Effect of Electroacupuncture on gut Microbiota in Participants with Knee Osteoarthritis

Tianqi Wang
Beijing University of Chinese Medicine https://orcid.org/0000-0003-0508-9481
Tian-Qi Wang
Beijing University of Chinese Medicine
Jing-Wen Yang
Beijing University of Chinese Medicine
Guang-Xia Shi
Beijing University of Chinese Medicine
Li-Qiong Wang
Beijing University of Chinese Medicine
Ling-Ru Li
Beijing University of Chinese Medicine
Hui Hu
Beijing University of Chinese Medicine
Zhi-Shun Liu
China Academy of Chinese Medical Sciences Guanganmen Hospital
Jun Wang
Beijing University of Chinese Medicine
Yong Yuan
Chinese academy of traditional Chinese medicine
Wen-Rui Jia
Beijing University of Chinese Medicine
Hua Li
Beijing University of Chinses Medicine
Xin-Wei Wang
China Academy of Chinese Medical Sciences Guanganmen Hospital
Bin Wu
Beijing University of Chinese Medicine
Cun-Zhi Liu (lcz_tg@126.com)
https://orcid.org/0000-0001-8031-5667

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Abstract

Objective A close relationship between knee osteoarthritis and gut microbiota disorders has recently been described. Herein, we aim to investigate the effect of electroacupuncture on gut microbiota in participants with knee osteoarthritis.

Methods We conducted a study of 60 participants with KOA and 30 matched healthy controls. Sixty participants with KOA were allocated to either electroacupuncture (EA) group (n=30) or sham acupuncture (SA) group (n=30). EA group was treated by five obligatory acupoints and three adjunct acupoints. Eight non-acupoints that were separated from conventional acupoints or meridians were used for the SA group. Both groups received 24 sessions within eight weeks. Faecal microbial analyses were carried out after collecting stools at T_0 and T_8 weeks, and microbiomes were analyzed by 16S ribosomal RNA gene sequencing.

Results There was a significant structure difference of gut microbiota between KOA participants and healthy controls (HCs). Difference was observed in β-diversity analysis. Thirteen genera separated KOA from HCs, out of which *Escherichia-Shigella*, *Streptococcus* and *Eubacterium_hallii group* were increased in KOA participants, while *Bacteroides*, *Blautia*, *Bifidobacterium*, *Faecalibacterium*, *Subdoligranulum*, *Prevotella*, *Lactobacillus, Ruminococcus, Agathobacter* and *Megamonas* were decreased. After treatment, the amount of *Streptococcus* and *Escherichia-Shigella* in EA group had statistically decreased while *Ruminococcus* increased compared with SA group (all $P<0.05$). EA group decreased more in WOMAC pain, WOMAC total score (all $P<0.05$) and NRS score ($P=0.002$) than those in SA group at eight weeks. Adverse events were low and similarly distributed between groups. *Ruminococcus* and the total score of WOMAC was negatively correlated ($P=0.044$). *Streptococcus* was positively correlated with WOMAC total score ($P=0.044$) and *Escherichia-Shigella* was positively correlated with NRS score (P=0.024).

Conclusion This study presents a comprehensive landscape of gut microbiota in KOA. EA could reduce pain by regulating the intestinal flora of KOA participants. Dysbiosis was found in the gut microbiome in KOA and partially relieved by electroacupuncture. Our study suggests that gut microbiota could be a potential therapeutic target and diagnostic biomarker for KOA.

Introduction

Knee osteoarthritis (KOA) is one of the most common chronic conditions and forms of arthritis worldwide, which features as a protracted course of disease, especially among elderly patients [1-3]. KOA is the leading cause of lower extremity disability among older adults [4]. The prevalence of symptomatic KOA was higher in women (10.3%) compared with men (5.7%) [5]. With increasing life expectancy, osteoarthritis is anticipated to become the fourth leading cause of disability by the year 2020 [6].

The most common symptom of KOA patients is chronic knee pain, which leads to a decrease in the amount of activity, and the body of KOA patients is chronically inflammatory in the system. In normal humans, these intestinal microbes interact to maintain the stability of the intestinal microecology, so that

the body is in a healthy state. It will produce corresponding symptoms and even disease once the intestinal microstable is imbalanced [7]. Its imbalance is an important trigger for the increase of inflammation level in the body, and it is also involved in the occurrence of OA. According to clinical studies, patients who have adopted green-lipped mussels and glucosamine have improved the symptoms of KOA, and the structure of the intestinal flora has changed [8]. The role of the bacterial axis in the pathogenesis of OA suggests that metabolic inflammation may accelerate the pathological process of OA [9]. At the same time, follow-up studies suggested that the abundance of *Streptococcus* species was associated with increased knee pain, for this association was driven by local inflammation in the knee joint. The results indicate the microbiome is a possible therapeutic target for osteoarthritis-related knee pain [10].

Acupuncture, used in China and other Asian countries for the past 3,000 years [11], has the potential to manage chronic pain with effectiveness, especially among KOA [12]. It was found that acupuncture can improve the diversity of gut microbiome and the content of beneficial flora through different acupoints, so as to achieve the purpose of adjusting gut microbiome. It is also recognized that the gut microbiome can have a profound influence on systemic inflammation and chronic disease [13]. At present, there are few reports on whether acupuncture can improve knee joint function and reduce inflammatory response by regulating the amount and structure of intestinal flora in patients. In-depth discussion in this field has certain practical significance to reveal the internal mechanism of acupuncture to improve function and relieve pain of KOA.

Methods

Study cohort

There were three study groups. We first conducted a cross-sectional study of 60 KOA participants and 30 matched healthy controls. The 60 KOA participants were recruited from a large randomized clinical trial included 480 KOA participants. Faecal samples were collected twice before and after treatment by signing an informed consent. Sixty KOA participants were enrolled in a 1:1 allocation ratio to EA or SA group from five hospitals (Beijing Hospital of Traditional Chinese Medicine Affiliated to Capital Medical University, Dongzhimen hospital of Beijing university of Chinese medicine, Dongfang hospital of Beijing university of Chinese medicine, Guang'anmen hospital and Chinese academy of traditional Chinese medicine acupuncture hospital). Participants were recruited from the community through media, outpatient and poster paper advertisements at three hospital centers (April 2018 to January 2019). The inclusion criteria were as follows: age from 45 to 75; Kellgren-Lawrence [14] grade II or III (mild or moderate) radio graphically-confirmed KOA on one or both knees; duration of more than six months and pain intensity≥4 on a 10 numerical rating scale (NRS). The exclusion criteria included history of knee surgery or arthroscopy; pain in the knee caused by floating cartilage, joint effusion, inflammatory, malignant, or autoimmune disease; serious acute or chronic organic disease or mental disorder; pregnancy or breastfeeding; history of bleeding disorder. Participants were also not included if they had had acupuncture treatment or participated in other clinical trials in the past three months.

