Original Article
Male sexual health and dysfunction

pISSN: 2287-4208 / eISSN: 2287-4690
World J Mens Health 2024 Apr 42(2): 363-372
https://doi.org/10.5534/wjmh.220278

The World Journal of MEN's HEALTH

Characteristics of Gut Microbiota in Patients with Erectile Dysfunction: A Chinese Pilot Study

Jiaqi Kang*, Qihua Wang*, Shangren Wang, Yang Pan, Shuai Niu, Xia Li, Li Liu, Xiaoqiang Liu

Department of Urology, Tianjin Medical University General Hospital, Tianjin, China

Purpose: Little is known about the role of gut microbiota in the pathogenesis of erectile dysfunction (ED). We performed a study to compare taxonomic profiles of gut microbiota of ED and healthy males.

Materials and Methods: A total of 43 ED patients and 16 healthy controls were enrolled in the study. The 5-item version of the International Index of Erectile Function (IIEF-5) with a cutoff value of 21 was used to evaluate erectile function. All participants underwent nocturnal penile tumescence and rigidity test. Samples of stool were sequenced to determine the gut microbiota.

Results: We identified a distinct beta diversity of gut microbiome in ED patients by unweighted UniFrac analysis (R^2=0.026, p=0.036). Linear discriminant analysis effect size (LEfse) analysis showed *Actinomyces* was significantly enriched, whereas *Coprococcus_1*, *Lachnospiraceae_FCS020*_group, *Lactococcus*, *Ruminiclostridium_5*, and *Ruminococcaceae_UCG_002* were depleted in ED patients. *Actinomyces* showed a significant negative correlation with the duration of qualified erection, average maximum rigidity of tip, average maximum rigidity of base, tip tumescence activated unit (TAU), and base TAU. *Coprococcus_1*, *Lachnospiraceae_FCS020*_group, *Ruminiclostridium_5*, and *Ruminococcaceae_UCG_002* were significantly correlated with the IIEF-5 score. *Ruminiclostridium_5* and *Ruminococcaceae_UCG_002* were positively related with average maximum rigidity of tip, average maximum rigidity of base, ΔTumescence of tip, and Tip TAU. Further, a random forest classifier based on the relative abundance of taxa showed good diagnostic efficacy with an area under curve of 0.72.

Conclusions: This pilot study identified evident alterations in the gut microbiome composition of ED patients and found *Actinomyces* was negatively correlated with erectile function, which may be a key pathogenic bacteria.

Keywords: Actinomyces; Erectile dysfunction; Microbiota; RNA, ribosomal, 16S

INTRODUCTION

As a common sexual disorder in males, erectile dysfunction (ED) is defined as the inability to achieve and maintain sufficient penile erection to complete satisfactory sexual intercourse [1]. ED prevalence ranged from 37.2% to 48.6% in a survey of eight countries [2]. ED not only represents a troublesome issue in terms of qual-

Received: Dec 29, 2022 **Revised:** Mar 14, 2023 **Accepted:** Mar 30, 2023 **Published online** Jun 1, 2023
Correspondence to: Li Liu https://orcid.org/0000-0002-4970-2834
Department of Urology, Tianjin Medical University General Hospital, No. 154, Anshan Road, Heping District, Tianjin 300052, China.
Tel: +86-18622208373, **Fax:** +86-60361614, **E-mail:** 1005973741@qq.com
Correspondence to: Xiaoqiang Liu https://orcid.org/0000-0003-2681-4160
Department of Urology, Tianjin Medical University General Hospital, No. 154, Anshan Road, Heping District, Tianjin 300052, China.
Tel: +86-13920915038, **Fax:** +86-60361614, **E-mail:** xiaoqiangliu@aliyun.com
*These authors contributed equally to this work as co-first authors.

ity of life but also increases the risk of cardiovascular disease (CVD) events and deaths [3,4]. Therefore, ED should be regarded as an early manifestation of CVD, which has drawn increasing attention from urologists.

Since the rapid development of sequencing technologies in recent years, the pivotal role of gut microbiota in human health has been increasingly recognized. Growing evidence has demonstrated a close connection between gut microbiota and multiple disorders, such as obesity [5], diabetes [6], atherosclerosis [7], anxiety, and depression [8]. Accordingly, we hypothesized that ED, as one of the complications of the above disorders, may be regulated by gut microbiota to some extent.

A recent review proposed a "funnel" model including five levels from correlation studies to molecular mechanistic studies for evaluating evidence connecting the microbiome to human diseases [9]. The first level of associative studies refers to finding the prevalent microbes in diseased *versus* healthy individuals. However, rare studies investigated the microbial composition of ED patients to date. A community-based study recently examined the relationship between gut microbiota and ED [10]. In detail, they found that the abundance of *Alistipes* and *Clostoridium* XVIII was significantly correlated with poor erectile function. Given the above, we performed the study to further explore the characteristics of gut microbiota in ED patients, and identify key aberrant taxa correlated with male erectile function.

MATERIALS AND METHODS

1. Patient recruitment

1) Patient recruitment

The 5-item version of the International Index of Erectile Function (IIEF-5) with a cutoff value of 21 was used to diagnose ED [11]. Each patient had to meet the following inclusion criteria: (1) have a regular sexual partner for more than three months; (2) IIEF-5 score ≤21; (3) between 18–60 years of age; (4) no history of radical prostatectomy, pelvic trauma, or surgery; (5) no severe mental illness. Healthy controls had to meet the following inclusion criteria: (1) have a regular sexual partner for more than three months; (2) IIEF-5 score >21; (3) between 18–60 years of age; (4) have good health with no significant medical diseases. Participants with oral antibiotic use in the prior 2 weeks or personal history of inflammatory bowel disease (IBD), irritable bowel syndrome, autoimmune diseases, liver diseases, diarrhea, and malignant tumors were excluded from the study.

