Journal of Translational Medicine

RESEARCH

Open Access

The *Lachnospiraceae*-butyric acid axis and its role in glucocorticoid-associated osteonecrosis

Mingbin Guo[1,2,3†], Shuai He[1,2,3†], Wei Song[1,2,3], Jianbin Mai[1,2,3], Xinwei Yuan[1,2,3], Yixuan Huang[1,2,3], Hongzhong Xi[1,2,3], Guangquan Sun[1,3], Yugen Chen[1,2,3], Bin Du[1,2,3*] and Xin Liu[1,3*]

Abstract

Glucocorticoids (GCs) are key inducers of osteonecrosis, yet not all patients treated with GCs develop glucocorticoid-associated osteonecrosis (GAON). The factors mediating this relationship are unclear. Studies have shown that gut microbiota and their metabolites influence bone metabolism, but their role in GAON is unclear. This study aimed to explore the connection between GAON and gut microbiota. Through bidirectional Mendelian randomization analysis, we identified 14 gut microbial taxa, including *Lachnospiraceae* (IVW, $P=0.011$), associated with GAON. RNA-seq analysis revealed that GAON differentially expressed genes (DEGs) were enriched for intestinal inflammatory response mechanisms. We then compared patients who developed GAON (17 cases), those who did not (GAnON, 15 cases), and those untreated with GCs (Blank, 15 cases) for gut microbiota composition, short-chain fatty acids (SCFAs), and serum inflammatory factors. Our findings indicated a decrease in *Lachnospiraceae* abundance (GAON 17.13%, GAnON 12.51%, Blank 24.52%) in GC-treated patients. Serum inflammatory factors (IL-17 A, IL-33, and TNF-α) associated with GAON (59.603 ± 12.147, 89.337 ± 20.714, 42.584 ± 9.185) showed significant differences between Blank (1.446 ± 0.683, 11.534 ± 4.705, 4.682 ± 1.48) and GAnON (25.353 ± 8.181, 32.527 ± 7.352, 12.49 ± 3.217) groups, with a negative correlation between these factors and *Lachnospiraceae* levels. Butyric acid levels in SCFAs varied among groups ($P<0.01$) and correlated with *Lachnospiraceae* and inflammatory factors. Controlled experiments in GAON rats demonstrated butyric acid's osteoprotective role in GAON development ($P<0.01$). In conclusion, our study suggests that reduced *Lachnospiraceae* and butyric acid levels, along with increased inflammation due to GCs use, contribute to GAON. Butyric acid may mediate the effects of *Lachnospiraceae* and inflammation. Butyrate supplementation could potentially reduce GAON incidence, offering a novel approach for its clinical management.

Keywords Glucocorticoid associated osteonecrosis, Gut microbiota, *Lachnospiraceae*, Inflammatory immune response, Butyric acid, Mendelian randomization, Metagenomics, Host-microbiome interactions

†Mingbin Guo and Shuai He contributed equally to this work.

*Correspondence:
Bin Du
fsyy00608@njucm.edu.cn
Xin Liu
liuxinsurg@163.com

[1]Department of Orthopedics, Affiliated Hospital of Nanjing University of Chinese Medicine, 155 Hanzhong Road, Nanjing, Jiangsu Province 210029, China
[2]Nanjing University of Chinese Medicine, Nanjing 210029, China
[3]Jiangsu Province Hospital of Chinese Medicine, Nanjing 210029, China

Introduction

Due to its excellent anti-inflammatory, anti-allergic and immunosuppressive effects, GC is widely used in the treatment of various inflammatory diseases, allergic diseases, rheumatic and autoimmune diseases. However, the use of GC has also brought many adverse events. GAON occures in 9–40% of patients receiving long-term treatment [1, 2]. GC is now recognized as the most essential trigger for non-traumatic osteonecrosis. GAON causes severe joint pain and activity limitation, and usually affects adults under 50 years old. It is a leading cause of joint replacement surgeries among young individuals [3]. The joint most commonly affected by GAON is the hip (femoral head), followed by the knee and shoulder (humeral head) [4]. Various theories have been proposed by scholars regarding the pathogenesis of GAON, including lipid metabolism disorder, hemodynamic disorder, increased intraosseous pressure, osteocyte apoptosis, gene polymorphism, and immune factors [5], yet the exact pathogenesis remains unclear.

The gut microbiota, often referred to as the human body's second gene pool, contains a vast amount of information and exhibits significant polymorphism. Numerous recent studies have demonstrated that the gut microbiota and related metabolites play a pivotal role in regulating bone metabolism [6, 7]. Alterations in intestinal microbiota can lead to local inflammation and osteoporosis in several ways [8]. Infiltration of chronic inflammation, loss of bone mass and decreased bone strength may be factors associated with GAON. CHEN [9] et al. showed that transplantation of gut microbiota from normal mice to diseased mice attenuated GAON. Oral administration of *Lactobacillus animalis* also alleviated GAON in mice. Compared with conventionally reared mice, germ-free mice had greater mass of trabecular and cortical bone, and the expression of inflammatory cytokines was lower in the bone and bone marrow of germ-free mice [10]. Gut microbiota can indirectly affect osteoblasts and osteoclasts by altering bone immune status, thereby influencing mice bone health [11]. Indeed, gut microbiota contain species and individual polymorphisms similar to single nucleotides, which may have a significant regulatory role and research value on bone metabolism. Taken together, exploring the regulation of the gut microbiota by GC may provide a new perspective for the prevention and treatment of GAON. However, it is worth noting that the above studies are mainly based on observational cross-sectional analyses or animal model experiments, which could not reveal the causal association between gut microbiota and GAON in humans.

Direct study of the role of specific flora on GAON is difficult because of the enormous amount of information contained in the gut microbiota, the synergistic and antagonistic inter- microbiota interactions, and the existence of the intestinal mucosal barrier. For both the gut and bone, changes in metabolite levels resulting from shifts in gut microbiota composition may serve as crucial mediators affecting bone metabolism [12]. Among the intestinal metabolites, the role of SCFAs in the pathological mechanisms of GAON is relatively well-understood [13]. Studies have established that SCFAs play significant regulatory roles, including promotion of calcium absorption, inhibition of inflammatory responses, and modulation of lipid metabolism [6, 14, 15], which are pertinent to several key pathogenic mechanisms implicated in GAON. Accordingly, there is substantial basis for our hypothesis that the gut microbiota and metabolic products play a significant role in the pathogenesis of GAON.

Mendelian randomization analysis is a statistical method based on genome-wide association study (GWAS) data used to reveal causal relationships. It uses genetic variants as instrumental variables to infer the causal relationship between exposure and outcome, effectively avoiding the confounding bias found in traditional epidemiology. In the present study, bidirectional MR analysis initially suggested a possible unidirectional causal relationship between special gut microbiota and GAON. RNA sequencing (RNA-seq) analysis of femoral head specimens also indicated that intestinal inflammatory immune responses play a significant role in the pathogenesis of GAON. Based on the above rationale, we prospectively collected stool and serum samples from GAON, GAnON, and normal subjects for study. 16 S rDNA sequencing and metabolomics helped us clearly demonstrate that GC can lead to alterations in gut *Lachnospiraceae* abundance, and that a decrease in *Lachnospiraceae* concurrent with an increase in inflammatory factors is significantly associated with GAON. Metabolomics confirmed that faecal butyric acid levels were significantly positively correlated with *Lachnospiraceae* abundance and significantly negatively correlated with inflammatory factor levels, suggesting that butyric acid may mediate the pathway in which GC-induced downregulation of *Lachnospiraceae* and upregulation of inflammation contribute to GAON. Ultimately, the vital osteoprotective role of butyric acid in the pathogenesis of GAON was validated through in vivo experiments in rats. The technical route is depicted in Fig. 1. Our research could potentially pave the way for novel strategies and methodologies in the clinical management of GAON, offering fresh perspectives for both prevention and therapeutic intervention.

Materials and methods

MR analysis

Study design

To get reliable results, the instrumental variables (IVs) must satisfy three assumptions [16], as shown in Fig. 2.