After a brief telephone screening, participants were scheduled to visit one of the three participating sites to sign an informed consent statement and undergo a brief rheumatologic examination (including radio graphic examination of affected knees) by an orthopedist. Eligibility to participate was determined initially by the research investigators at each site involved. Thirty participants matched healthy controls were also recruited at the same time. They were responsible for completion of the medical assessment and to check eligibility criteria. Eligibility data were entered on a secure online database and were monitored centrally before confirmation of study participation. The subject's demographic data and medical history were obtained at baseline.

Prior to the study, the study process was explained to participants during recruitment. Participants were informed that participation in the trial was absolutely voluntary and that they could withdraw from the trial at any time. In the event of their withdrawal, collected data would not be deleted and were used in the final analyses. Otherwise, research investigators had to comply with Good Clinical Practice (GCP) guidelines in the study. No participant was recruited without full, written informed consent being first obtained. The study was approved by the medical ethical review committee of Beijing Hospital of Traditional Chinese Medicine affiliated to Capital Medical University (2017BL-077-01) and was prospectively registered at ClinicalTrials.gov (Registration No. NCT 03366363) on 20 November 2017, prior to recruitment of the first participant.

Study treatment

All acupuncturists participant in the study have Chinese medicine practitioner licenses; they have at least three years clinical experience. Huatuo brand disposable, sterile steel needles (size 0.30×40 mm, manufactured by Suzhou Medical Appliance in Jiangsu, China) were used. Acupuncture treatment was semi-standardized: all participants were treated with a selection of local and distant points chosen by the acupuncturists according to the principles of traditional Chinese medicine. Participants were treated by use of 6-7 local acupuncture points which including ST34 (Liangqiu), ST35 (Dubi), ST36 (Zusanli), EX-LE2 (Heding), EX-LE5 (Neixiyan), GB33 (Xiyangguan), GB34 (Yanglingquan), SP9 (Yinlingquan), SP10 (Xuehai), LR7 (Xiguan), LR8 (Ququan), and 2-3 distal points which including GB31 (Fengshi), GB36 (Waiqiu), GB39 (Xuanzhong), GB41 (Zulinqi), ST40 (Fenglong), ST41 (Jiexi), LR3 (Taichong), BL60 (Kunlun), SP6 (Sanyinjiao), KI3 (Taixi). In the process of treatment, we used syndrome differentiation according to the participants' different situation. If pain occurred on the outside of the affected knee joint, gallbladder meridian points would be mainly selected. If pain occurred in front of the affected knee joint, stomach meridian points would be selected. If pain occurred in the interior of the affected knee joint, spleen, liver and kidney meridian points would be chosen. If pain occurred in the rear of the affected knee, bladder meridian points would be used. Needles were stimulated manually for 10 seconds to achieve "De Qi" sensation. Both EA and SA therapies consist of 24 sessions of 30 minutes, administered over eight weeks (usually three sessions per week).

EA group

An electrical apparatus (HANS-200A acupoint nerve stimulator, Nanjing Jisheng Medical Co, Ltd. production, density wave with frequency of 2/100Hz) would be then connected to the needles with alligator clips to stimulate the needles in pairs ST36-GB34 and ST34-SP10. The fixed current intensity was uniformly 0.2mA.

SA group

Eight non-acupoints that are separate from conventional acupoints or meridians will be used for the SA group. The schedule, electrode placements and other treatment settings are the same as for the EA group but with superficial skin penetration (2~3 mm in depth) and no electricity output or needle manipulation for de qi.

Outcome measures

Primary outcome measurement

The response rate was calculated according to a change of 50% from baseline in Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) total scores (pain, stiffness and function) at eight weeks.

Secondary outcome measurement

Knee pain were assessed by WOMAC pain subscale (five items, scored from 0 to 20) and NRS (scored from 0 to 10, 0 represent no pain, 10 represent unbearable pain). Stiffness and function were assessed by WOMAC stiffness (two items, scored from 0 to 8) and function subscale (17 items, scored from 0 to 68). The standard 12-item Short Form Health Survey (SF-12, 0-100, higher scores representing better quality of life), an abbreviated form of the SF-36 that yields the physical and mental health summary (PCS and MCS), were used to assess the health-related quality of life of the participants.

Sample collection and DNA extraction

Faecal samples were collected at the hospital and frozen at -80℃ within three hours after sampling. DNA extraction was performed using a QIAamp Fast DNA Stool Mini Kit (Qiagen, California, USA). The concentration of bacterial DNA was measured using Nano drop 2000 (Thermo Scientific, USA). Demographics and clinical variables were collected during the clinic visits.

16S ribosomal RNA gene sequencing

The V3-V4 region of the bacteria's 16S ribosomal RNA (rRNA) gene was amplified by PCR with barcode-indexed primers (338F and 806R), using FastPfu Polymerase. Amplicons were then purified by gel extraction (AxyPrep DNA Gel Extraction Kit, Axygen Biosciences, Union City, California, USA) and were quantified using QuantiFluor-ST (Promega, USA). The purified amplicons were pooled in equimolar concentrations, and paired-end sequencing was performed using an Illumina MiSeq instrument (Illumina, San Diego, California, USA).

Microbial analysis

The 16S rRNA sequencing data were processed using the Quantitative Insights Into Microbial Ecology platform (V.1.9.1). Sequencing reads were demultiplexed and filtered. Operational taxonomic units (OTUs) were picked at 97% similarity cut-off, and the identified taxonomy was then aligned using the Greengenes database (V.13.8). Chimeric sequences were identified and deleted. OTUs with a number of sequences<0.005% of the total number of sequences were removed from the OTU table. After filtering, an average of 34661 reads per sample was obtained (min: 23994; max: 42940). In addition, rarefaction was performed on the OTU table to prevent methodological artefacts arising from varying sequencing depth. Alpha-Diversity was measured by species richness from the rarefied OTU table. Beta-Diversity was estimated by computing unweighted UniFrac and was visualized with principal coordinate analysis. In efforts to dissect possible species for OTUs, we performed MegaBLAST search to align the reads of OTUs against reference sequences in the National Center for Biotechnology Information (NCBI) 16S rRNA database.

Statistical analysis

The results were analyzed using the SPSS software (SPSS V.12.0 KO for Windows). A value of $P<0.05$ would be considered statistically significant. Measurement data were expressed by mean and standard deviation (SD), enumeration data expressed as a percentage. Mean between-group differences and two-sided 95% CI are also presented to assess superiority.

All statistical analyses were performed using R packages (V.2.15.3). For the comparison of continuous variables, Mann-Whitney U test (Kruskal-Wallis test for more than two groups) and One-way ANOVA test were used. For correlation analysis, spearman's rank test was performed. Multiple hypothesis tests were adjusted using Benjamin and Hochberg false discovery rate (FDR), and significant association was considered below an FDR threshold of 0.05.

Results

Characteristics of treatment groups at baseline are presented in Table 1. There were no significant differences among the treatment conditions on any baseline demographic and clinical characteristics (all $P>0.05$).

Primary outcome in WOMAC Index at the end of week 8

After eight weeks of treatment, the response rates (a change of 50% from baseline in WOMAC total scores) were 60% for EA group and 30% for SA group by intention-to-treat analysis in Table 2. Analysis showed a statistically significant difference in response rate between the two groups ($P<0.05$).