Clinical information gathered included age, body mass index (BMI), lipids, serum uric acid (UA) and testosterone, alcohol and smoking use, and comorbidities (hypertension and diabetes mellitus). Alcohol drinking was defined as drinking alcohol more than once a week.

2) Ethics statement

The present study protocol was reviewed and approved by the Ethics Committee of the Tianjin Medical University General Hospital (IRB2021-KY-060). Informed consent was submitted by all subjects when they were enrolled.

2. Nocturnal penile tumescence and rigidity (NPTR) test

All subjects underwent NPTR tests for at least one night with more than 6 hours of sleep. The RigiScan® Plus rigidity assessment system (GOTOP Medical, Inc.) was applied to monitor and record the penis rigidity and tumescence of each subject during the night. The NPTR parameters include qualified erection times, duration of qualified erection, average maximum rigidity, duration of tip rigidity >60%, the increase of tumescence (ΔTumescence), rigidity activated unit (RAU), and tumescence activated unit (TAU) were collected and analyzed. If the tumescence of penis increased by 20% compared with the baseline and lasted for more than 3 minutes, then return to the baseline for at least 5 minutes, this active period of penile erection is considered one qualified erection. RAU and TAU are measured values of time intensity introduced in 1994 to explain the time dependency of erectile rigidity and tumescence. An RAU is calculated by multiplying the elapsed time during an erectile event by the rigidity of that event, whereas TAU is calculated by multiplying the elapsed event time by the percent increase in tumescence over baseline [12].

3. Specimen collection, DNA extraction, and PCR amplification

All fecal samples were collected using sterile collectors and immediately stored frozen to -80 ℃. We extracted total genome DNA from samples using cetyltrimethylammonium bromide/sodium dodecyl sulfate

and monitored DNA concentration and purity on 1% agarose gels. According to the concentration, DNA was diluted to 1 ng/μL using sterile water. 16S rRNA genes were amplified using the specific primer with the barcode (V4-V5: 515F-907R). All PCR reactions used 15 μL of Phusion® High-Fidelity PCR Master Mix (New England Biolabs), 0.2 μM each of the forward and reverse primers, 10 ng of template DNA, and a reaction concentration of 30 μL. Initial denaturation at 98 ℃ for 1 minute was followed by 30 cycles of denaturation at 98 ℃ for 10 seconds, annealing at 50 ℃ for 30 seconds, and elongation at 72 ℃ for 60 seconds. The same volume of 1X loading buffer (containing SYB green) should be mixed with PCR products and electrophoresed. For further experiments, samples with a bright main strip between 400–450 bp were chosen. PCR products were mixed in equal density. The mixture of PCR products was then purified using GeneJET Gel Extraction Kit (Thermo Scientific).

4. Library preparation and sequencing

We generated sequencing libraries using NEB Next®UltraTMDNA Library Prep Kit for Illumina (NEB) following manufacturers' instructions. Qubit@ 2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 systems were used to assess library quality. Finally, 250 bp/300 bp paired-end reads were generated from the library using an Illumina MiSeq platform.

5. Bioinformatics analysis

The sequence analysis was conducted using the UPARSE software package. Sequences with ≥97% similarity were assigned to the same operational taxonomic unit (OTU). The RDP classifier is used to annotate taxonomic information for each representative sequence for each OTU. We rarify the OTU table and calculate four metrics to compute alpha diversity: an estimate of species abundance is derived from the Chao1 index and the observed OTUs; an estimate of species diversity is derived from Shannon indexes and Simpson indices. Principal Coordinate Analysis was performed based on unweighted UniFrac distance calculated by the QIIME software. Adonis test was performed to compare distance dissimilarities. To find biomarkers between groups, linear discriminant effect size (LEfSe) analysis was performed with a threshold of 2 for linear discriminant analysis (https://huttenhower.sph.harvard.edu/lefse/).

6. Random forest classifier

Based on the relative abundance of taxa at the genus level, a random forest model was used to discriminate samples of healthy participants and ED patients. The cross-validation error curve was obtained by using five trials of the ten-fold cross-validation. The smallest number of OTUs with the minimum cross-validation error was chosen as the optimal set. The construction of random forest model was performed by the randomForest package. With the pROC package, the receiver operating characteristic curves (ROC) were plotted and the area under curve (AUC) was calculated.

7. Statistical analysis

Continuous variables with normal distribution were presented as mean±standard deviation; non-normal variables were reported as median (interquartile range). Categorical data were reported as number (percentage). Means of 2 continuous normally distributed variables were compared by independent samples Student's t-test. Mann–Whitney U test was used to compare the means of 2 groups of variables not normally distributed. Correlation between genera abundances and clinical data was performed with Spearman correlation. All tests were two-sided, and $p<0.05$ was considered significant. Versions 8 and 3.6.1 of GraphPad Prism and R software were used to conduct statistical analyses.