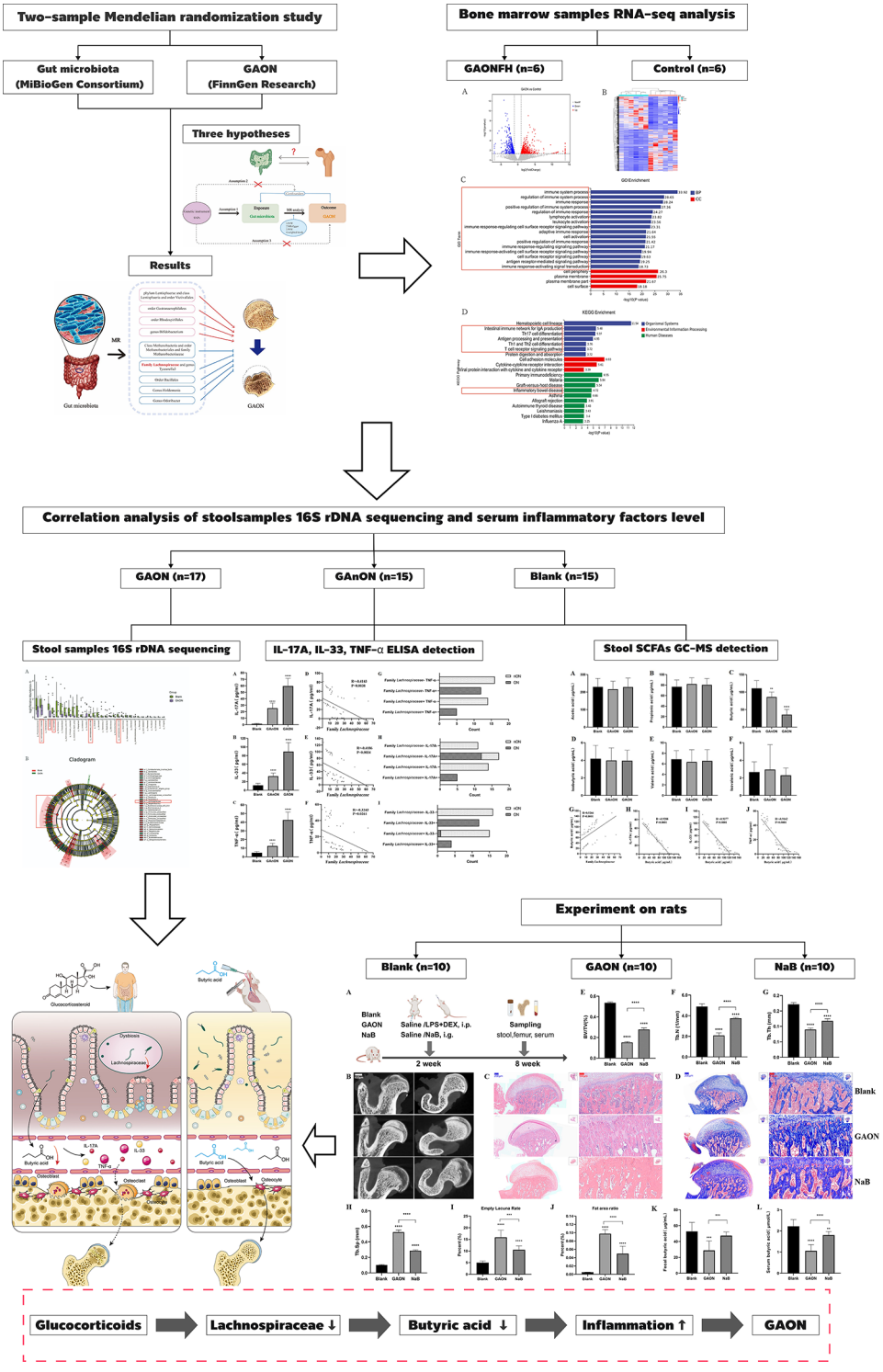

Fig. 1 Technology roadmap

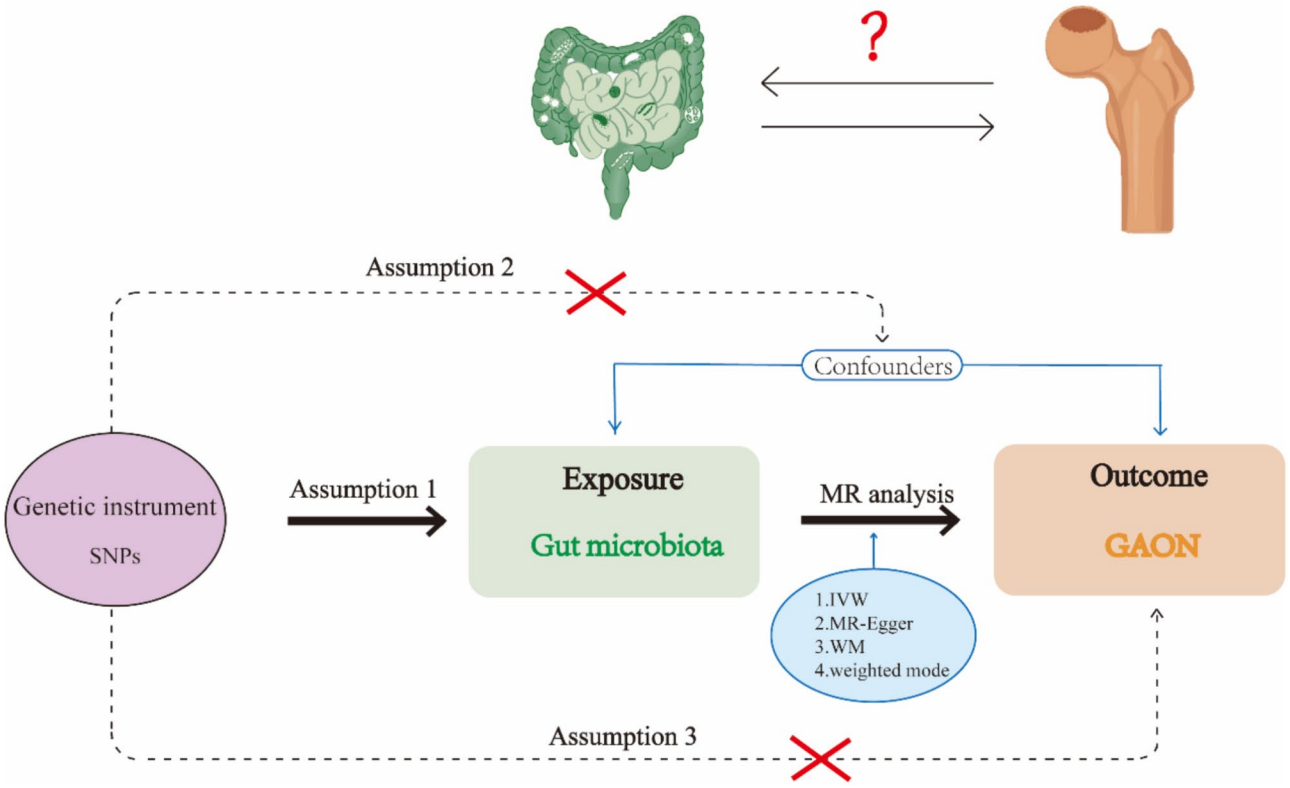

Fig. 2 Three assumptions of MR analysis. Assumption 1: IVs must be strongly associated with exposures. Assumption 2: IVs must not be associated with confounders of the exposure-outcome relationship. Assumption 3: IVs must influence the outcome only through the exposure

First, it was demonstrated whether gut microbiota alterations could precipitate GAON: MR analysis was used to analyze the causal relationship between 211 gut microbial taxa and GAON. Subsequently, a reverse MR analysis was performed to rule out the reverse potential effects of GAON on the gut microbiota. STROBE-MR was employed as the reporting standard [17]。.

Data sources

The GWAS data of gut microbiota were obtained from MiBioGen consortium [18] (https://mibiogen.gcc.rug.nl), containing 18,340 individuals and 122,110 SNPs, 131 genera, 35 families, 20 orders, 16 classes, and 9 phyla. The GAON statistics were obtained from the data released by FinnGen Research [19] (https://r9.finngen.fi/). GAON diagnostic criteria are based on the ICD-10 criteria, and the statistics contain 198,622,275 variants from 264 cases and 377,013 controls.

The selection of IVs

To obtain more comprehensive results, SNPs associated with exposure were selected as potential IVs at the locus-wide significance level ($P<1\times10^{-5}$) and clumping procedure ($R^2<0.001$, window size=10,000 kb). 103 SNPs (phylum), 179 SNPs (class), 217 SNPs (order), 341 SNPs (family), and 1,196 SNPs (genus) were selected as IVs. IVs with $F<10$ were excluded. After IVs those may be related to confounding factors of GAON, including smoking, body mass index, blood lipid, radiation therapy [20], were screened and removed through PhenoScanner [21], MR analysis was performed again.

Mendelian randomization analysis

The study employed several methods, including inverse variance weighted (IVW), MR-Egger, weighted median estimator, and weighted mode, to explore the causal relationship between exposure and outcome. Heterogeneity was assessed using Conchrane's Q-test. Horizontal pleiotropy was assessed by MR-Egger intercept, and outliers were detected and excluded by Outlier-corrected in the MR-PRESSO method. $P< 0.05$ was deemed to be statistically significant. The primary result was based on the IVW method in the absence of horizontal pleiotropy. In the event that heterogeneity was detected ($P<0.05$), the random-effects IVW model was utilized to obtain a more conservative estimate. Other MR analysis methods were utilized to complement the IVW method and generate wider confidence intervals [22]. To assess whether GAON has a potential causal effect on gut microbiota, reverse MR analysis was performed. All data were analysed using R software version 4.2.3.