Secondary outcomes in WOMAC Index

After eight weeks of treatment in table 3, the total WOMAC score, WOMAC pain subscale, WOMAC stiffness subscale, WOMAC function subscale, and NRS score of the knee joint in the electroacupuncture group (n=30) and the sham acupuncture group (n=30) all decreased to varying degree. The quality of life score of SF-12 was improved both physically and mentally. At week 8, the NRS score of electroacupuncture group was significantly lower than that of the sham group (P=0.002, P<0.01), the total WOMAC score and WOMAC pain score of electroacupuncture group were lower than that of the sham group (all P<0.05).

Adverse events were uncommon and did not occur more frequently in either group. There was no significant bleeding in either group.

During this trial, no participants took rescue medicine in both groups.

Changes of faecal microbial diversities in KOA

As shown in the Figure 1, the Sob index calculation at the genus level showed that there was no significant difference in Alpha-diversity of intestinal flora between KOA groups and healthy controls. The composition analysis chart of intestinal bacteria group manager in the groups of KOA participants and healthy controls shows that the horizontal axis represents the first principal component (contribution rate is 18.26%) and the vertical axis represents the second principal component (contribution rate is 12.79%). By comparing the distance of each point, healthy controls were arranged and clustered in the PCA diagram, indicating that there were significant differences between the intestinal bacteria of KOA participants and healthy controls.

Correlation analysis of environmental factors

According to Figure 2, compared with healthy subjects in Phylum level, the ranking of environmental factors affecting the incidence of KOA participants was: C (course of disease), > T (body mass index), > A (age), > X (gender). The horizontal axis represents the first principal component (contribution rate is 5.19%) and the vertical axis represents the second principal component (contribution rate is 3.11%) on RDA/CCA analysis. We know that the main environmental factor affecting the incidence of KOA participants was the course of disease.

Disease status discrimination with the 13-genera microbiome signature

On the genus level in One-way ANOVA test bar plot, 13 gut microbiota were found to have a changed relative abundance between KOA participants and Healthy controls (all P<0.05), in which *Escherichia-Shigella* (P=0.019), *Streptococcus* (P=0.028) and *Eubacterium_hallii group* (P=0.015) were increased in KOA participants while *Bacteroides* (P=0.011), *Blautia* (P=0.029), *Bifidobacterium* (P=0.013), *Faecalibacterium* (P=0.011), *Subdoligranulum* (P=0.020), *Prevotella* (P=0.010), *Agathobacter* (P=0.014), *Lactobacillus* (P=0.012), *Ruminococcus* (P=0.010) and *Megamonas* (P=0.014) were decreased (Figure 3(A)).

Electroacupuncture treatment partially ameliorates gut dysbiosis of KOA

After treatment, the amount of *Streptococcus* (P=0.039) in EA group had statistically decreased while *Agathobacter* (P=0.006), *Faecalibacterium* (P=0.029), *Prevotella* (P=0.031) and *Ruminococcus* (P=0.030) were increased on the genus level in One-way ANOVA test bar plot. No differences were observed in SA group (all P>0.05) (Figure 3(B) and (C)).

Correlations between the gut microbe and KOA clinical indices

At the genus level, *Bacteroides* were negatively correlated with WOMAC stiffness (P<0.01), and negatively correlated with WOMAC total score, function and pain (all P<0.05). *Escherichia-shigella* was positively correlated with NRS score (P<0.05), and negatively correlated with SF-12 psychology (P<0.001). *Streptococcus* and WOMAC total score were positively correlated (P<0.05). *Faecalibacterium* was negatively correlated with WOMAC, WOMAC stiffness and pain (all P<0.01), negatively correlated with WOMAC function and NRS score (all P<0.05), and positively correlated with SF-12 psychology (P<0.05). *Agathobacter* was significantly negatively correlated with WOMAC total score and pain (all P<0.001), and significantly negatively correlated with NRS score, WOMAC function and stiffness (all P<0.01). The total scores of *Ruminococcus* and WOMAC were negatively correlated (P<0.05, Figure 4).

Correlation between specific microbiota in KOA patients and response rate

The content of *Bacteroides*, a beneficial bacterium closely related to the clinical efficacy of KOA patients, in the intestinal tract of the patients whose WOMAC total score decreased by more than 50% in the electroacupuncture group was higher than that of the patients whose WOMAC total score decreased by less than 50% in the electroacupuncture group, and the difference was statistically significant (P=0.036).

COG pathway analysis

According to the function prediction of COG, it can be seen that: Carbohydrate transport and metabolism, Amino acid transport and metabolism, General function prediction only, Transcription, Replication Recombination and repair, Cell wall/membrane/envelope biogenesis, Translation, ribosomal structure and biogenesis , Inorganic ion transport and metabolism, Energy production and conversion, Signal transduction mechanisms were the top ten metabolic functions of gene number in healthy controls and patients with knee osteoarthritis, and healthy controls are slightly higher than KOA patients in abundance genes among these ten functions.

Discussion

To our knowledge, this study was first a clinical trial, aimed to evaluate the effectiveness and safety of electroacupuncture following a randomized, controlled design. In this randomized clinical trial, the results suggested that EA was more effective than SA for pain associated with KOA in eight weeks. In our study, the primary outcome measurement was the response rate, which was calculated by the proportion of patients whose total score of WOMAC decreased by 50%. After treatment, the response rate of EA group

was 60% and that of SA group was 30%, illustrating that the response rate of EA was 30% higher than that of SA. We analyzed the effect of EA as a complementary therapy of knee osteoarthritis, with respect to relieve pain during treatment compared two groups in secondary outacome measurements. The treatment of acupuncture might modify the composition of the microbiome which is consistent with the results of the previous study [15].

In our current study, we applied a 16S rRNA sequencing approach to a unique KOA cohort in addition to a study of a subset of participants analyzed before and after acupuncture therapy, demonstrating that KOA was associated with altered composition and function of gut microbiota. In the prospective study, two months of acupuncture use was found to partially mitigate the microbial dysbiosis. The gut microbiome of KOA reflected a significant shift in the overall microbial diversity. No obvious differences were found within-individual or between-individual on Alpha-diversity in study of microbiomes due to a small cohort of KOA participants whereas differences could be found on Beta-diversity, which was different from healthy controls. Therefore, our findings might optimize a large cohort to ensure adequate statistical power for capturing microbial diversities.