RESULTS

1. Clinical characteristics of the participants

In total, we recruited 43 men with ED and 16 healthy controls in the study (Fig. 1). The clinical characteristics of the participants are shown in Table 1. In general, no significant difference was observed in almost clinical parameters, including age (31 [5.5] *vs.* 28 [8], p=0.336). BMI (24.69 [3.41] *vs.* 24.01 [4.23], p=0.953), smoking (19 *vs.* 4, p=0.263), drinking (26 *vs.* 7, p=0.377), hypertension (3 *vs.* 0, p=0.556), and diabetes mellitus (2 *vs.* 0, p>0.999). In terms of laboratory tests, the two groups showed insignificant differences in total cholesterol (5.04±0.77 *vs.* 4.36±0.85, p=0.051), triglyceride (1.63±0.78 *vs.* 1.48±0.83, p=0.394), low-density lipoprotein cholesterol (1.20±0.24 *vs.* 1.26±0.23, p=0.428), high-density lipoprotein cholesterol (3.06±0.62 *vs.* 2.69±0.78, p=0.252), UA (410.83±86.05 *vs.* 435.75±95.12, p=0.344), and testosterone (548.21±191.38 *vs.* 573.66±250.32, p=0.680). Therefore, baseline clini-

cal characteristics were comparable between the two groups.

The IIEF-5 score and NPTR test results of the two groups were summarized in Table 2. The IIEF-5 score was significantly lower for the ED group than for the control group (p<0.001). In terms of NPTR test results, parameters except for qualified erection times and Δtumescence of tip between ED and controls were statistically significant (p<0.05). In summary, patients in the ED group showed a decrease in erectile function.

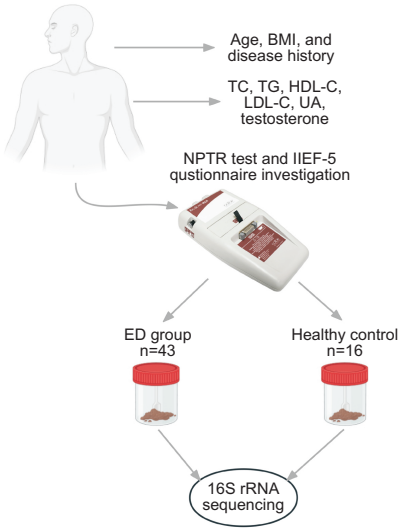

Fig. 1. Schematic representation of the study design. BMI: body mass index, TC: total cholesterol, TG: triglyceride, LDL-C: low-density lipoprotein cholesterol, HDL-C: high-density lipoprotein cholesterol, UA: uric acid, NPTR: nocturnal penile tumescence and rigidity, IIEF-5: the 5-item version of the international index of erectile function, ED: erectile dysfunction.

Table 1. The demographics and serum characteristics of patients with ED and healthy controls

Cohort characteristic	ED group	Control group	p-value
Age (y)	31 (5.5)	28 (8)	0.336
BMI (kg/m^2)	24.69 (3.41)	24.01 (4.23)	0.953
Smoking (%)	19 (44.19)	4 (25.00)	0.263
Drinking (%)	26 (60.47)	7 (43.75)	0.377
Hypertension (%)	3 (6.98)	0 (0)	0.556
Diabetes mellitus (%)	2 (4.65)	0 (0)	>0.999
TC (mmol/L)	5.04±0.77	4.36±0.85	0.051
TG (mmol/L)	1.63±0.78	1.48±0.83	0.394
LDL-C (mmol/L)	1.20±0.24	1.26±0.23	0.428
HDL-C (mmol/L)	3.06±0.62	2.69±0.78	0.252
UA (μmol/L)	410.83±86.05	435.75±95.12	0.344
Testosterone (ng/dL)	548.21±191.38	573.66±250.32	0.680

Continuous variables with normal distribution were presented as mean±standard deviation; non-normal variables were reported as median (interquartile range). Categorical data were reported as number (percentage).
ED: erectile dysfunction, BMI: body mass index, TC: total cholesterol, TG: triglyceride, LDL-C: low-density lipoprotein cholesterol, HDL-C: high-density lipoprotein cholesterol, UA: uric acid.

2. Altered diversity of gut microbiome in patients with ED

Known as within-community diversity, alpha diversity examines the number of species in a local uniform habitat to reflect microbial diversity and abundance. No difference was observed for alpha diversity between the ED and control groups, which includes species richness (Chao1 and Observed OTUs) and diversity (Shannon and Simpson indices) of the gut microbial community (Fig. 2A). Overall, there was a decreasing trend for alpha diversity indices of ED patients. Beta diversity measures the diversity among groups. As shown in Fig. 2B, unweighted UniFrac distances showed a significant difference (R^2=0.026, p=0.036), which suggested a notable change in the gut flora between the two groups.

Table 2. The IIEF-5 score and parameters of NPTR test of patients with ED and healthy controls

Parameter	ED group	Control group	p-value
IIEF-5 score	11 (7)	24 (3)	<0.001
Qualified erection times	5.05±2.48	5.50±2.42	0.532
Duration of qualified erection (min)	78.76±46.06	134.42±58.43	<0.001
Average maximum rigidity of tip (%)	89.3 (13.65)	100 (4.05)	0.001
Average maximum rigidity of base (%)	78.6 (19.7)	90.2 (12.52)	0.020
Duration of tip rigidity >60% (min)	28 (47.75)	60.5 (64.63)	0.017
Duration of base rigidity >60% (min)	13 (25.75)	30.5 (78)	0.014
ΔTumescence of tip (cm)	2.09±0.80	2.10±0.79	0.958
ΔTumescence of base (cm)	2.20 (0.75)	2.7 (0.75)	0.025
Tip RAU	44.06±27.59	71.81±39.79	0.004
Base RAU	38.24±23.39	67.38±35.63	0.001
Tip TAU	31.24±20.88	50.06±27.09	0.006
Base TAU	31.00 (23.50)	48.50 (27.25)	<0.001

Continuous variables with normal distribution were presented as mean±standard deviation; non-normal variables were reported as median (interquartile range).
IIEF-5: the 5-item version of the International Index of Erectile Function, NPTR: nocturnal penile tumescence and rigidity, ED: erectile dysfunction, RAU: rigidity activated unit, TAU: tumescence activated unit.

