in dimension reduction, while unsupervised learning such as PCA cannot use prior knowledge of categories.

2) LDA is better than PCA when the sample classification information depends on mean value instead of variance.

The main disadvantages of LDA algorithm are as follows:

1) LDA is not suitable for dimensionality reduction of non-Gaussian distribution samples, and PCA also has this problem.

2) LDA dimension reduction can be reduced to the dimension of category number K-1 at most. If the dimension reduction is greater than K-1, LDA cannot be used.There are some evolutionary versions of the LDA that can circumvent the problem.

3) When the sample classification information depends on variance instead of mean value, LDA has a poor effect of dimension reduction.

4) LDA may overfit the data.

Discussion

Normal erectile function is a complex phenomenon affected by sex hormones, psychology, nerves and hemodynamics, such as hormonal disorders, obesity, stress, anxiety, hypertension, diabetes, etc. Markle confirmed that the intestinal microbiota of sterile mice was regulated by microbiota,and the intestinal microbiota of normal male mice was implanted into female nude mice, which resulted in the sustained increase of testosterone levels in females and the regulation of their autoimmunity. It has also been shown that when female mice were treated with normal intestinal microbiota obtained from donor male mice in the context of impaired reproductive capacity. Testosterone levels were significantly increased (10).Microbiot-based therapies have also been used for diseases of the immune system, such as type 1 diabetes, which is linked to abnormal levels of sex hormones (11).In a well-designed study, Yurkovetskiy showed that germ-free 5-week-old mice had higher plasma testosterone concentrations than non-pathogen free (SPF) mice. Whereas SPF mice had elevated testosterone levels after 12 weeks. In addition, mice transplanted with filamentous bacteria or proteobacteria similar to Shigella escherichia coli showed higher serum testosterone concentrations at 12 weeks of age than mice without bacteria. However, the probiotic mixture did do not increase testosterone levels (12).In common with the above, the microbiome can be positively and negatively regulate sex hormone levels based on host physiology, bacterial strains and other factors. These findings strongly indicate that the gut microbiota can improve sexual function by regulating sex hormone levels. Reiter probiotics had positive effects on testicular size and serum testosterone levels in aging mice (13). Thus it can be observed that using probiotics to regulate human microbiota is another method to improve sexual ability. Normal erectile function depends on normal functional penile blood vessels and cavernous blood vessels. It was found that the growth of gut

microbiota in ED patients with diabetes was inhibited, the number of probiotics was significantly reduced, but the number of opportunistic pathogens was increased (14).Cho found that serum TMAO was associated with intestinal germicides (15).Spalding found that TMAO could promote vascular endothelial inflammation and enhance smooth muscle cell proliferation, eventually leading to vascular wall fibrosis and vascular function destruction (16).Sun found that TMAO could lead to vascular endothelial dysfunction through ROS mediated oxidative stress, as well as increased adhesion of monocytes in human umbilical cord vessels and endothelial repair dysfunction (17). Therefore, this study reviewed the association between gut microbiota distribution and erectile dysfunction.

Numerous studies have demonstrated that the pathogenesis of ED is related to the inflammatory response. The streptococci causes inflammation mostly, such as Group A Streptococcus (GAS), which is A gram-positive pathogen and can cause A range of diseases, including deadly invasive infections. And Tomomi study showed that Streptococcus haemal and Streptococcus are associated with systemic diseases such as infective endocarditis and abscess, and increased expression levels of the inflammation-related factor IL-1 mRNA when mice were attacked by Streptococcus hemoglobin (18).The Roxella is comprised of gram-positive anaerobes and is one of the common bacteria that produce short-chain fatty acids (SCFAs), especially butyric acid, which affects colon motility and has immunomaintenance and anti-inflammatory effects (19). Bertillon's found that Pruplatella can induce TLR2 signaling pathways and low levels of P65-mediated inflammation. The results of this study showed that streptococci were up-regulated and Rosteria were down-regulated, and both Streptococcus and Rosteria were associated with inflammatory response, and ED was involved in inflammation, therefore, streptococcus and Rosteria were associated with ED.

Obesity increases the likelihood of erectile dysfunction (20). Studies have further shown a link between human gut flora and obesity. Modification of the characterization of Roxella may affect various metabolic pathways, resulting in various diseases such as irritable bowel syndrome, obesity, type 2 diabetes, neurological diseases and allergies (19).Miao Ping confirmed that the rats with metabolic syndrome had obvious gut microbiota imbalance, and the content of opportunistic pathogens such as streptococcus increased (21).The results of this study showed that the number of Streptococcus in ED patients was up-regulated, while the number of Rosteria was down-regulated.