Interestingly, the KOA-enriched genera were relatively rare in the normal human gut. It is recognized that potentially pathogenic bacteria that are normally present in low abundance can thrive and contribute to inflammation or autoimmunity under inflammatory conditions. An uncommon genus in the family of *Proteobacteria* revealed the most significant association with KOA patients while the major genera in the samples of healthy controls belong to *Firmicutes* family, indicating that gut microbiota signature can be used to discriminate between KOA participants from healthy controls. Obesity-mediated gastrointestinal-microbiome changes are postulated to affect low-grade systemic and local inflammation in OA [16,17]. Nevertheless, in our study the effect of *Streptococcus* on knee WOMAC pain is not fully driven by BMI. This suggests a direct role for the gastrointestinal microbiome in OA related knee pain and inflammation [18]. We postulate that greater *Streptococcus* abundance leads to higher knee WOMAC pain through local joint inflammation. Our observation is in line with the previous study that *Streptococcus* abundance was significantly associated with effusion severity in the knee joints[10]. This leads to believe that *Streptococcus* might also be involved in other inflammatory joint pain disorders. Involved in the occurrence of inflammation, and the inflammatory response is closely related to the occurrence of OA [19]. The rate of inflammatory response in OA population is much greater than that in non-OA population [20]. It is now established that activation of inflammation in obesity is also caused by shifts in the gut microbiome [21, 22]. Particularly, *Faecalibacterium*,which produces high amounts of butyrate and exhibits anti-inflammatory effects, has shown potential as a probiotic in the treatment of type 2 diabetes [23] and hence may be considered as a crucial ecosystem service providers for human health.

Primarily diet, the composition of the gut microbiome, which is dependent on both intrinsic and extrinsic factors [24], plays a large role in digestion and shaping the immune system [25]. Many anaerobic intestinal microbiota such as species in *Ruminococcus*, *Bacteroides*, *Blautia*, *Bifidobacterium*, *Faecalibacterium* and *Subdoligranulum* are known to produce short-chain fatty acids (SCFA) by fermentation of dietary fibres [26,27,28]. SCFA is known to exert a beneficial effect on health through the

anti-inflammatory effects. Decreased production of SCFA by microbiota raises luminal oxygen concentration in mice, resulting in the expansion of facultative anaerobes [29]. Acupuncture is considered as a secure and powerful tool for repelling pain [30]. We speculate that the possibility that improvements in EA may through shaping the structure of gut microbiome, especially through reducing *Streptococcus* abundance, thereby achieving rapid reduction in signs and symptoms of osteoarthritis of the knee. Moreover, mechanisms on regulating intestinal flora may be explained that stimulation of EA increases the content beneficial bacterium of *Ruminococcus*, *Prevotella*, *Bacteroides, Faecalibacterium* and *Agathobacter*, which microbiota have been shown to produce different amounts and profiles of SCFA from the same carbohydrate substrates [31]. SCFA-producing bacteria with elevated fecal SCFA concentrations may promote the energy intake from fibers, inhibit opportunistic pathogens and protect the hosts against inflammation and colonic diseases [32].

According to TCM theory, acupuncture produces therapeutic effects by the retention of needles at acupoints through acquiring“Deqi”manually. Deqi is a specific needle sensation, referring to the response to stimulations such as the thrusting, lifting, or rotating of the needle after insertion. It has been asserted to be a criterion to determine the appropriateness of acupuncture stimulation [33]. According to the syndrome differentiation of channels and collaterals, the lesions of 60 KOA patients in this study were mainly distributed on 4 channels, among which the lesions with the syndrome of Foot Yangming Stomach Meridian were the most common (44 cases), accounting for 73.33%. In the selection of acupoints, the frequency of Dubi and Zusanli acupoints in Foot Yangming Stomach meridian was the highest. In the electroacupuncture group, both Dubi and Zusanli acupoints were selected for acupuncture treatment. Dubi acupoints is commonly known as "external knee eye", which is a local point of the knee joint, a Tongli joint, which is mainly for knee pain and has unfavorable joint flexion and extension [34]. Zusanli (stomach meridian, ST36) is an acupoint located at 3 cm below the knee joint on the anterior aspect of the leg. ST36, based on the TCM theory, could harmonize the spleen and stomach, tonify and replenish the middle qi, unblock the meridian and free the collateral vessels, disperse wind and transform dampness, reinforce the healthy qi, and eliminate the pathogenic factors. Adding other acupoints, in TCM theory, could synergize to protect the multiple organs and systems, such as cardiac, brain, lungs, kidneys, and liver [35]. In the past decade, studies reported the potential of acupuncture at ST36 for infectious diseases due to its numerous effects, such as anti-inflammatory, improving micro-circulatory disturbance and accelerating the recovery of various gastrointestinal disorders [36]. This study suggested that acupuncture at ST36 may increase the number of beneficial bacteria. After beneficial adjustment, intestinal flora improved intestinal barrier function, reduces inflammation and oxidative stress, and then inhibited the occurrence of chronic low-grade inflammation, which indirectly reduced the degree of joint pain.

Although our investigations attempt to provide a comprehensive insight into the potential contribution of the gut microbiome in KOA, there are several limitations to be addressed in future studies. First, we were not able to observe variations among participants at different disease stages, probably due to a relatively small number of participants at moderate or advanced stage, as most participants in our cohort were Kellgren-Lawrence grade II or III (mild or moderate) radio graphically-confirmed KOA on one or both knees.

Second, our pathway analysis was based on the inferred meta-genome from 16S rRNA sequence. Although inference of a metagenome approach (PICRUSt) has been commonly used in 16S rRNA studies, short-gun sequencing for metagenomics and metatranscriptomics may reveal more accurate microbial community composition and function [37]. Third, the small sample size in this exploratory trial increases the possibility of a type II error (i.e., a real effect of acupuncture being missed because of insufficient power). For future trials, sample size estimation could be calculated, for example, using PASS software, based on the data derived from this trial. Fourth, we did not validate the characteristics of the flora in an independent set of patients with KOA, and further investigation is needed to verify in a small independent cohort. Nonetheless, our comprehensive investigation of the gut microbiome in KOA reveals compositional and functional dysbiosis in participants that are partially alleviated by electroacupuncture. The identified KOA microbial signature needs further validation in larger cohorts.

Conclusion

In conclusion, our study suggests that structural alterations of gut microbiota, induced by electroacupuncture (EA), are associated with the anti-inflammatory effects of EA. In particular, this treatment reduced the number of pathogenic bacteria, such as *Streptococcus* and increase the content of beneficial bacteria including *Faecalibacterium* and *Agathobacter* in the gut. Although it is still unclear whether changes of gut microbiota by EA directly contribute to the improvement of relieving pain in KOA, our clinical study provides circumstantial evidence that gut microbiota might be involved.

Declarations

Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Availability of data and materials

To view data and materials please visit the journal online (https://cloud.majorbio.com/project/index).

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Author contributions

Study concept and design: C.-Z. Liu, T-Q. Wang, and L.-R. Li. Acquisition, analysis, or interpretation of data: C.-Z.Liu, L.-Q.Wang, J.-W. Yang, G.-X.Shi, Z.-S. Liu, H. Hu, J. Wang, and Y. Yuan. Drafting of the manuscript: T.-Q. Wang, J.-W.Yang, L.-Q. Wang, and W.-R. Jia. Critical revision of the manuscript for important intellectual content: J.-W. Yang and L.-Q. Wang. Obtaining of funding: C.-Z. Liu. Study supervision: C.-Z. Liu.

Ethics approval

Beijing Hospital of Traditional Chinese Medicine is affiliated with Capital Medical University. Patient informed consent will be obtained.