3. Taxonomic changes of gut microbiota at phylum and genus level in patients with ED

We next compared the community composition of gut microbiota at the phylum and genus levels. As expected, it was conserved at the phylum level, which is populated most predominantly by *Firmicutes*, followed by *Proteobacteria*, *Actinobacteria*, and *Bacteroidetes* (Fig. 3A, 3B). Genera present in less than 0.1% relative

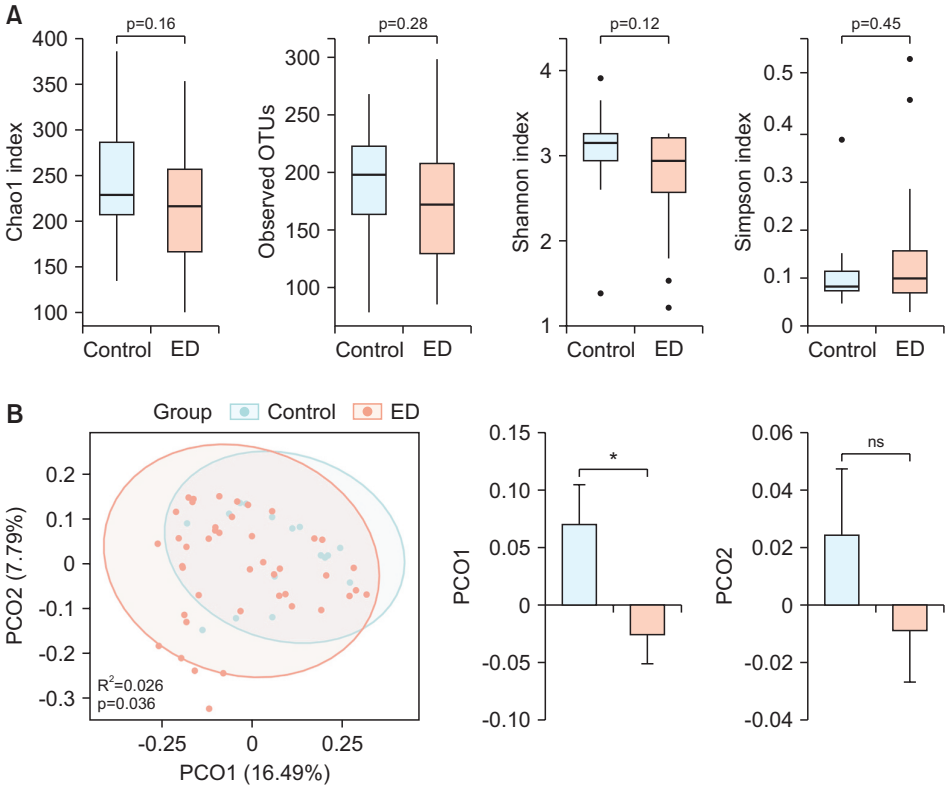

Fig. 2. Comparison of microbial diversity between ED and control groups. (A) Alpha diversity, including the observed Chao1 index, observed OTUs, Shannon index, and Simpson index; and (B) a principal component analysis diagram based on an unweighted UniFrac distance matrix. ED: erectile dysfunction, OTU: operational taxonomic units, PCO: principal coordinate analysis, ns: not significant (p>0.05). *Statistically significant (p<0.05).