RNA-seq analysis of bone tissue

Study participants

Study participants were recruited in the context of routine medical care at Jiangsu Provincial Hospital of Traditional Chinese Medicine (approved by the Ethics Committee, number: 2023NL-037-01). The first selection criterion for participation in the study was the need for primary artificial hip arthroplasty (HA) as determined by X-ray plain films, CT and MRI. The experimental group was GAONFH patients with an ARCO score of grade III or IV. The control group were patients diagnosed with traumatic femoral neck fracture, and patients with pathological fractures due to tumors and other severe osteopenia were excluded. Referencing the literature on sample size requirements [23–25], after rigorously selecting eligible patients, a total of 12 patient specimens (GAONFH group: 6 cases, control group: 6 cases) were collected for the study. See Supplementary Material 2 for details.

Sample collection

Surgical procedure of HA was performed as usual in the orthopedic operating room. The femoral head sample was taken during the operation and divided into two parts with scalper. Small samples of the necrotic bone area were extracted and placed in RNALater preservation solution of Beyotime for 8 h at 4℃, and transferred to -80℃ refrigerator until further processing. Samples from the control group were obtained and stored in the same way.

Data sources and preprocessing

Total RNA was isolated using the Trizol Reagent (Invitrogen Life Technologies), after which the concentration, quality and integrity were determined using a NanoDrop spectrophotometer (Thermo Scientific). Three micrograms of RNA were used as input material for the RNA sample preparations. Firstly, mRNA was purified from total RNA using poly-T oligo-attached magnetic beads. To select cDNA fragments of the preferred 400–500 bp in length, the library fragments were purified using the AMPure XP system (Beckman Coulter, Beverly, CA, USA). Products were quantified using the Agilent high sensitivity DNA assay on an Agilent Bioanalyzer 2100 system. The sequencing library was then sequenced on Illumina NovaSeq 6000 platform. High-quality clean data was obtained by filtering the raw data through fastp (0.22.0) software. Clean reads were mapped to the reference genome using HISAT2 (v2.1.0). Read counts were calculated using HTSeq (v0.9.1) and normalized to fragments per kilobase of transcript per million mapped (FPKM). For more details, see Supplementary Material 3.

Identification of DEGs and functional enrichment analysis

Gene differential expression analysis was performed using DEseq software (v1.38.3), and the differentially expressed genes (DEGs) were identified with threshold value of $|\log_2\text{FoldChange}| > 1$, and p-value < 0.05. GO enrichment analysis was performed using topGO (v2.50.0), and the p-value was calculated using the hypergeometric distribution method. KEGG pathway enrichment analysis was performed using Clusterprofiler software (v4.6.0), focusing on significantly enriched pathways with p-value < 0.05.

Stool and serum indicator tests

Study participants

In this study, 47 patients were prospectively included and divided into three groups: experimental group, control group and blank group. 17 patients in the experimental group (GAON group) developed osteonecrosis using GC; 15 patients in the control group (GAnON group) did not develop osteonecrosis using GC; and 15 patients in the blank group were normal subjects. Patients in the GAON group met the ARCO diagnostic criteria. Patients in the GAnON group had a history of prolonged GC use for 3 months or more without GAON. All participants were from the same dietary habit area, and none had travel history and been exposed to any drugs or gut microbiota modulators that could potentially alter the gut microbiota within one month prior to specimen collection. For more details, see Supplementary Material 2.

Fecal sample collection and 16 S rDNA sequencing analysis

The faeces of the subjects were collected and stored at -80℃. Genomic DNA was extracted using the OMEGA Stool DNA Kit (D4015) according to the manufacturer's instructions. The quantity and quality of extracted DNAs were measured using NanoDrop NC2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and agarose gel electrophoresis, respectively. PCR amplification of bacterial 16 S rDNA V3-V4 region was performed using forward primer 341 F (5'-CCTACGGGGNGGCWGCAG-3') and reverse primer 805R (5'-GACTACHVGGGTATCTAATCC-3'). Sequencing was performed using the Illumina NovaSeq platform to obtain raw data. Microbiome bioinformatics analysis was mainly performed using QIIME2. See Supplement material 3 for details.

Detection of fecal SCFAs

The mixed standard reserve solution of 6 SCFAs (acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid and isovaleric acid) was prepared with ether, which was appropriately diluted and prepared as the working solution. The samples were extracted with phosphoric acid solution and ether, and 4-methylvaleric acid solution

was used as the internal standard. And the working solution was added to the equal volume of the internal standard solution. Subsequently, the samples were vortexed for 1 min and centrifuged at 12,000 rpm for 10 min at 4 °C. The supernatants were transferred to vials and then analysed by gas chromatography-mass spectrometry (GC-MS).

Blood sample collection and ELISA for serum inflammatory markers

Blood samples were collected in anticoagulation tubes and left to stand for 20 min at room temperature, then centrifuged at 1000 rpm for 10 min at 4 °C, and the supernatant was taken and stored at -20 °C. Inflammatory factors (IL-17 A, IL-33, TNF-α) were detected using Solarbio ELISA kit. According to the kit instructions, standards and test samples were added in steps, and finally the OD value was measured at 450 nm by Microplate Reader.

In vivo rat experiments

Experimental animal grouping and modeling

Under the approval of the Animal Ethics Committee of Nanjing University of Chinese Medicine (202306A059), male Sprague Dawley (SD) rats (6 to 8 weeks of age) weighing 200 to 250 g were used in this study. The animals were raised in a controlled temperature, humidity and 12 h/dark environment. After one-week adaptive rearing, male SD rats were randomly divided into three groups (10 rats in each group): Blank group, GAON group and NaB group (same as the GAON group modelled, sodium butyrate gavage). Administration dose and time: GAON modeling: The rats were injected intraperitoneally with lipopolysaccharide (LPS, 200 μg/kg/day) once a day for 2 days. The second injection of lipopolysaccharide was followed by intraperitoneal injection of dexamethasone solution (DEX, 20 mg/kg/day) 24 h later, three times a week for 6 weeks. The Blank group was injected intraperitoneally with equal amount of 0.9% saline. NaB group was given sodium butyrate gavage (100 mg/kg/day) along at the same time of modelling. Blank and GAON groups were given equal amount of saline gavage. Throughout the research process, we always paid attention to protecting animal welfare. Under the supervision of the ethics committee, we maintained a clean and hygienic environment for animal housing, ensured ample diet and water, and used small-sized needles when administering drugs.

Stool, serum and femoral head sample collection and analysis

Rat stools were collected and stored at -80 °C for inspection. One week after the last administration, rats were anaesthetized with pentobarbital, and blood was collected from the abdominal aorta so that the rats died of shock. The blood was centrifuged at 1000 rpm for 10 min at 4℃, and the supernatant was taken and stored at -20℃. Stool and serum were subjected to targeted metabolomics based on GC-MS. Rat femoral head samples were collected for Micro-CT scanning and quantitative bone microstructure analysis. After scanning, the femoral head samples were fixed with 4% paraformaldehyde for 24 h and decalcified with 10% EDTA solution for 4 weeks. After paraffin embedding and section, HE and Masson staining were performed, and microscope observation and image collection were conducted.

Statistical analysis

GraphPad Prism software was applied for statistical analysis. Before comparing differences between two groups, perform an F test for variance homogeneity. If the p-value is less than 0.05, indicating significant variance difference, use Welch's t-test; if the p-value is 0.05 or higher, indicating no significant variance difference, use the two independent samples t-test. Pearson's Correlation test was used to analyse the correlation between faecal and serum indices. $P<0.05$ was considered statistically significant.

Results

MR analysis

Effect of gut microbiota to GAON

14 gut microbial taxa were examined to have suggestive associations with GAON (Fig. 3 and supplementary Table 1). We found that the phylum *Lentisphaerae*, class *Lentisphaeria*, order *Gastranaerophilales*, order *Rhodospirillales*, order *Victivallales*, genus *Bifidobacterium* were positively correlated with GAON. On the contrary, class *Methanobacteria* and its child taxon (order *Methanobacteriales* and family *Methanobacteriaceae*), family *Lachnospiraceae* and its child taxon (genus *Tyzzerella3*), order *Bacillales*, genus *Holdemania*, genus *Odoribacter* were negatively correlated with GAON. MR-Egger intercept test and Cochrane's Q test in sensitivity analysis did not offer any proof of pleiotropy and heterogeneity, as shown in supplementary Tables 2 and Fig. 4. Moreover, the MR-PRESSO global test did not detect any horizontal pleiotropy or outlier, further validating the robustness of our findings.