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Tables

Table 1: Demographic and baseline characteristics

Characteristic	EA group (n=30)	SA group (n=30)	P Value
Age (years), mean ± SD	64.73 ± 5.39	66.10 ± 7.42	0.418
Women, n (%)	19 (63.3%)	20(66.7%)	0.787
BMI (kg/m2), mean ± SD	26.04 ± 2.92	25.86 ± 4.02	0.846
Duration of disease(months), mean ± SD	85.73 ± 74.15	104.37 ± 96.45	0.475
Education background (years), mean ± SD	12.07 ± 2.97	10.87 ± 2.87	0.117
WOMAC total points (0-96), mean ± SD	34.62 ± 10.39	33.96 ± 11.26	0.12
Physical health (SF-12)a, mean ± SD	33.19 ± 6.84	33.58 ± 5.65	0.798
Mental health (SF-12)a, mean ± SD	41.62 ± 6.95	42.41 ± 8.24	0.704
NRS score(0-10), mean ± SD	6.00 ± 1.34	6.13 ± 1.33	0.7
Bristol classification of stools	4.17 ± 0.65	4.57 ± 0.57	0.419

EA group: Electroacupuncture group; SA group: Sham acupuncture group

BMI：Body mass index was calculated as weight in kilograms divided by height in meters squared;

WOMAC: Western Ontario and McMaster Universities Arthritis Index;

SF-12: Medical Outcomes Study 12-item Short-Form Health Survey;

NRS：Numerical rating Scale/Score.

[a] Higher values indicate better status.

Table 2: Response rate in WOMAC Index between EA and SA group at 8 weeks

Outcome	Intention-to-treat		
	EA group (n=30)	SA group (n=30)	P Value
Effective, n (%)	18 (60)	9 (30)	0.037
Non-effective, n (%)	12 (40)	21(70)	

EA group: Electroacupuncture group; SA group: Sham acupuncture group

Table 3: Outcomes at 4, 8, 16 and 26 weeks (intention-to-treat analysis)

Outcome	EA group (n=30)	SA group (n=30)	*P* value
WOMAC total points			
Week 4	24.97 ± 12.26	23.50 ± 12.58	0.649
Week 8	14.77 ± 7.11	20.23 ± 12.62	0.043
Week 16	19.33 ± 8.09	19.17 ± 11.07	0.947
Week 26	20.27 ± 8.06	19.33 ± 9.31	0.680
WOMAC pain			
Week 4	4.87 ± 2.91	4.83 ± 2.98	0.965
Week 8	2.73 ± 1.72	4.17 ± 2.98	0.026
Week 16	3.73 ± 1.87	4.13 ± 2.71	0.509
Week 26	3.63 ± 1.67	4.03 ± 2.24	0.436
WOMAC stiffness			
Week 4	2.03 ± 1.22	1.97 ± 1.33	0.840
Week 8	1.40 ± 1.00	1.87 ± 1.28	0.121
Week 16	1.50 ± 0.78	1.93 ± 1.11	0.085
Week 26	1.63 ± 0.96	1.73 ± 1.08	0.707
WOMAC function			
Week 4	18.07 ± 8.94	16.70 ± 9.15	0.561
Week 8	10.63 ± 5.88	14.20 ± 9.65	0.089
Week 16	14.10 ± 5.96	13.00 ± 7.68	0.538
Week 26	15.00 ± 6.58	13.57 ± 6.52	0.400
NRS score (0–10)			
Week 4	4.00 ± 1.82	4.10 ± 1.75	0.829
Week 8	2.00 ± 1.60	3.83 ± 2.53	0.002
Week 16	3.30 ± 1.20	3.70 ± 1.93	0.340
Week 26	3.13 ± 1.36	3.50 ±1.70	0.359
SF-12 physical score			

Week 4	34.63 ± 6.64	33.86 ± 6.69	0.647
Week 8	37.13 ± 5.57	35.22 ± 6.31	0.220
Week 16	37.05 ± 5.40	35.22 ± 6.85	0.241
Week 26	36.05 ± 4.31	35.33 ± 7.09	0.615
SF-12 mental score			
Week 4	43.29 ± 6.22	43.04 ± 5.37	0.868
Week 8	43.86 ± 7.60	43.50 ± 6.74	0.856
Week 16	43.92 ± 5.96	42.90 ± 7.09	0.556
Week 26	44.09 ± 6.90	42.84 ± 6.41	0.484

EA, electroacupuncture; SA group: Sham acupuncture group; WOMAC, Western Ontario and McMaster Universities osteoarthritis index; SF-12, Medical Outcomes Study 12-item short-form health survey; NRS, Numerical rating scale/score.

Figures

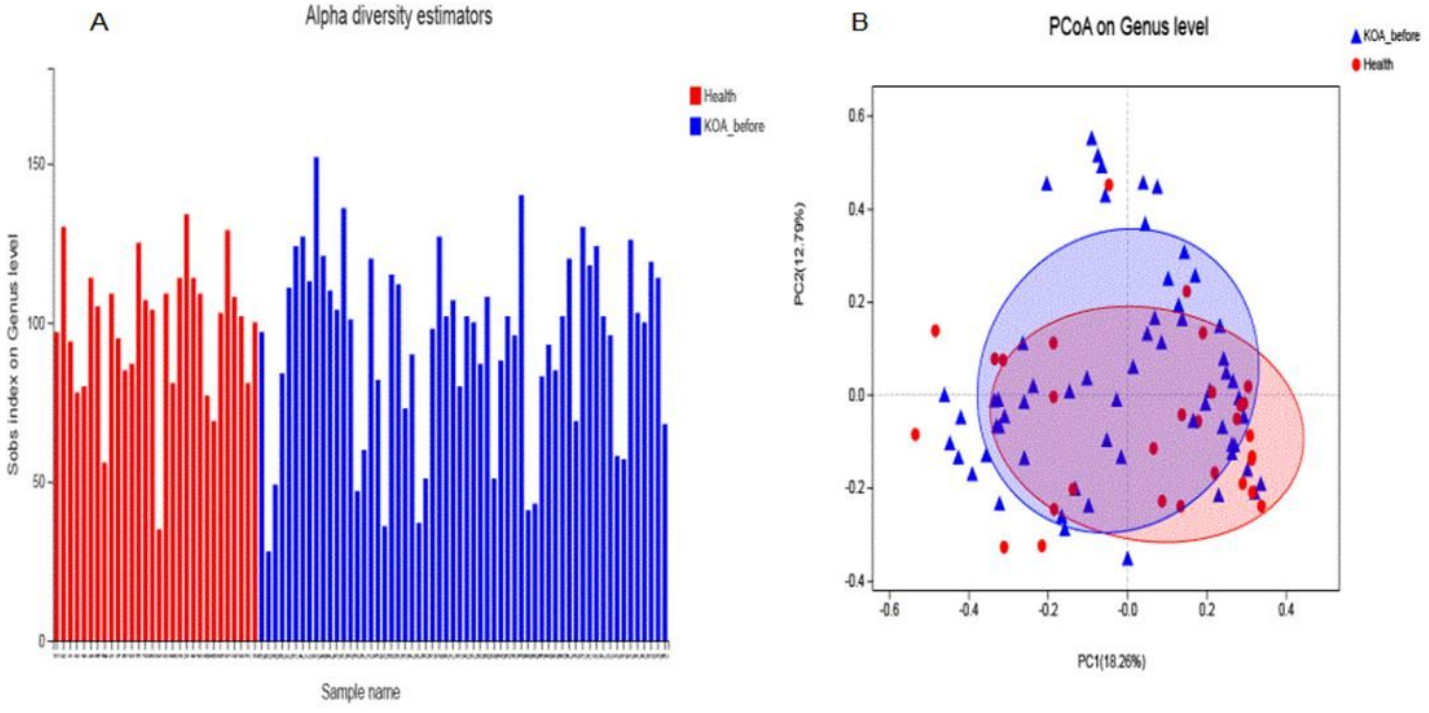

Figure 1

Changes of faecal microbial diversities in knee osteoarthritis (KOA) (n=60) compared with healthy controls (n=30). (A) Alpha-Diversity, illustrated by microbiota richness (number of observed operational