In this study, patients were selected to detect the fecal bacterial diversity of the with functional ED and HD groups. Results of Alpha diversity analysis showed that the ED group had lower bacterial diversity. It was further confirmed that there were significant differences between the two groups in the level of colony and genus.Level analysis, and also found that the number of Streptococcus (Streptococcus) and Subdoligranulum (rare small cocci) genera in the ED group increased, while the number of Prevotella_9 (Prevola_9), Blautia (Brautia), Lachnospiraceae NK4A136 group (NK4A136 group of Trichospiraceae) and Roseburia (Rhodoria) decreased.

In summary, erectile dysfunction is associated with colonic microbial diversity in men. Gut microbiota can regulate male erectile function by regulating hormone levels, inflammatory mediators and various factors.

Gut microbiota is a promising and promising option for the treatment of erectile dysfunction in men in the future.

Declarations

Author Contributions

Shaofeng Chen, Geng Qiang, and Jun Guo conceived and designed the study, analyzed the data and wrote the manuscript. Fu Wang, and Guojin Yu designed, performed and analyzed the experiments shown in Figure 1-3 and table 1. Zhong Li , Yu Zhao , Yuan Sun, and Xiuchuan Yan designed, performed and analyzed the experiments shown in Figures 4 and 7.

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Figures

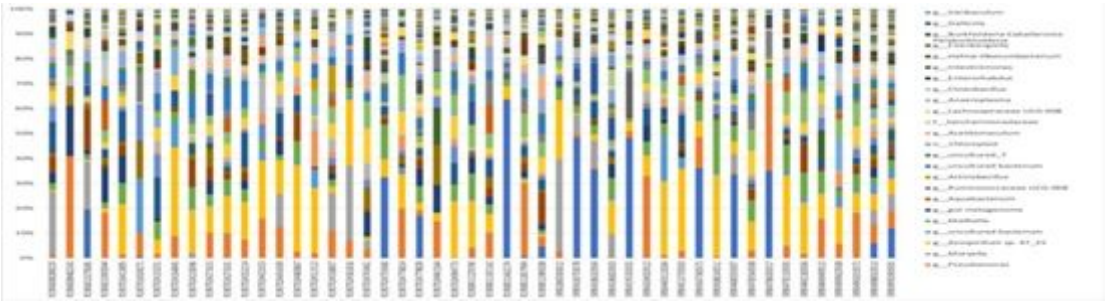

Figure 1

The distribution of abundance bacteria and core bacteria in ED and HD groups. Note：There was no difference between the genera of the ED-HD group. Alloprevotella was statistically significant.

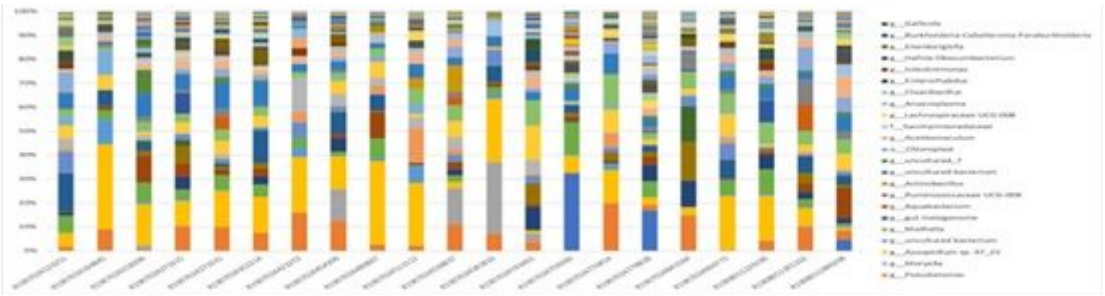

Figure 2

The distribution of abundance bacteria and core bacteria in ED groups. Note：Alloprevotella was not identified only in the ED group.