Reverse MR analysis

Reverse analytical approach was deemed necessary to ensure the integrity and validity of the results obtained. Supplementary Table 3 shows the reverse MR outcomes and there was no evidence of a significant reverse causality between GAON and the fourteen identified statistically significant gut microbiota taxa above. There was no horizontal pleiotropy or heterogeneity in the MR-Egger

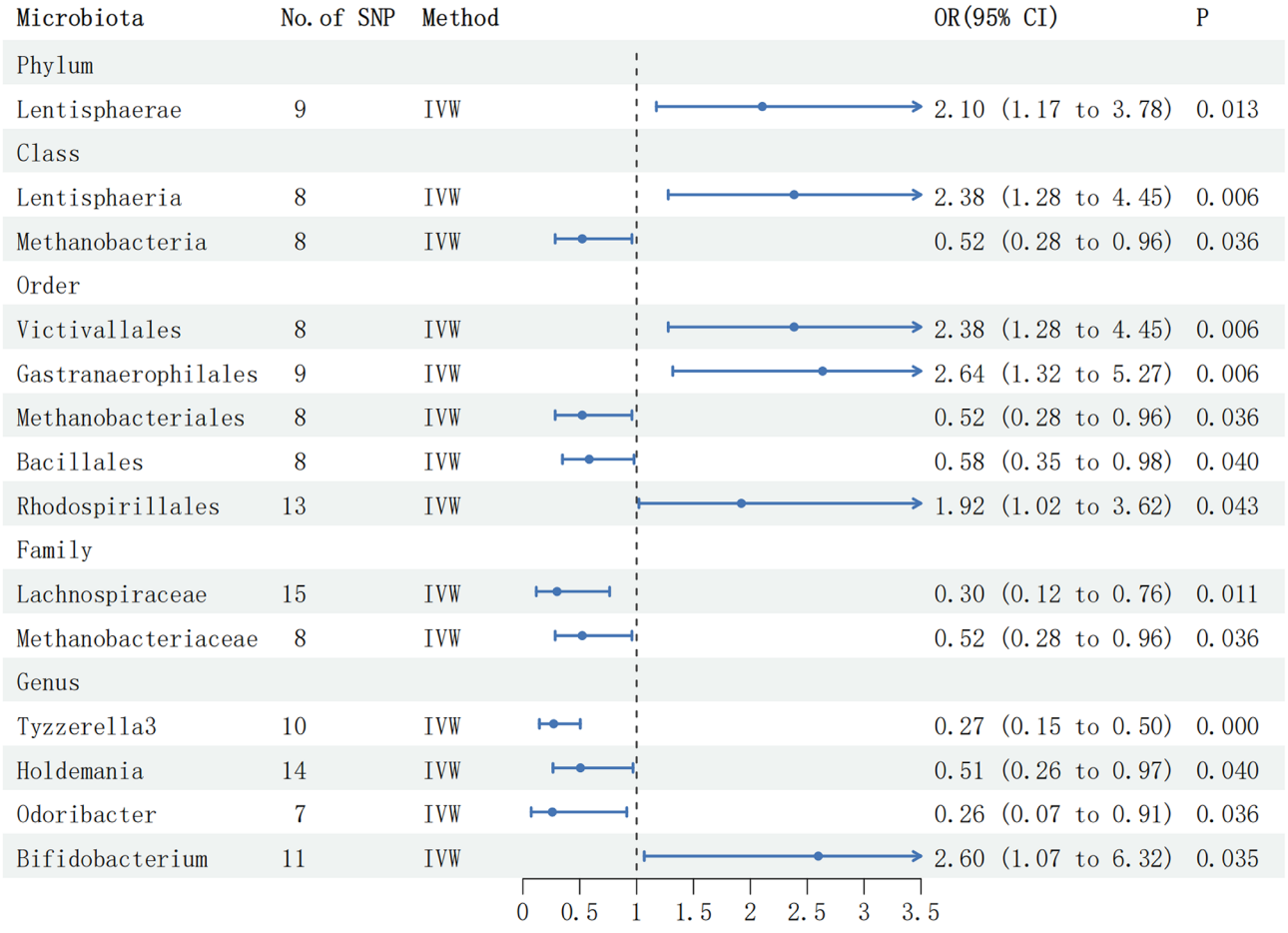

Fig. 3 Forest plot of suggestive causal effects of 14 gut microbial taxa on GAON(genus Tyzzerella3 $P<0.0001$)

intercept and Conchrane's Q test. Figure 5 shows the result of the MR analysis.

RNA-seq analysis of bone tissue

A total of 12 patients were included in the study (Control group: 6 patients, GAONFH group: 6 patients). Typical specimens of Control and GAONFH group are shown in Figure S1(A-D). Based on our transcriptome sequencing results, a total of 1,413 DEGs were identified between GAONFH and Control group, including 788 up-regulated genes and 625 down-regulated genes (Fig. 6(A)). The heatmap of the DEGs (Fig. 6(B)) showed that the DEGs could distinguish GAONFH samples from Control samples. The DEGs were obtained for GO and KEGG functional enrichment analysis. GO enrichment analysis showed that the biological functions of up-DEGs enrichment mainly involved immune system process, immune response, lymphocyte activation and leukocyte activation (Fig. 6(C)). The biological functions of down-DEGs enrichment mainly involved the anatomical structure morphogenesis and lipid metabolic process (Figure S2(A)). KEGG enrichment analysis showed that up-DEGs were mainly enriched in intestinal immune network for IgA production, inflammatory bowel disease, Th17, Th1 and Th2 cell differentiation (Fig. 6(D)); down-DEGs were mainly enriched in PPAR signaling pathway (Figure S2(B)). This suggests that the activation of intestinal inflammatory immune system alterations is closely related to GAON.

Analysis of faecal and serum indices

Analysis of gut microbiota

Species composition and difference analyses among different groups were carried out based on the species abundance table at phylum, class, order, family, genus and species levels, with parameter thresholds: confidence > 0.7. The 30 communities with the highest relative abundance at each taxonomic level were clustered and presented in heat maps, as in Figure S3(A-F). Comparing the results with the MR analysis, we found that the abundance in the GAON, GAnON and Blank groups were phylum *Lentisphaerae* (0%, 0.00149%, 0.00105%), class *Lentisphaeria* (0%, 0.00149%, 0.00105%), order *Gastranaerophilales* (0.0092%, 0%, 0.03172%), family *Lachnospiraceae* (17.12596%, 12.50607%, 24.51633%), genus *Bifidobacterium* (13.15974%. 14.86774%, 3.12511%). The

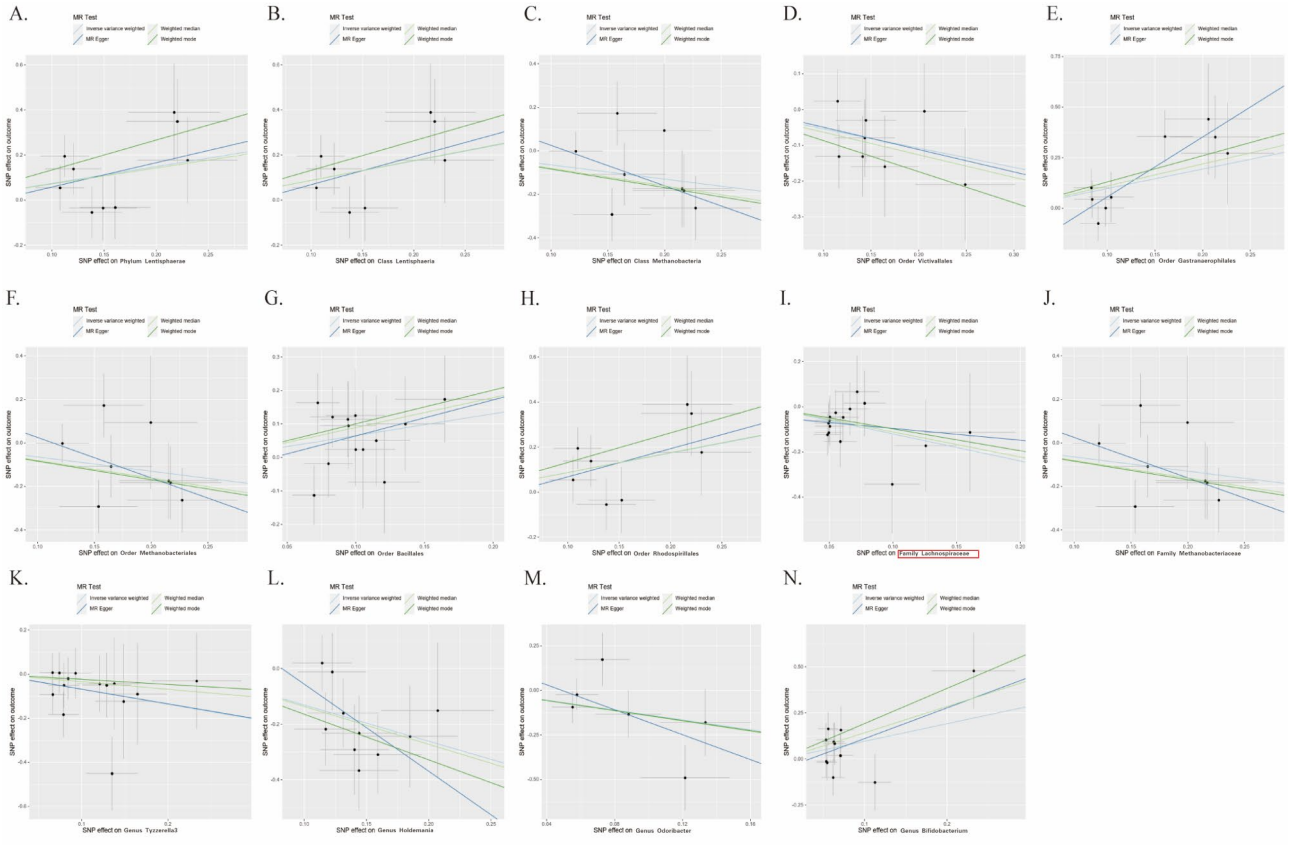

Fig. 4 Scatter plots for causal effects of gut microbiota on GAON. A-N plots include four methods: the IVW method, MR-Egger method, weighted median method, and weighted mode method. The slopes of the straight lines derived from four methods are consistent, suggesting that exposures correlate with outcome