taxonomic unit (Genus)). (B) Principal coordinate analysis (PCoA) of unweighted UniFrac analysis demonstrated that individuals with KOA were significantly different from healthy controls (Beta-Diversity).

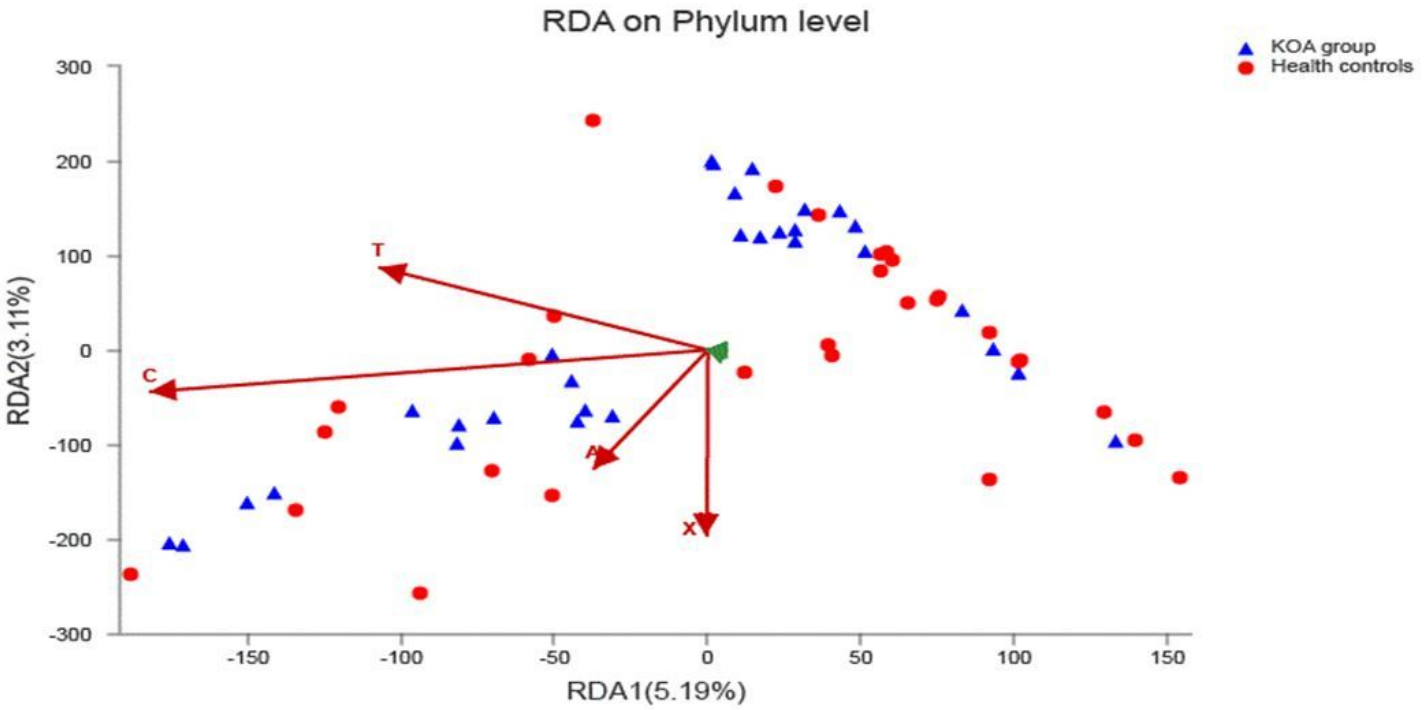

Figure 2

The four environmental factors were C – course of disease; T – body mass index; A – the age; X – gender, the length of the environmental factor arrow can represent the degree of environmental factor's influence on the species data.