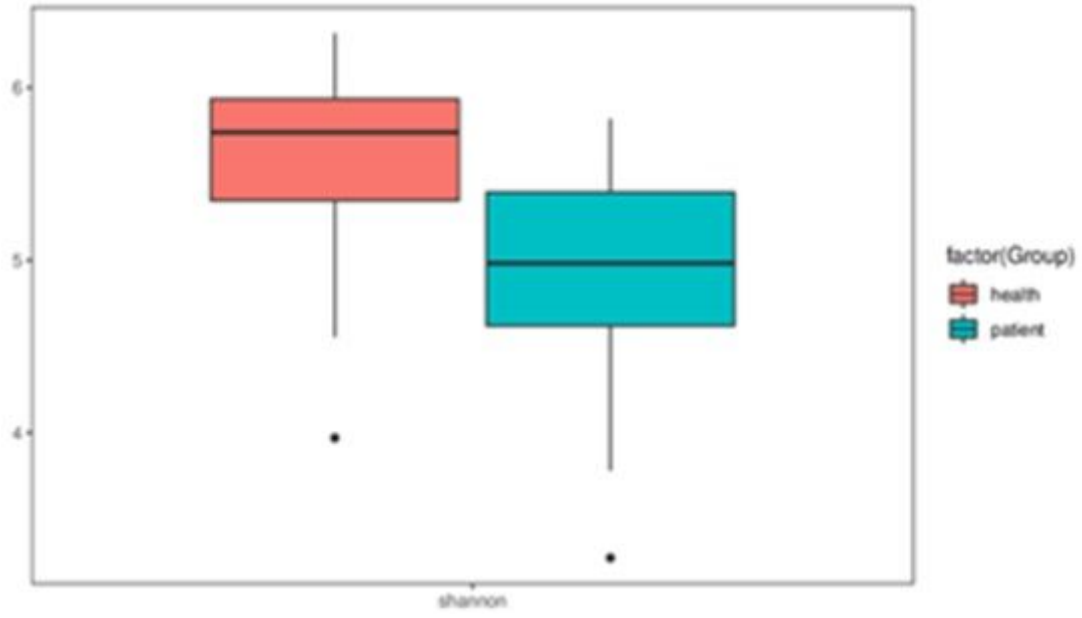

Figure 3

Median Shannon index . Note: Compared with HD group, ED group had lower bacterial diversity.

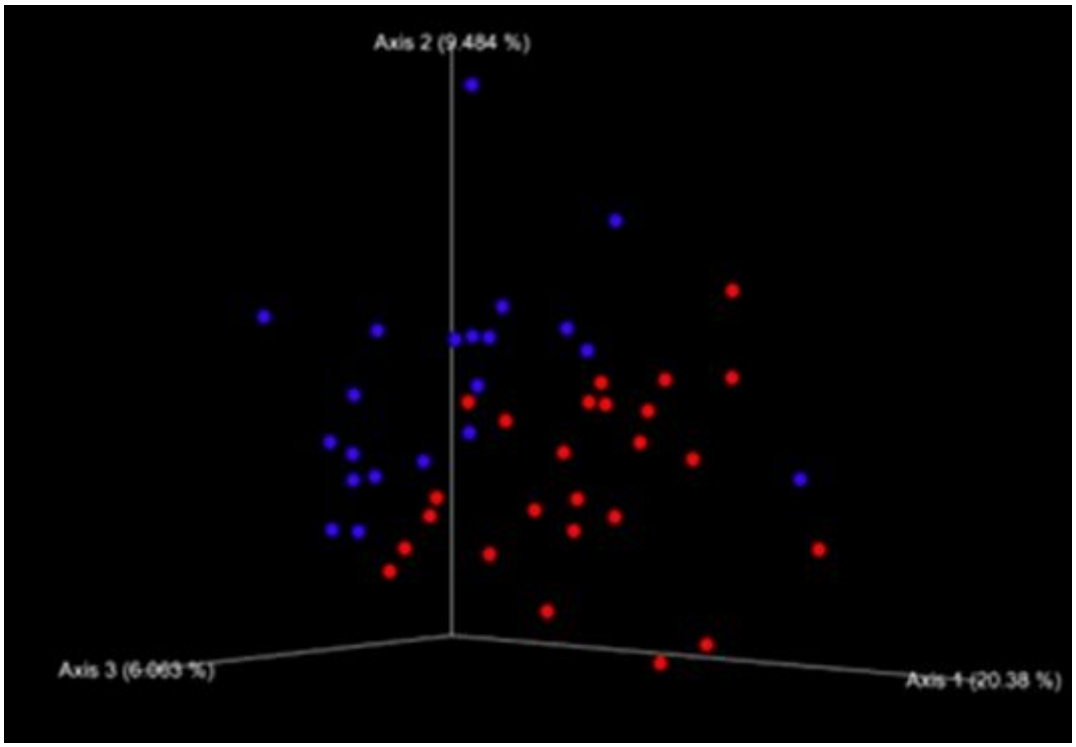

Figure 4

Median Shannon index. Note: the bacterial structure of the ED-HD group was different.