Kruskal-Wallis test was used to analyze the species significance difference. As shown in Fig. 7(A), at the genus level, in the GAON group compared with the Blank group, *Lachnospira*, *Lachnospiraceae_UCG-010*, *Lachnospiraceae_unclassified*, *Ruminococcus_1*, *Ruminococcaceae_UCG-013*, *Odoribacter*, *Roseburia* significantly decreased, *Catenibacterium* and *Tyzzerella_4* significantly increased, and the difference was statistically significant ($P<0.05$). LEfSe (LDA Effect Size) analysis plots more visually show different species at all levels. In Fig. 7(B), we could find that Family *Lachnospiraceae* is differential between GAON and Blank Group, which is consistent with the result of MR analysis.

Correlation analysis between Lachnospiraceae and serum inflammatory indexes

As shown in Fig. 8(A, B, C), the levels of IL-17 A, IL-33 and TNF-α in GAON group were significantly higher than those in GAnON group and Blank group, and GAnON group was also slightly higher than Blank group. The difference was statistically significant ($P<0.0001$), indicating that the inflammatory response was significantly up-regulated in GAON. Then Pearson's Correlation analysis was performed on *Lachnospiraceae* abundance and inflammatory index levels in three groups, and the results (Fig. 8(D, E, F)) showed that *Lachnospiraceae* abundance was significantly negatively correlated with the levels of IL-17 A, IL-33, and TNF-α, that is, the down-regulated *Lachnospiraceae* abundance might be the cause of the up-regulated serum inflammatory factors. Afterwards, we conducted subgroup analysis based on the levels of serum inflammatory factors (IL-17 A, IL-33, TNF-α) and the abundance of *Lachnospiraceae*. Lower than the average level of the corresponding group was the down-regulated group (Family *Lachnospiraceae*-, IL-17 A-, IL-33-, TNF-α-), and greater than or equal to the average level of the corresponding group was the up-regulated group (Family *Lachnospiraceae* +, IL-17 A +, IL-33 +, TNF-α +). As results in Fig. 8(G, H, I), inflammatory factor levels exceeded the mean value in almost all GAON patients, and the proportion of GAON was significantly higher in patients with down-regulated *Lachnospiraceae* abundance and up-regulated serum inflammatory factor levels than in other subgroups.

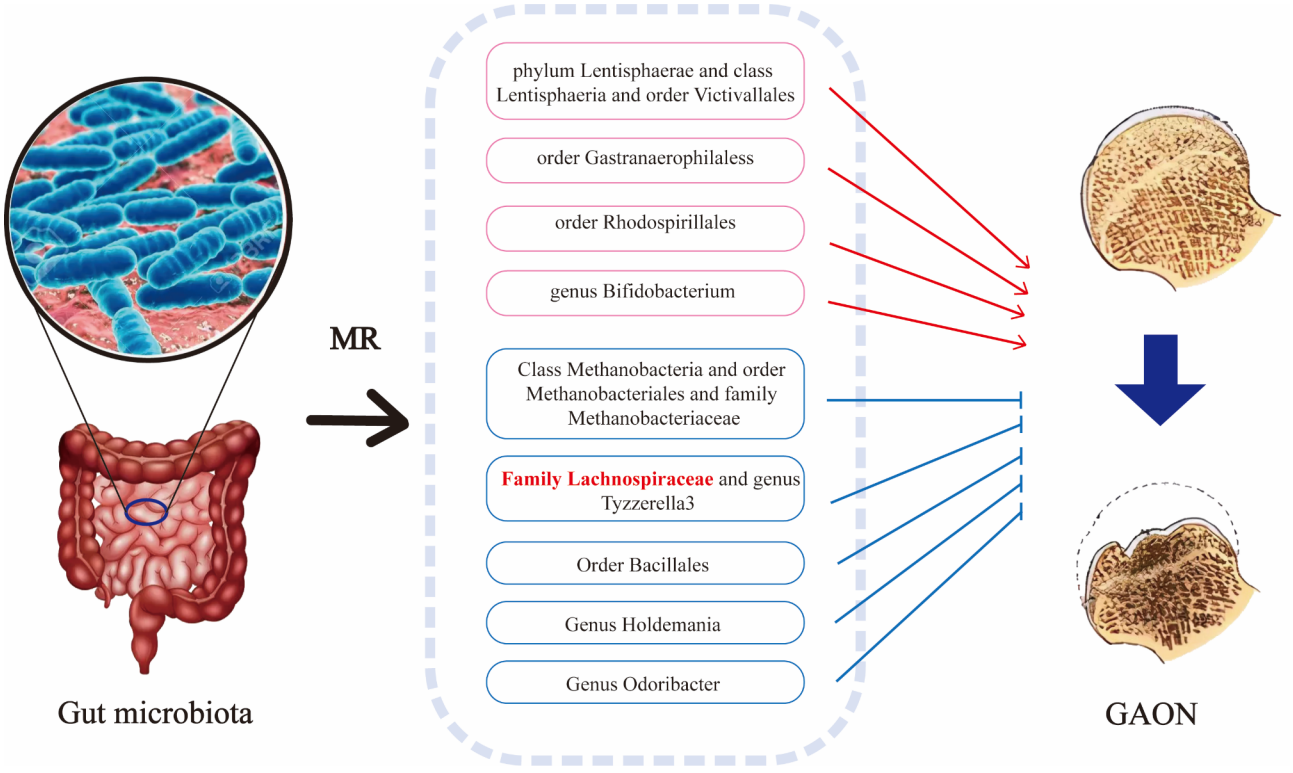

Fig. 5 The result of MR analysis

Association analysis of SCFAs with Lachnospiraceae and serum indicators of inflammation

As shown in Fig. 9 (A-F), butyric acid content was highest in the Blank group and lowest in the GAON group with statistically significant differences, while no statistically significant differences were found in the content of acetic, propionic, isobutyric, valeric and isovaleric acids. Then Pearson's Correlation analysis of butyric acid with *Lachnospiraceae* and serum inflammatory factors was carried out, and the results of Fig. 9(G-J) showed that the abundance of *Lachnospiraceae* was significantly positively correlated with butyric acid content, and butyric acid content was significantly negatively correlated with the levels of IL-17 A, IL-33, and TNF-α, that is, butyric acid may be the mediating factor of the up-regulated serum inflammatory factors by down-regulated *Lachnospiraceae*.

In vivo rat experiments

Micro-CT and staining results in Fig. 10(B-D) showed that in the Blank group, the cartilage of the femoral head was thick; the subchondral bone trabeculae were arranged neatly and densely; there were abundant haematopoietic cells and no adipocyte hyperplasia and hypertrophy in the medullary cavity. Compared with the Blank group, both the GAON and NaB groups showed decreased osteocytes between the bone trabeculae and an increased number of empty osteocytic lacunae, and nuclear pyknosis. Differently, compared with the NaB group, the GAON group had a significant increase in fibrous tissue between bone trabecular, a decrease in the number of hematopoietic cells, and an increase in the area of adipose and some hypertrophic fusion into a capsule. Bone microstructure quantitative analysis (Fig. 10(E-H)) revealed that BV/TV, Tb.N, Tb.Th in GAON and NaB groups were significantly lower than Blank group, and Tb.Sp was significantly higher than Blank group, whereas the overall condition of NaB group was better than that of GAON group, and the difference was statistically significant ($P<0.05$). The rate of empty bone lacunae and the percentage of fat area were calculated based on the HE staining results, as shown in Fig. 10(I-J), and the differences among the three groups were statistically significant ($P<0.05$). Analysis of faecal and serum butyric acid content in Fig. 10(K-L) showed that butyric acid content was significantly lower in the GAON group compared to the Blank group, while it was elevated in the NaB group compared to the GAON group, with a statistically significant difference ($P<0.05$). In conclusion, osteonecrosis features were present in both GAON and NaB groups, while the degree of osteonecrosis in the NaB group with higher butyric acid level was significantly reduced compared with the GAON group, which demonstrated that the reduction of butyric acid content was an important factor in promoting GAON.