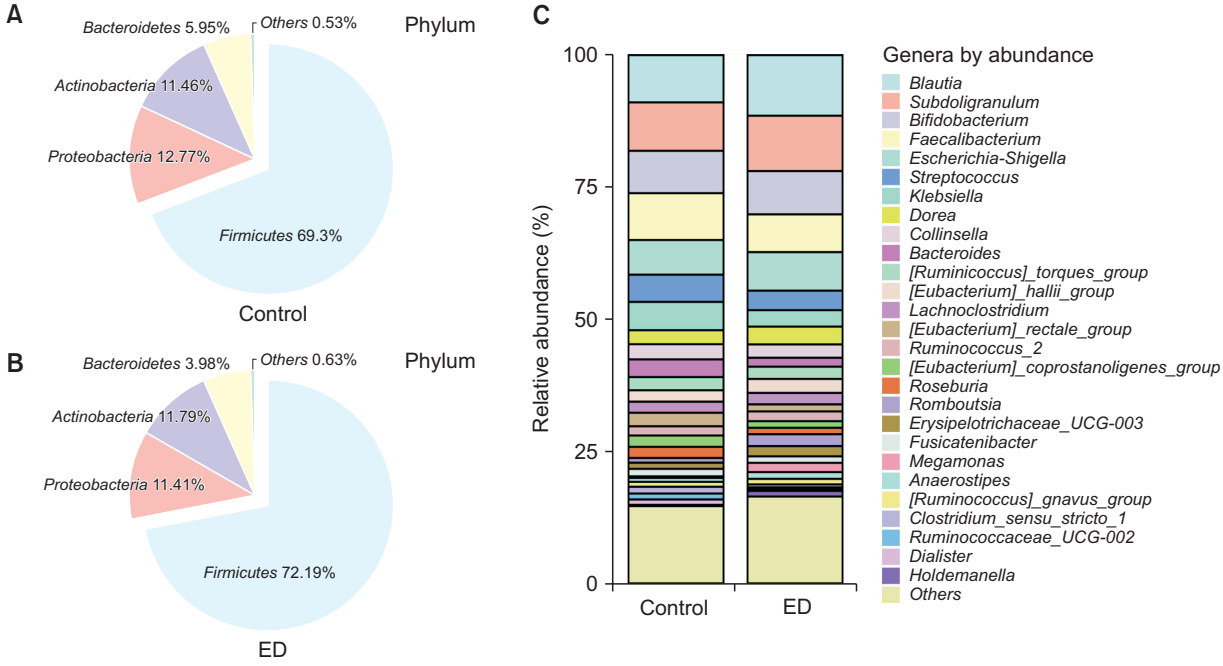

Fig. 3. Comparisons of the gut microbial community compositions between ED and control groups. The sector graph of the compositions at the phylum level in the control group (A) and ED group (B), and the percent stacked column chart of the composition at the genus level in two groups (C). ED: erectile dysfunction.

The World Journal of MEN's HEALTH

abundance were grouped in the category "Others". The top five most abundant genera found in ED patients were *Blautia* (11.47%), *Subdoligranulum* (10.48%), *Bifidobacterium* (8.20%), *Faecalibacterium* (7.14%), and *Escherichia-Shigella* (7.29%) (Fig. 3C). Based on the beta diversity analysis, the compositions of the gut microbial communities were different between the groups.

4. Identification of key gut microbes between the ED and control groups

LEfSe analysis was performed to identify which bacterial taxa were different between the ED and control groups. Totally, we identified 34 gut microbes showing significant differences, 9 of which had a high abundance in the ED group and 25 of which had a high abundance in the control group (Fig. 4A). Additionally, the abundance comparisons of predominant genera showed that *Actinomyces* was significantly enriched, whereas *Coprococcus_1*, *Lachnospiraceae_FCS020_group*, *Lactococcus*, *Ruminiclostridium_5*, and *Ruminococcaceae_UCG_002* were depleted in patients with ED (Fig. 4B–4G). The above results demonstrated impressive changes in the gut flora composition of the ED group, revealing the importance of intestinal microbiota in disease development.

5. Correlation analysis between the differential taxa and clinical indices of patients with ED

A Spearman correlation analysis calculated for all patients was conducted to better investigate the relationship between differential genera and clinical characteristics. These key genera showed significant