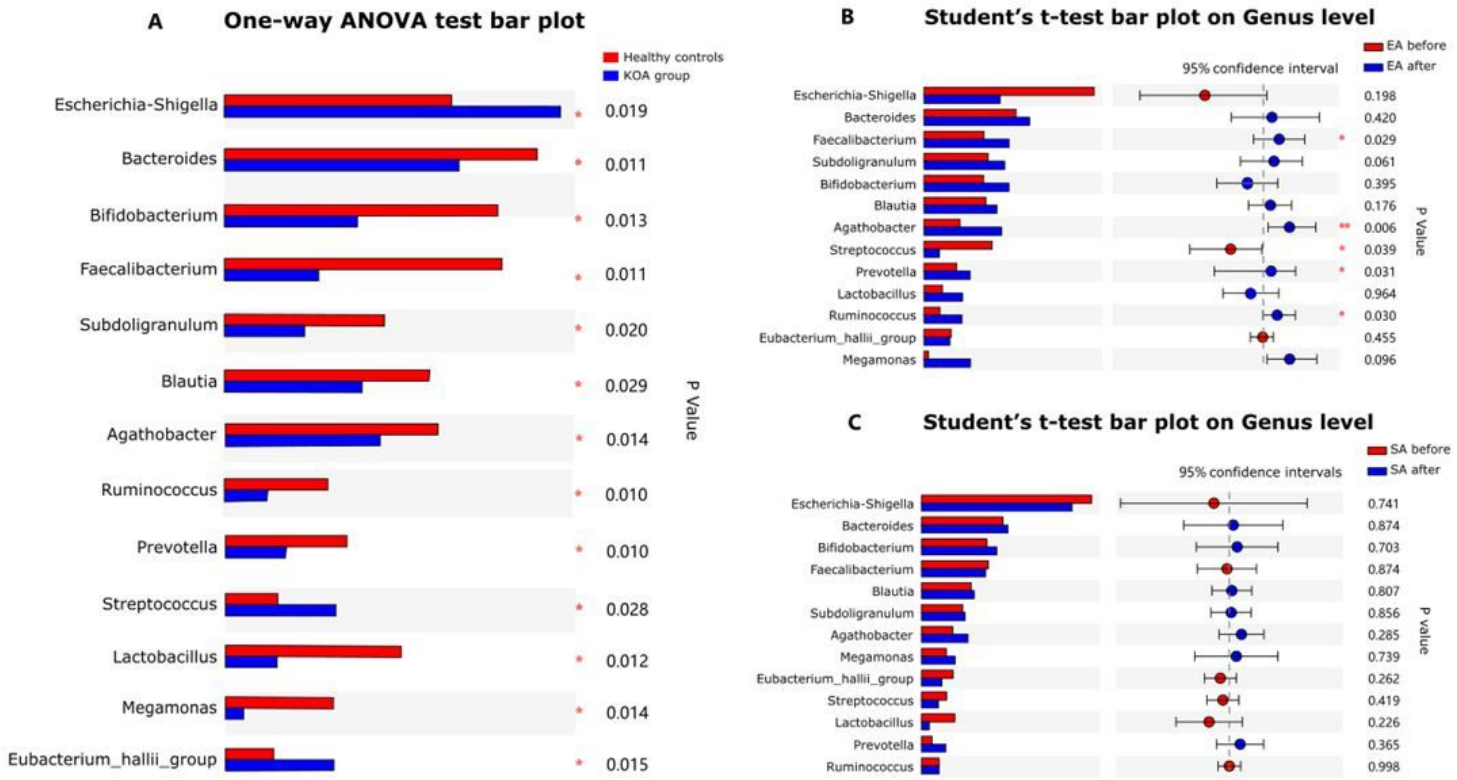

Figure 3

Microbiome alterations at the genus level in knee osteoarthritis (KOA). (A) The relative abundance of 13 genera were significantly different between KOA participants (n=60) and healthy controls (n=30) after correcting for confounding variables. (B) The microbiome composition was partially mitigated in electroacupuncture (EA) group. Five of the KOA-associated genera were reversed in KOA after EA treatment, compared with baseline (n=30, * P <0.05, ** P <0.01, paired Student's t-test). (C) After sham acupuncture (SA) treatment, no differences were found in the KOA-associated genera (n=30, all P>0.05, Student's t-test).

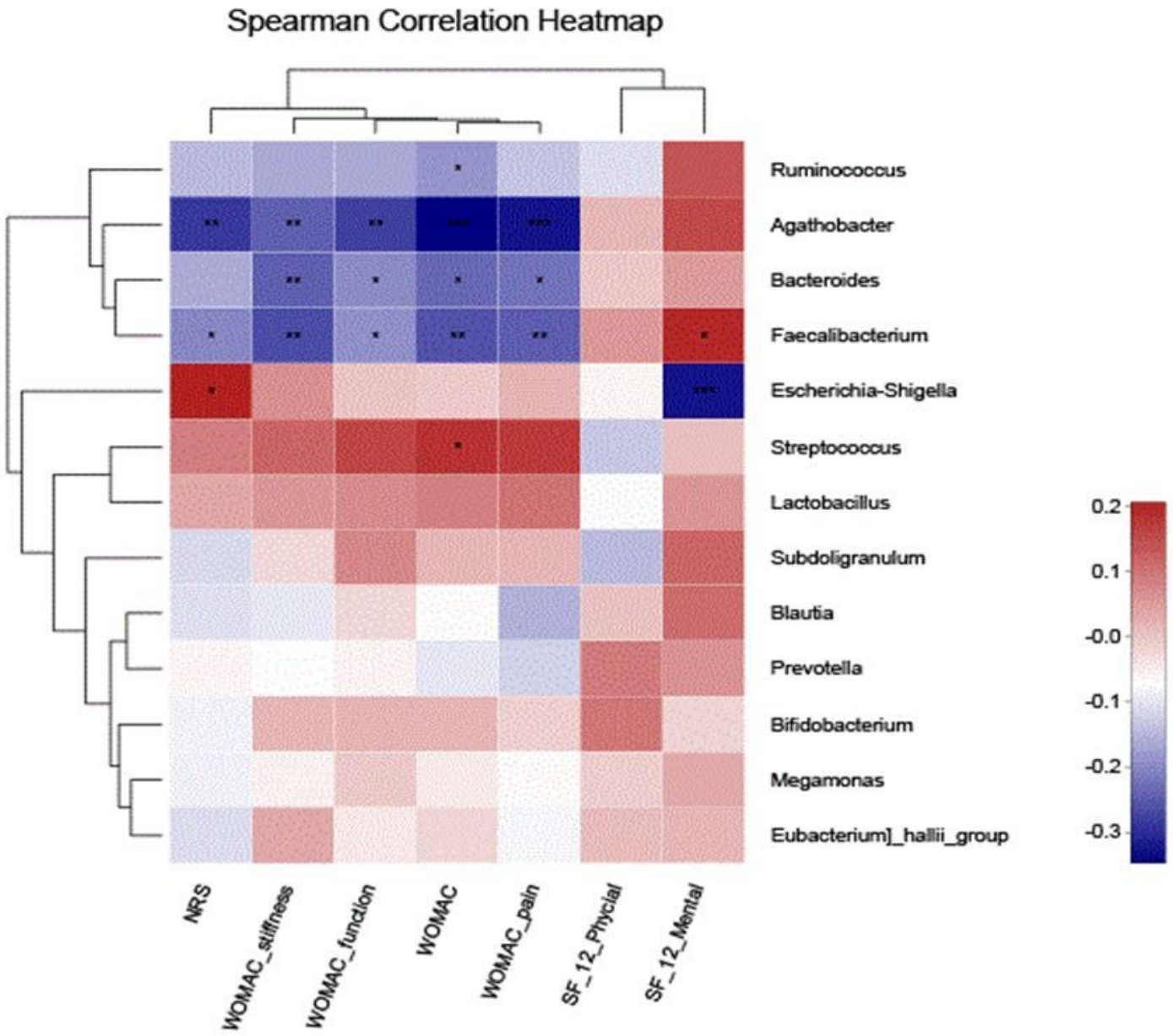

Figure 4

Spearman correlations between the 13 KOA-associated genera and clinical indices that included NRS: Numerical rating scale/score; WOMAC: Western Ontario and McMaster Universities osteoarthritis index; SF-12: Medical Outcomes Study 12-item short-form health survey, *P<0.05, **P<0.01, ***P<0.001.