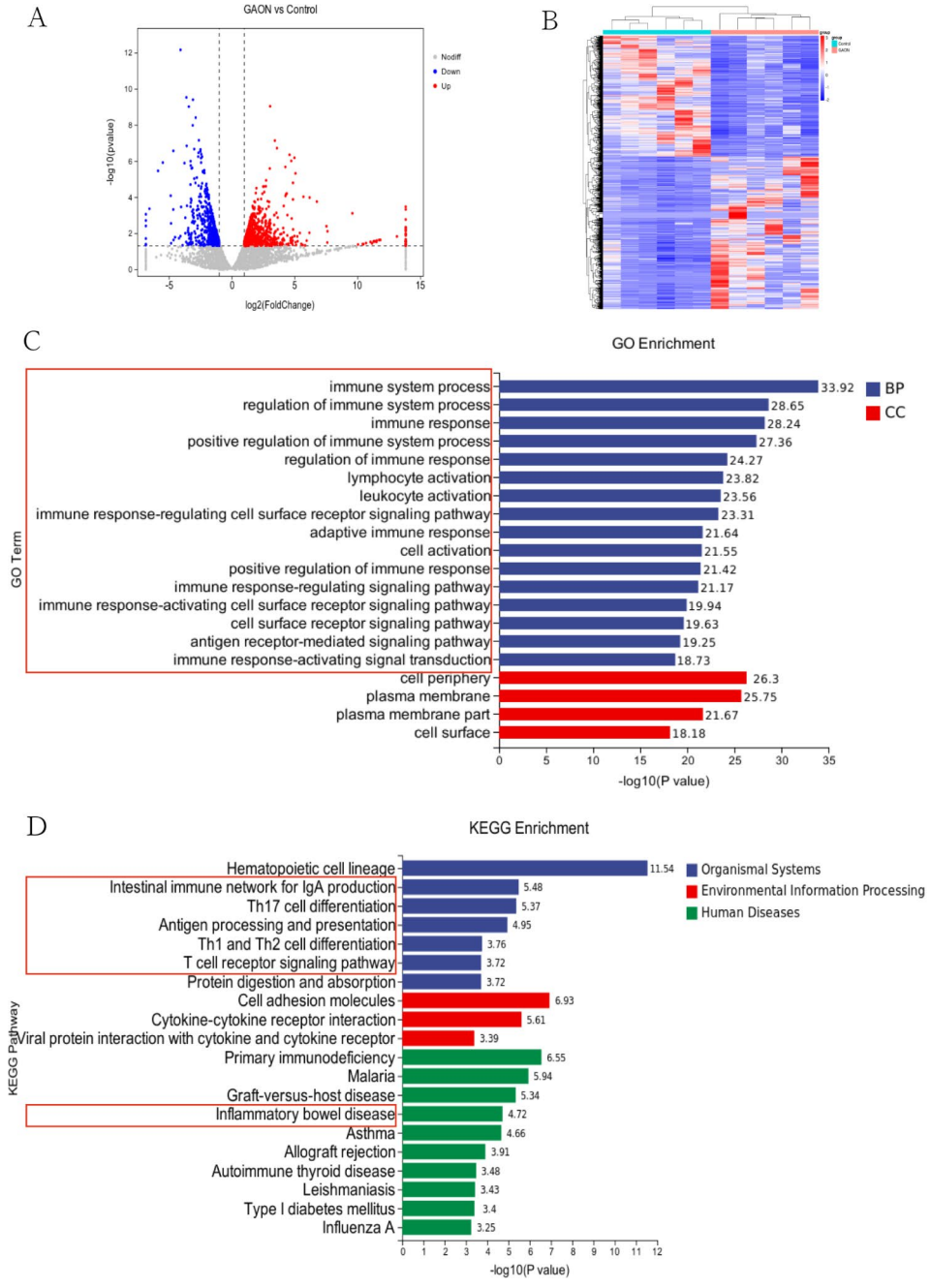

Fig. 6 DEGs analysis results. (**A**) Volcano map of DEGs. (**B**) Heatmap of DEGs. (**C**) GO terms and (**D**) KEGG pathways enriched by up-DEGs

Discussion

Due to the widespread use of GC in clinical practice, GAON affects a wide range of people and mostly affects youngsters. The disability rate of GAON is high, with the majority of patients eventually requiring artificial joint replacement. However, the limited lifespan of artificial joint prostheses can significantly reduce the long-term quality of life for patients, making early prevention and treatment of GAON extremely important. The onset of GAON is insidious, necessitating the identification of high-risk factors or precursor states for early intervention. The pathogenesis of GAON remains a subject of debate, as GC use can lead to multiple physiological and pathological changes simultaneously, making it difficult to clarify the causal relationships with GAON. The use of GC is closely related to the inflammatory immune response. The gut, however, is an important target organ of the body's inflammatory response, in which the key role of gut microbiota is often overlooked by GAON researchers. Several recent studies have shown that GC

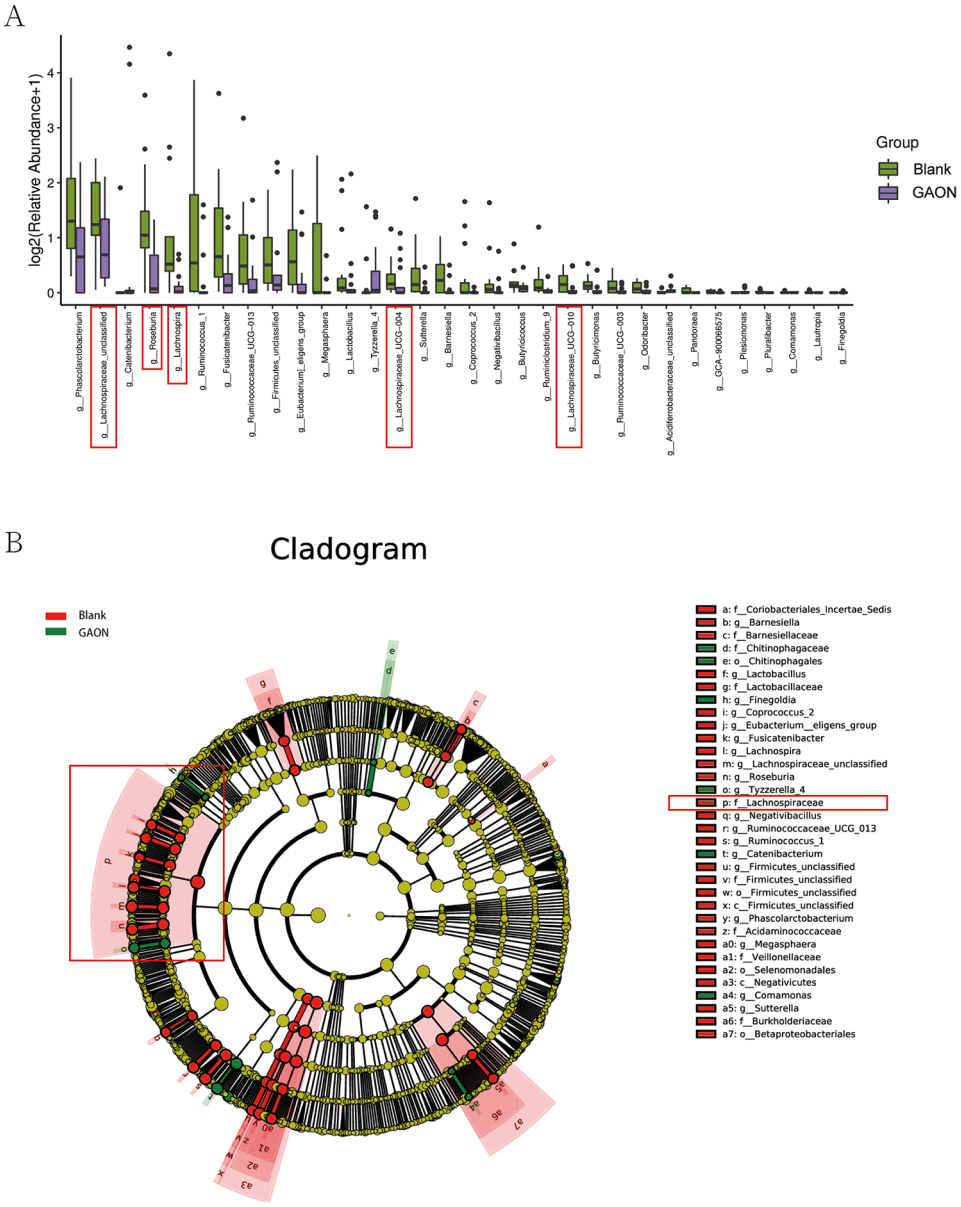

Fig. 7 Result of gut microbiota analysis. (**A**) Significant difference analysis chart; (**B**) LEfSe analysis diagram

can lead to changes in the gut microbiota of humans and animals [26, 27]. Given the significant impact of intestinal microbiota on bone metabolism, which has been widely reported in recent years, we propose the scientific hypothesis that GC-induced dysbiosis of gut microbiota may promote GAON.