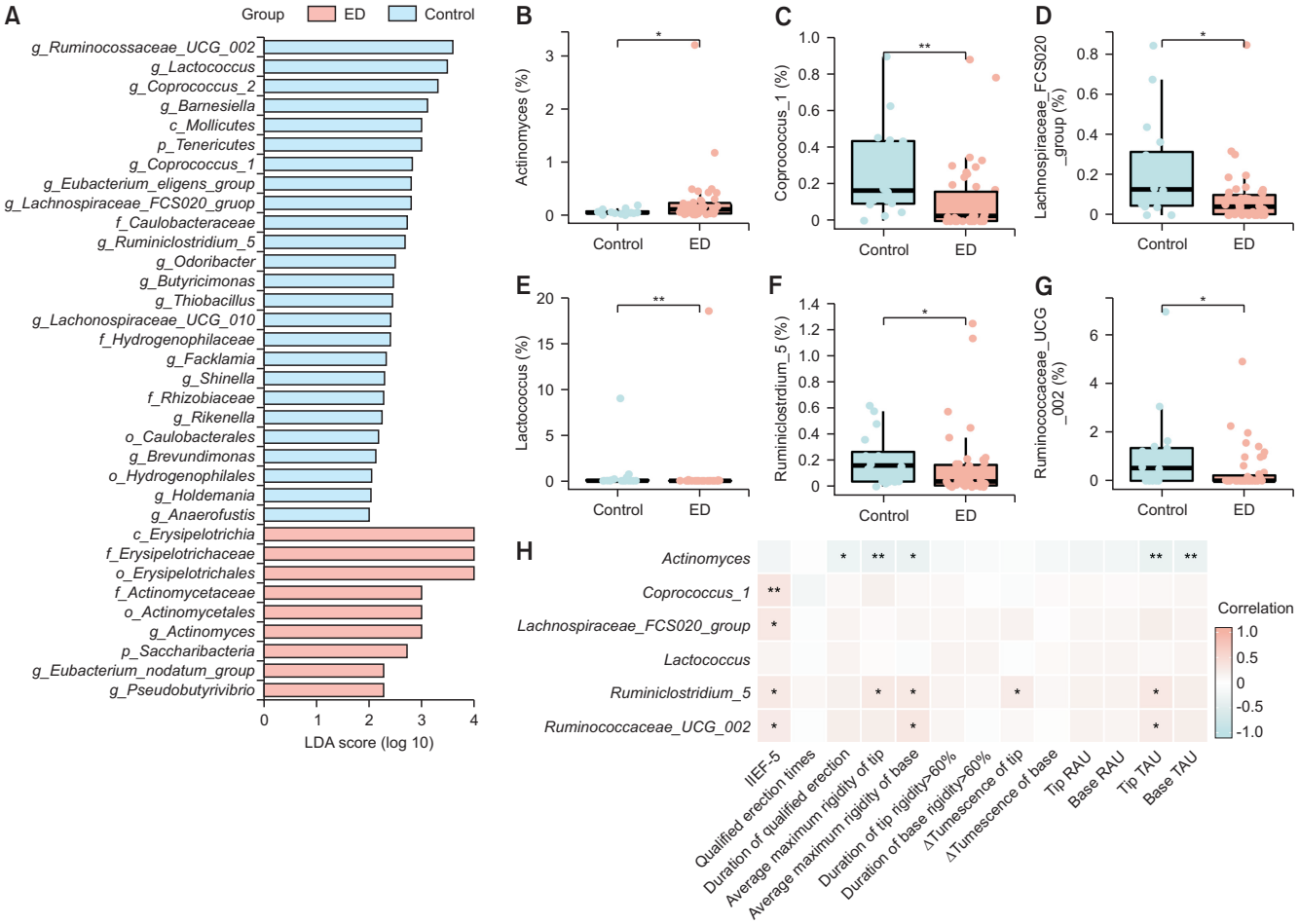

Fig. 4. Linear discriminant analysis effect size (LEfse) analysis of the gut microbiota. (A) Abundances of the gut microbiota in both groups; (B–G) Boxplots of the relative abundance of one significantly increased and five decreased gut microbiota; and (H) Correlation analysis between clinical indicators and differential genera. ED: erectile dysfunction, IIEF-5: 5-item version of the International Index of Erectile Function. Statistically significant (*p<0.05, **p<0.01).

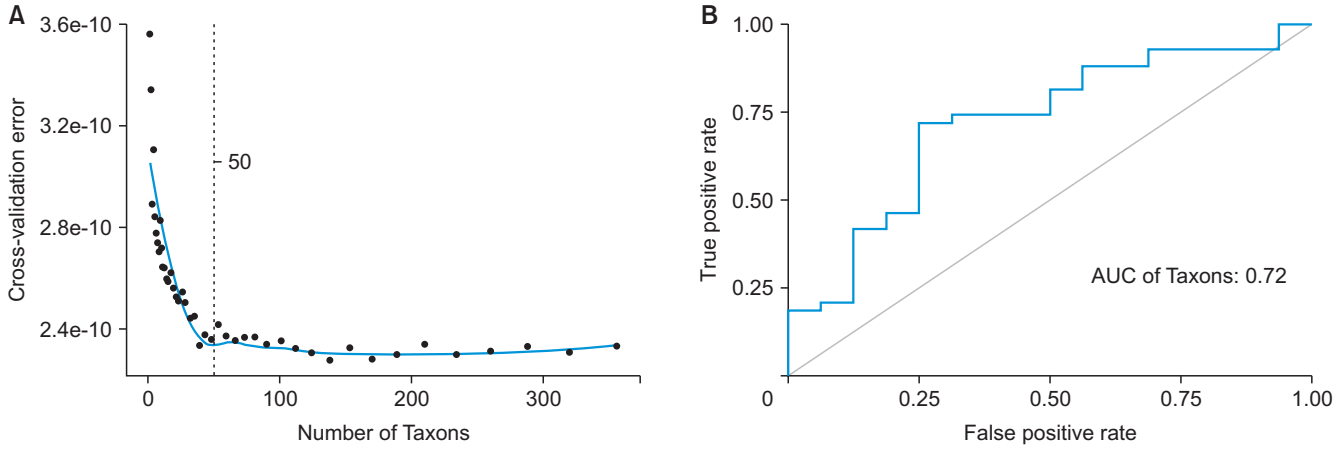

Fig. 5. The gut microbiota classifier for ED. (A) The 10-fold cross-validation on a random forest model with 50 genera as optimal features. (B) The ROC curve reveals the potential diagnostic efficacy of the model with a relatively high AUC. ED: erectile dysfunction, ROC: receiver operating curve, AUC: area under curve.

correlations with the IIEF-5 score and almost NPTR parameters. In this regard, *Actinomyces* showed a significant negative correlation with the duration of qualified erection, average maximum rigidity of tip, average maximum rigidity of base, Tip TAU, and base TAU (Fig. 4H). Of note, *Coprococcus_1*, *Lachnospiraceae_FCS020*_group, *Ruminiclostridium_5*, and *Ruminococcaceae_UCG_002* were all significantly correlated with the IIEF-5 score. Additionally, a significant positive correlation existed between *Ruminiclostridium_5*, *Ruminococcaceae_UCG_002* and some NPTR parameters including average maximum rigidity of tip, average maximum rigidity of base, ΔTumescence of tip, and Tip TAU. Whereas no significant correlations were detected between *Lactococcus* and clinical parameters.

6. Gut microbes discriminate ED patients from healthy participants

To explore the potential role of intestinal microbes as the biomarker of ED, a random forest classifier was developed to discriminate ED patients from healthy participants with outstanding sensitivity and specificity. Firstly, 50 genera were selected as the optimal features by 10-fold cross-validation on a random forest model (Fig. 5A). A relatively high AUC value of 0.72 suggested the potential diagnostic efficacy of the model (Fig. 5B), which indicated the potential of the intestinal microbes as a biomarker for ED.

DISCUSSION

In the present study, we identified evident alterations in the gut microbiome composition of ED patients. Specifically, *Actinomyces* was significantly enriched, whereas *Coprococcus_1*, *Lachnospiraceae_FCS020_* group, *Lactococcus*, *Ruminiclostridium_5*, and *Ruminococcaceae_UCG_002* were depleted in ED patients. Spearman analysis showed a significant negative correlation of *Actinomyces* with the results of the NPTR test, which suggests the higher *Actinomyces*, the worse erectile function. *Coprococcus_1*, *Lachnospiraceae_FCS020*_group, *Ruminiclostridium_5*, and *Ruminococcaceae_UCG_002* were all positively correlated with the IIEF-5 and NPTR results. Further, a random forest classifier based on the relative abundance of taxa showed good diagnostic efficacy with an AUC of 0.72. The gut microbes showed the potential role of discriminating patients with ED from healthy controls, which may function as a promising biomarker of ED.