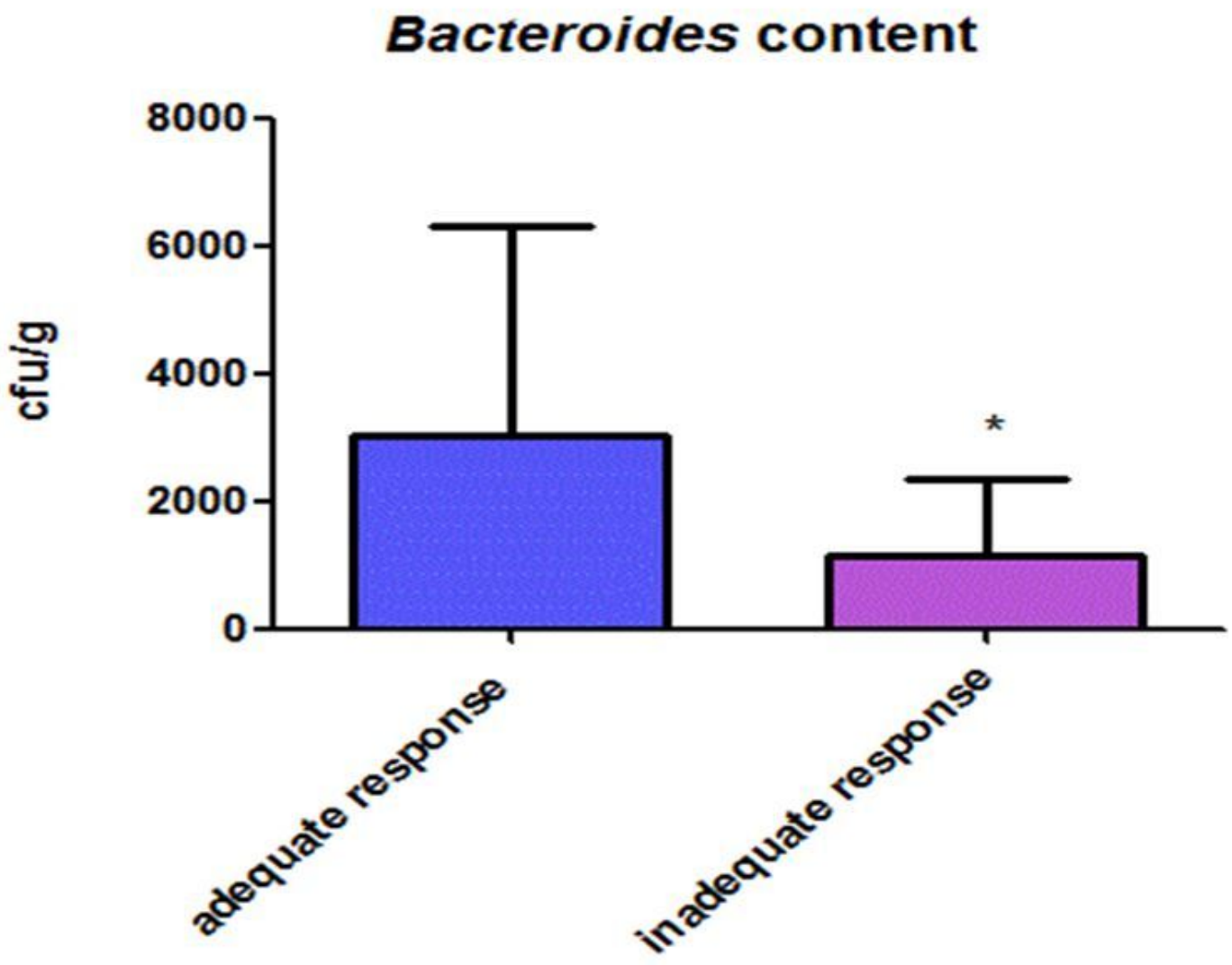

Figure 5

After EA treatment, the KOA-associated genera Bacteroides was more abundant in patients who demonstrated adequate response than in those with inadequate response (n=18 and 12, respectively; P=0.036, *P<0.05, Wilcoxon rank-sum test).

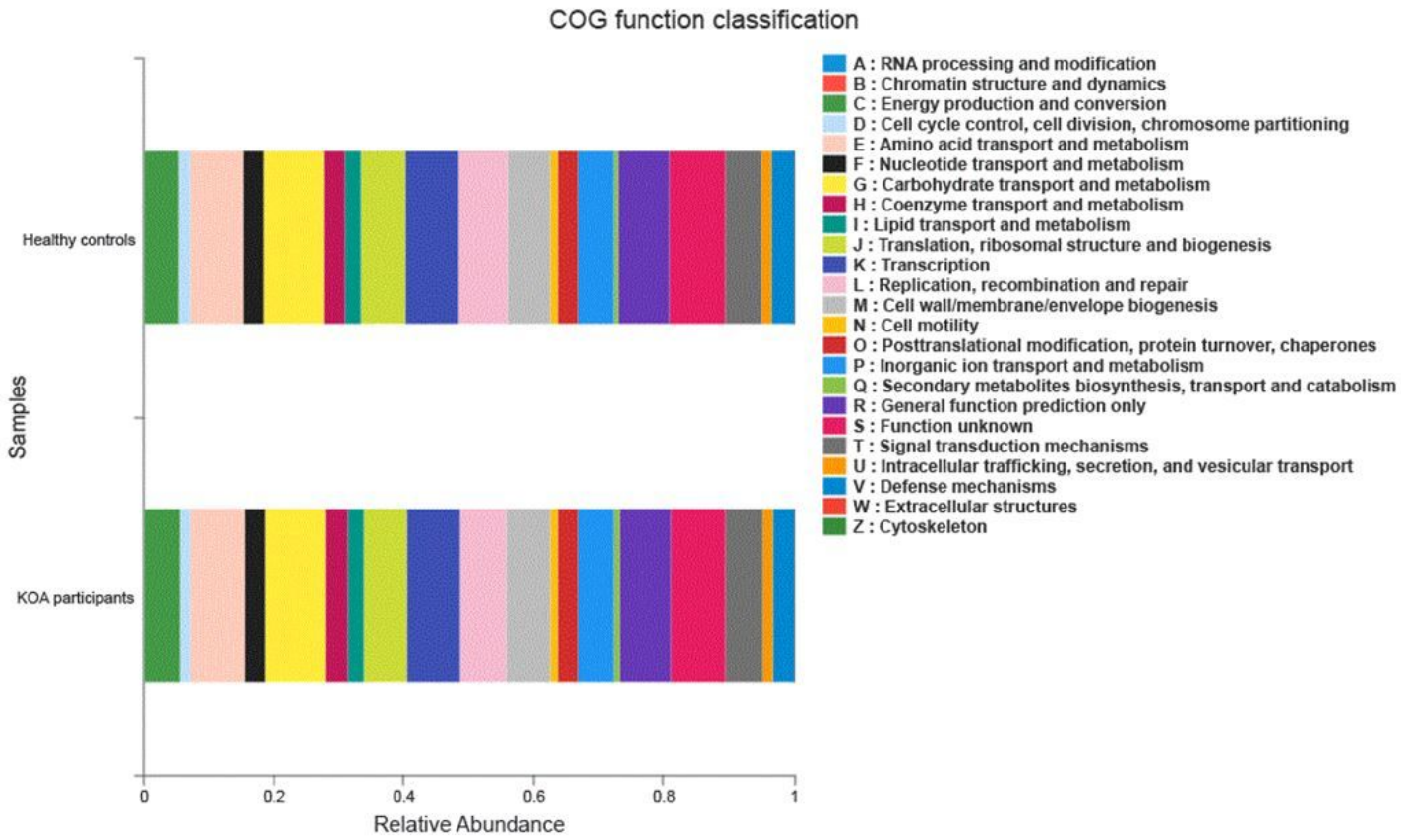

Figure 6

The differentially abundant predicted COG pathways in KOA participants (n=60) and healthy controls (n=30).