In this study, we first performed a two-sample MR analysis to investigate the correlation between the gut microbiota and GAON. Our analysis revealed that the phylum *Lentisphaerae*, order *Gastranaerophilales*, order *Rhodospirillales*, genus *Bifidobacterium* were positively correlated with GAON, and that family *Lachnospiraceae* and its child taxon (genus *Tyzzerella3*), genus *Holdemania*, genus *Odoribacter* were found to be negatively correlated with GAON, and the results of reverse MR analysis were negative. The above results suggest that changes of gut microbiota may unilaterally contribute to the occurrence of GAON. Similar conclusions have been reached in several experimental studies. For instance, it has been shown that GC-induced bone loss in mice is significantly reduced or negligible with gut microbiota depletion, achieved through broad-spectrum antibiotic treatment, indicating that the gut microbiota plays a necessary role in GC-induced trabecular bone loss [28]. A study by Ho Jun Kang found that supplementation with *Lactobacillus* mitigated GC-induced pathological bone effects, such as ischemia, apoptosis, obesity, bone loss in mice [29]. However, it is evident that the gut microbiota

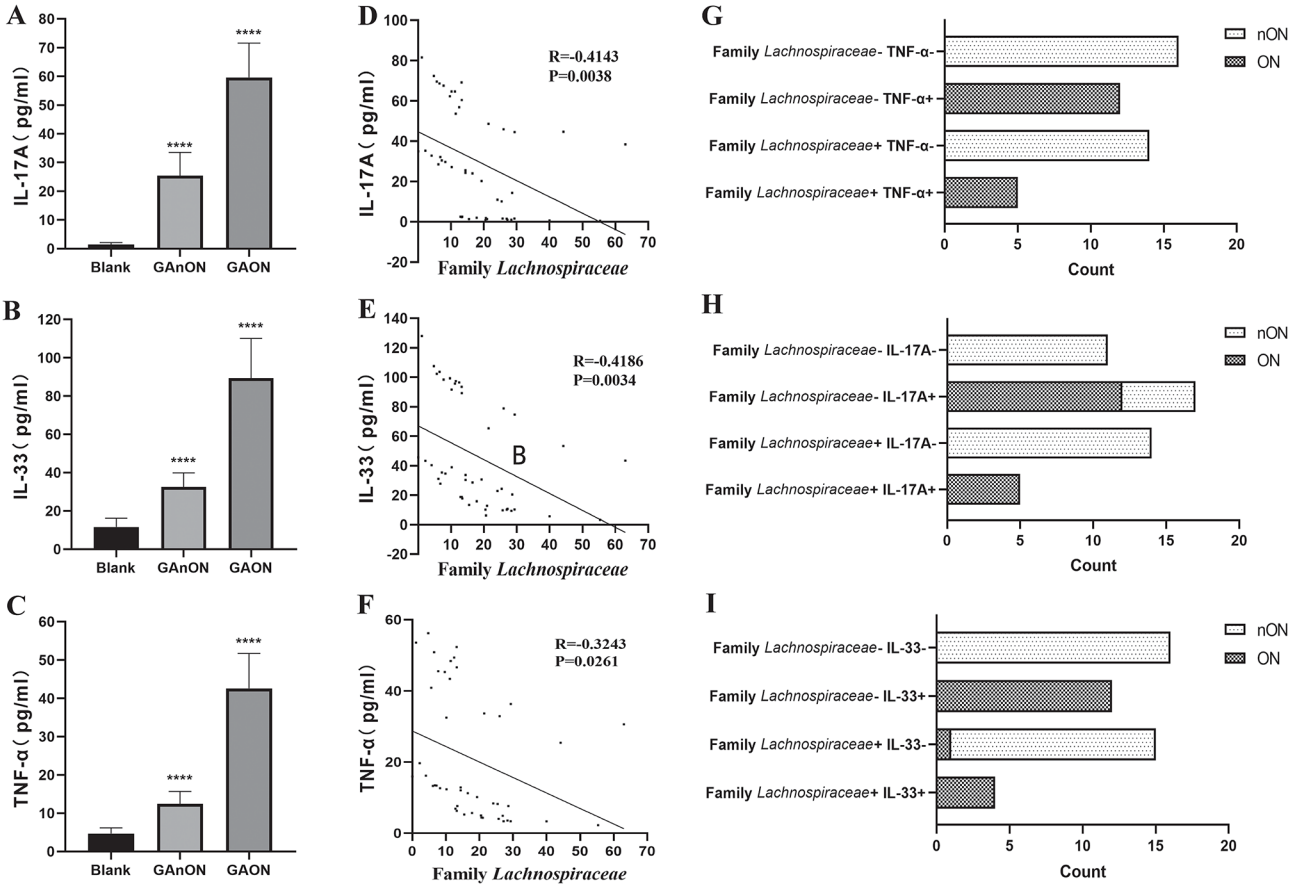

Fig. 8 (**A**-**C**)Bar graphs of IL-17 A (**A**), IL-33 (**B**), TNF-α (**C**) levels. The levels of serum inflammatory factors were significantly different among the three groups. (**D**-**F**) Correlation analysis between *Lachnospiraceae* and IL-17 A (**D**), IL-33 (**E**), TNF-α (**F**). There was a significant negative correlation between *Lachnospiraceae* abundance and the level of serum inflammatory factors. (**G**-**I**) *Lachnospiraceae* and TNF-α (**G**), IL-17 A (**H**), IL-33 (**I**) level subgroup analysis subject distribution bar chart. The proportion of GAON was significantly higher in patients with decreased *Lachnospiraceae* abundance accompanied by increased serum inflammatory factor levels than in other subgroups. ON: osteonecrosis. nON: no osteonecrosis. Significance levels are indicated as*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$

does not directly translocate to bone in diseased populations. The specific mediators and downstream mechanisms by which changes in gut microbiota contribute to GAON remain to be elucidated.

Therefore, to explore the potential mechanisms of GAON caused by gut microbiota, we conducted RNA-seq analysis on femoral head specimens from patients diagnosed with GAONFH, which is the most prevalent form of GAON, as well as from patients with traumatic femoral neck fractures. Enrichment analysis of DEGs revealed that the up-DEGs were predominantly associated with inflammatory immune responses and were significantly enriched in pathways such as IgA production by the intestinal immune network and inflammatory bowel disease. These findings not only substantiate the significant association between gut microbiota and GAON, but also imply that the inflammatory immune response could be a crucial mechanism linking gut microbiota to the development of GAON. Previous studies have shown that gut microbiota dysbiosis can trigger an increase in the secretion of pro-inflammatory cytokines, such as IL-6, IL-17 and TNF-α, leading to the upregulation of osteoclast activity and bone resorption [30]. Mingcan Yu et al. reported that alterations in gut microbiota can enhance the production of TNF-α and IL-17 by intestinal T-cells, promoting their migration from the intestines to the bone marrow, which results in bone loss [31]. Furthermore, the use of probiotics has been shown to reduce the levels of inflammatory cytokines, including TNF-α, IL-6, RANKL and IL-17, thereby inhibiting osteoclastogenesis and potentially preserving bone mass [32].

To further explore the link between gut microbiota and GAON, we conducted a prospective controlled study including three patient groups, differentiated by the presence or absence of GAON and GC usage. Microbial 16 S rDNA sequencing analysis of the fecal samples revealed differences in the abundance of *Lachnospiraceae* and *Bifidobacterium* between GAON and blank group, as well as between GAnON and blank group. *Lachnospiraceae* were less abundant, while *bifidobacterium* was more abundant