As previously described, few studies that investigated the microbial composition of ED patients are currently available. Okamoto et al [10] firstly compared the gut microbiota composition of high IIEF-5 score patients (IIEF-5 >16) and low IIEF-5 score patients (IIEF-5 ≤16). They found the abundance of *Alistipes* decreased and the abundance of *Clostridium XVIII* increased in the low IIEF-5 score group. Multivariate analysis showed *Clostridium XVIII* was an independent risk factor of ED. Compared with this prior study, more differentially abundant microbiota was found in our study, but

there was no difference in *Alistipes* and *Clostridium XVIII* between patients and healthy people. This may be related to the difference in geographic location and clinical characteristics of the two populations, and the nature of inter-individual differences in gut microbiota.

Actinomyces, as an opportunistic pathogen, mainly exist in the upper digestive tract [13]. Forbes et al [14] analyzed the gut microbiome composition of a variety of inflammatory diseases, including IBD, multiple sclerosis, and rheumatoid arthritis. They found that the abundance of *Actinomyces* was higher in disease cohorts than in healthy controls, which suggested that it might be a crucial genus in inflammation-related diseases. Inflammation may be the pathological mechanism underlying endothelial dysfunction in ED [15]. Evidence has shown that increased circulating levels of inflammatory cytokines and endothelial-prothrombotic compounds are related to ED development [16]. Whether *Actinomyces* is involved in this pathophysiological process and impairs erectile function is worth exploring.

Coprococcus, classically considered a common commensal bacterium, can utilize dietary fiber ingested by the human body [17]. Coprococcus abundance has been associated with a low prevalence of metabolic diseases such as type 2 diabetes, hyperlipidemia, and obesity in previous studies [18-20]. Wei et al [21] reported a decreased abundance of *Coprococcus* in hyperuricemia. These metabolic diseases, as risk factors for ED, may promote the development of ED by regulating intestinal flora. The biological function of *Lachnospiraceae_FCS020*_group is currently unclear and it has recently been reported to correlate with cognitive function in middle-aged adults [22]. *Lactococcus* is well-known as a gut commensal bacteria with probiotic characteristics that play important roles in human health [23]. A lower abundance of intestinal *Ruminiclostridium_5* has been found in renal calculi patients [24], whereas specific dietary prebiotics can ameliorate intestinal ecological homeostasis by elevating the abundance of *Ruminiclostridium_5* [25]. *Ruminococcaceae* is thought to be associated with disease, which is decreased in patients with IBD [26]. However, the role of *Ruminococcaceae_UCG_002* is still not clear yet. In a word, the differential microorganisms found in this study are frequently reported in human health and diseases, but their biological function remains to be studied.

Limitations of the study need to be addressed. Firstly, the study is limited by the lack of information on the causes of ED. The intestinal flora of ED caused by different etiologies may be different to some extent. Based on this, factors affecting intestinal flora, such as IBD and antibiotic use, were excluded as far as possible during enrollment, and the effects of age, smoking and alcohol history, hypertension, hyperglycemia, and hyperlipidemia were also corrected in our study. Secondly, the number of cases included in this study was insufficient. Future inclusion of additional patients is needed to further substantiate the results of this study.

CONCLUSIONS

This pilot study identified evident alterations in the gut microbiome composition of ED patients and found *Actinomyces* was negatively correlated with erectile function, which may be a key pathogenic bacteria. The gut microbes showed the potential role of discriminating patients with ED from healthy controls, which may function as a promising biomarker of ED.

Conflict of Interest

The authors have nothing to disclose.

Funding

This research was supported by the National Natural Science Foundation of China (82171594).

Acknowledgements

None.

Author Contribution

Conceptualization: JK, X Liu. Data curation: JK, QW, SW, SN. Formal analysis: JK, QW, YP. Methodology: JK, YP. Software: JK. Supervision: X Li, LL, X Liu. Writing-original draft: JK, QW. Writing-review & editing: all authors.

Data Sharing Statement

The raw sequence data reported in this paper have been deposited in the Genome Sequence Archive in National Genomics Data Center, China National Center for Bioinformation/Beijing Institute of Genomics, Chinese Academy of Sciences (GSA: CRA009132) that are publicly accessible at https://ngdc.cncb.

ac.cn/gsa.