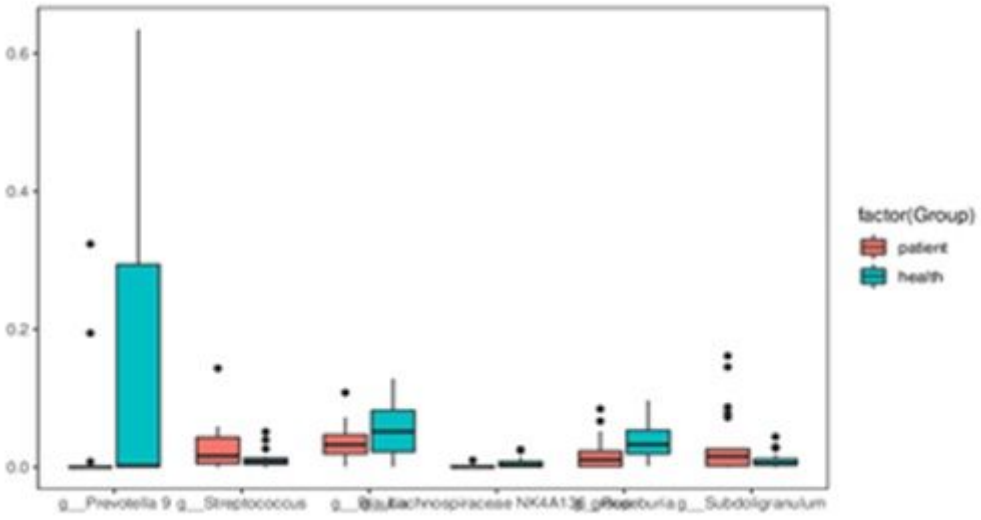

Figure 5

The Box figure of significant differences species between ED-HD groups

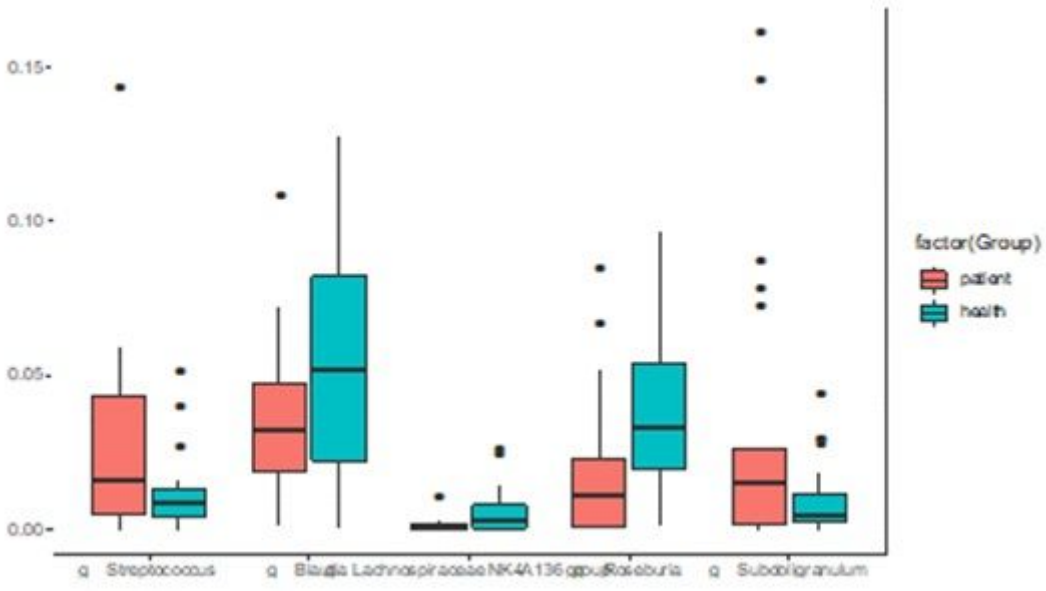

Figure 6

The Box figure of significant differences species between ED-HD groups(except Prevotella)

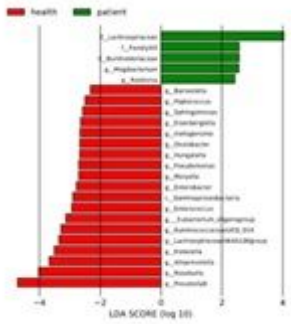

Figure 7

LDA value distribution histogram