REFERENCES

1. Hatzimouratidis K, Amar E, Eardley I, Giuliano F, Hatzichristou D, Montorsi F, et al.; European Association of Urology. Guidelines on male sexual dysfunction: erectile dysfunction and premature ejaculation. Eur Urol 2010;57:804-14.
2. Goldstein I, Goren A, Li VW, Tang WY, Hassan TA. Epidemiology update of erectile dysfunction in eight countries with high burden. Sex Med Rev 2020;8:48-58.
3. Vlachopoulos CV, Terentes-Printzios DG, Ioakeimidis NK, Aznaouridis KA, Stefanadis CI. Prediction of cardiovascular events and all-cause mortality with erectile dysfunction: a systematic review and meta-analysis of cohort studies. Circ Cardiovasc Qual Outcomes 2013;6:99-109.
4. Capogrosso P, Ventimiglia E, Boeri L, Capitanio U, Gandaglia G, Dehò F, et al. Sexual functioning mirrors overall men's health status, even irrespective of cardiovascular risk factors. Andrology 2017;5:63-9.
5. Le Chatelier E, Nielsen T, Qin J, Prifti E, Hildebrand F, Falony G, et al. Richness of human gut microbiome correlates with metabolic markers. Nature 2013;500:541-6.
6. Wu H, Tremaroli V, Schmidt C, Lundqvist A, Olsson LM, Krämer M, et al. The gut microbiota in prediabetes and diabetes: a population-based cross-sectional study. Cell Metab 2020;32:379-90.e3.
7. Meng Q, Ma M, Zhang W, Bi Y, Cheng P, Yu X, et al. The gut microbiota during the progression of atherosclerosis in the perimenopausal period shows specific compositional changes and significant correlations with circulating lipid metabolites. Gut Microbes 2021;13:1-27.
8. Simpson CA, Diaz-Arteche C, Eliby D, Schwartz OS, Simmons JG, Cowan CSM. The gut microbiota in anxiety and depression - a systematic review. Clin Psychol Rev 2021;83:101943.
9. Chaudhari SN, McCurry MD, Devlin AS. Chains of evidence from correlations to causal molecules in microbiome-linked diseases. Nat Chem Biol 2021;17:1046-56.
10. Okamoto T, Hatakeyama S, Imai A, Yamamoto H, Yoneyama T, Mori K, et al. The association between gut microbiome and erectile dysfunction: a community-based cross-sectional study in Japan. Int Urol Nephrol 2020;52:1421-8.
11. Rosen RC, Cappelleri JC, Smith MD, Lipsky J, Peña BM. Development and evaluation of an abridged, 5-item version of the International Index of Erectile Function (IIEF-5) as a diagnostic tool for erectile dysfunction. Int J Impot Res 1999;11:319-26.
12. Zou Z, Lin H, Zhang Y, Wang R. The role of nocturnal penile tumescence and rigidity (NPTR) monitoring in the diagnosis of psychogenic erectile dysfunction: a review. Sex Med Rev 2019;7:442-54.
13. Vasapolli R, Schütte K, Schulz C, Vital M, Schomburg D, Pieper DH, Vilchez-Vargas R, Malfertheiner P. Analysis of Transcriptionally Active Bacteria Throughout the Gastrointestinal Tract of Healthy Individuals. Gastroenterology. 2019 Oct;157(4):1081-1092.e3.
14. Forbes JD, Chen CY, Knox NC, Marrie RA, El-Gabalawy H, de Kievit T, et al. A comparative study of the gut microbiota in immune-mediated inflammatory diseases-does a common dysbiosis exist? Microbiome 2018;6:221.
15. Kaya-Sezginer E, Gur S. The inflammation network in the pathogenesis of erectile dysfunction: attractive potential therapeutic targets. Curr Pharm Des 2020;26:3955-72.
16. Vlachopoulos C, Rokkas K, Ioakeimidis N, Stefanadis C. Inflammation, metabolic syndrome, erectile dysfunction, and coronary artery disease: common links. Eur Urol 2007;52:1590-600.
17. Voreades N, Kozil A, Weir TL. Diet and the development of the human intestinal microbiome. Front Microbiol 2014;5:494.
18. Menni C, Hernandez MM, Vital M, Mohney RP, Spector TD, Valdes AM. Circulating levels of the anti-oxidant indolepropionic acid are associated with higher gut microbiome diversity. Gut Microbes 2019;10:688-95.
19. Breuninger TA, Wawro N, Breuninger J, Reitmeier S, Clavel T, Six-Merker J, et al. Associations between habitual diet, metabolic disease, and the gut microbiota using latent Dirichlet allocation. Microbiome 2021;9:61.
20. Olivares PDSG, Pacheco ABF, Aranha LN, Oliveira BDS, Santos AA, Santos PCMD, et al. Gut microbiota of adults with different metabolic phenotypes. Nutrition 2021;90:111293.
21. Wei J, Zhang Y, Dalbeth N, Terkeltaub R, Yang T, Wang Y, et al. Association between gut microbiota and elevated serum urate in two independent cohorts. Arthritis Rheumatol 2022;74:682-91.
22. Meyer K, Lulla A, Debroy K, Shikany JM, Yaffe K, Meirelles O, et al. Association of the gut microbiota with cognitive function in midlife. JAMA Netw Open 2022;5:e2143941.
23. Suez J, Zmora N, Segal E, Elinav E. The pros, cons, and many unknowns of probiotics. Nat Med 2019;25:716-29.
24. Tang R, Jiang Y, Tan A, Ye J, Xian X, Xie Y, et al. 16S rRNA gene sequencing reveals altered composition of gut microbiota in individuals with kidney stones. Urolithiasis 2018;46:503-14.
25. Thompson RS, Gaffney M, Hopkins S, Kelley T, Gonzalez A,

Bowers SJ, et al. Ruminiclostridium 5, Parabacteroides distasonis, and bile acid profile are modulated by prebiotic diet and associate with facilitated sleep/clock realignment after chronic disruption of rhythms. Brain Behav Immun 2021;97:150-66.

26. Vich Vila A, Imhann F, Collij V, Jankipersadsing SA, Gurry T, Mujagic Z, et al. Gut microbiota composition and functional changes in inflammatory bowel disease and irritable bowel syndrome. Sci Transl Med 2018;10:eaap8914.