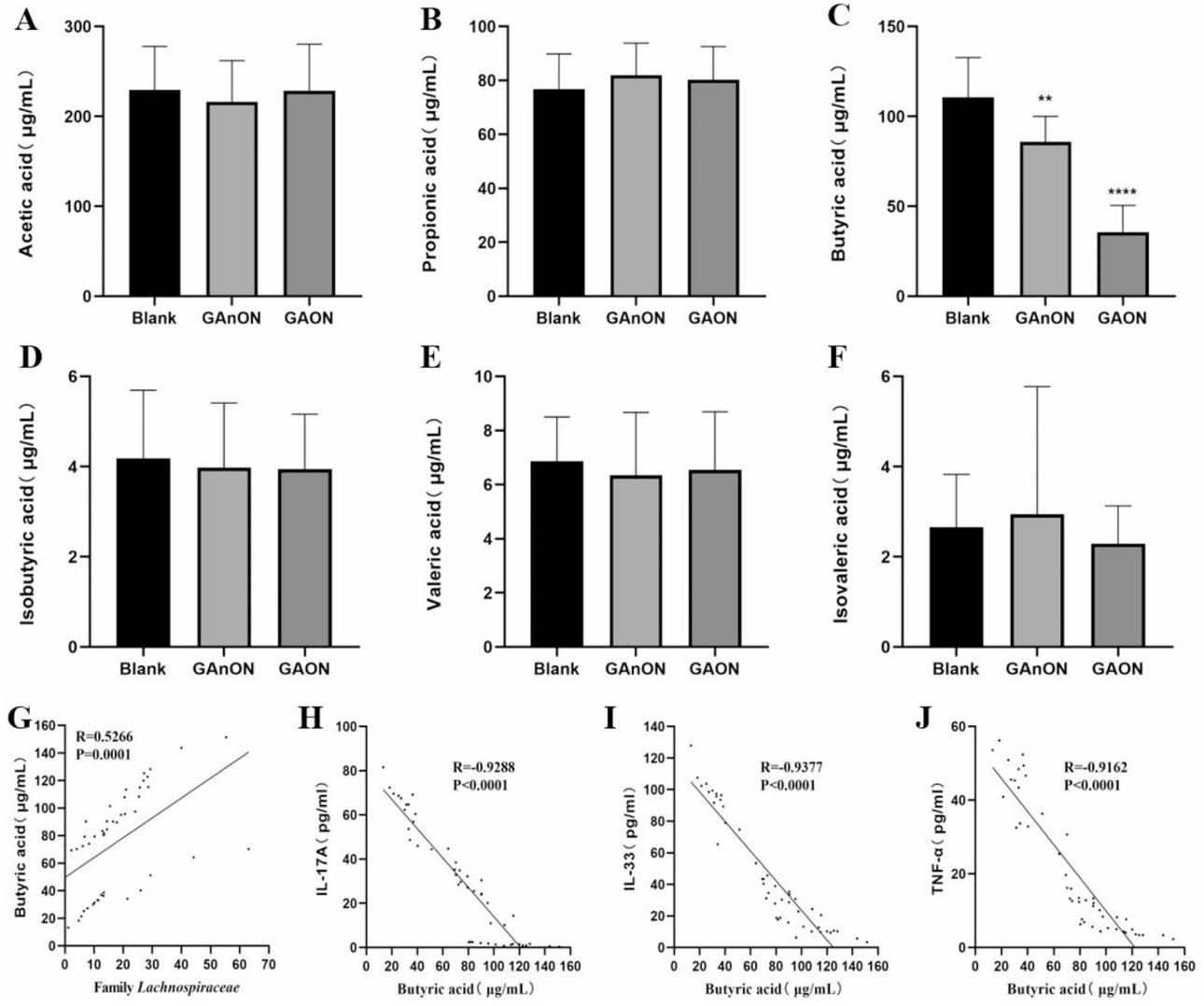

Fig. 9 (**A**-**F**): Bar graphs of acetic (**A**)、propionic (**B**)、butyric (**C**)、isobutyric (**D**)、valeric (**E**) and isovaleric acids (**F**) content. The butyric acid content was statistically different among three groups. (**G**) Correlation analysis between *Lachnospiraceae* abundance and butyric acid content. *Lachnospiraceae* abundance was significantly positively correlated with butyric acid content。(**H**-**J**) Correlation analysis of butyric acid with IL-17 A (**H**), IL-33 (**I**) and TNF-α (**J**) content. Butyric acid content was significantly negatively correlated with the levels of IL-17 A, IL-33, and TNF-α. Significance levels are indicated as $^{**}p < 0.01$, $^{****}p < 0.0001$

in both GAON and GAnON groups compared to blank group. Significant difference analysis and LEfSe analysis mapping showed a notable decrease in *Lachnospiraceae*, suggesting a close association with GAON. However, no significant difference in *Lachnospiraceae* abundance was observed when comparing GAON and GAnON groups. This finding implies that while GC use may decrease *Lachnospiraceae* abundance, this decrease alone may not necessarily result in GAON. The reduced abundance of *Lachnospiraceae* could be a necessary but not sufficient condition for GAON, suggesting that other factors likely interact with changes in gut microbiota to contribute to the disease's onset.

Given that inflammatory immune mechanisms were prominently enriched in our sequencing results, we hypothesize that the down-regulation of *Lachnospiraceae* may be an initiating factor that synergizes with other elements. To explore the potential synergistic role of the inflammatory response with the down-regulation of *Lachnospiraceae* in GAON, we conducted a correlation analysis of pro-inflammatory cytokines IL-17 A, IL-33, and TNF-α in blood samples from subjects with gut microbiota. Using ELISA, we discovered significant differences in the levels of these mediators among the Blank, GAnON, and GAON groups, with the highest levels observed in the GAON group. Notably, there was a significant negative correlation between the levels of these inflammatory mediators and the abundance of *Lachnospiraceae*. In almost all GAON patients, inflammatory factor levels were elevated above the mean, and

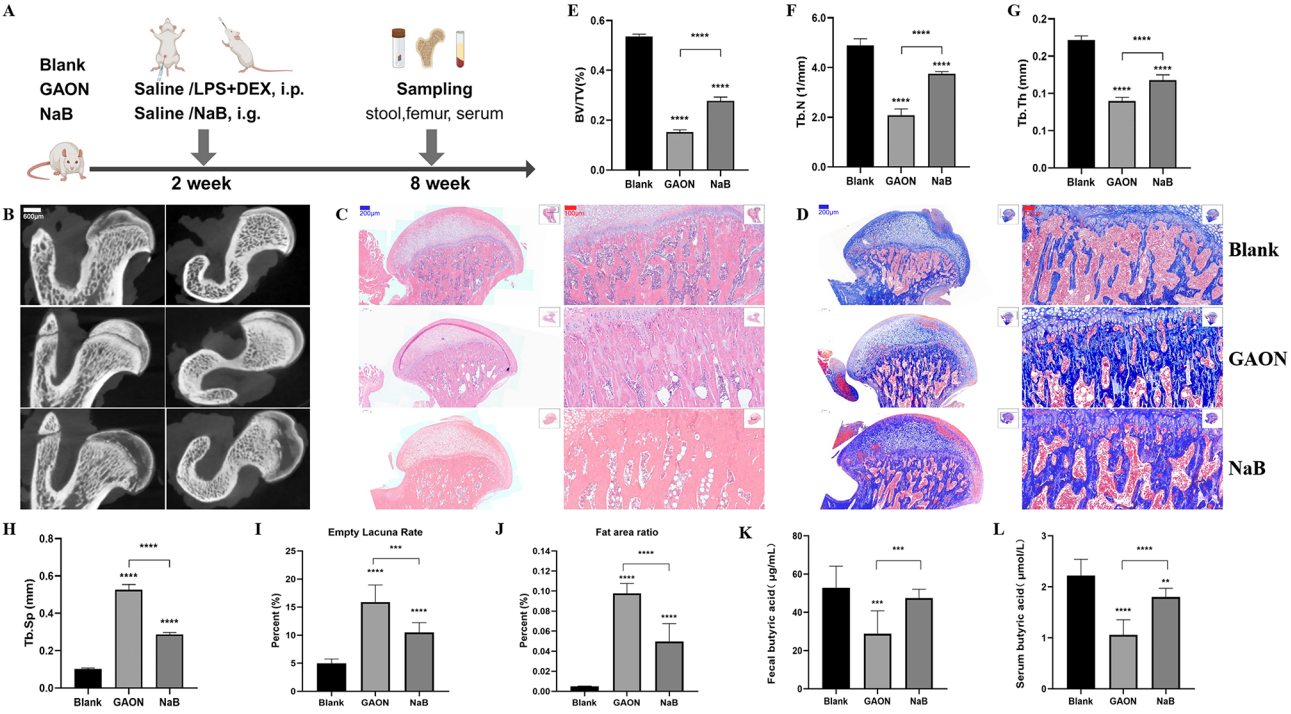

Fig. 10 (**A**) Flow chart of rat experiment. (**B**) Micro-CT images. From left to right are the coronal and horizontal planes. (**C**) HE staining results. (**D**) Masson staining results. Magnification from left to right is ×5 and ×200, respectively. (**E**-**J**) The quantification of BV/TV (**E**), Tb.N (**F**), Tb.Th (**G**), Tb.Sp (**H**), empty lacuna rate (**I**), fat area ratio (**J**). (**K**-**L**) Histogram of fecal (**K**) and serum (**L**) butyric acid content. Significance levels are indicated as $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$. A was created with BioRender.com

the highest proportion of osteonecrosis occurred in those with both down-regulated *Lachnospiraceae* and up-regulated serum inflammatory factors. These findings suggest that after *Lachnospiraceae* abundance is reduced by GC use, inflammatory mediator levels may determine the onset of GAON, indicating that inflammation could be a synergistic factor in GAON development following gut microbiota alterations. The link between inflammation and GAON is supported by other studies. For instance, a study by Ma et al. [33] reported that elevated plasma IL-33 levels are associated with the severity of hip osteonecrosis, with higher IL-33 levels in patients with more advanced disease stages. Zou et al. [34]demonstrated a positive correlation between increased IL-17 levels and pain severity in osteonecrosis patients. Furthermore, in early-stage GAON in mice, high levels of TNF-α were found to activate macrophages to the M1 phenotype, inhibiting bone formation and promoting bone resorption, thus accelerating osteonecrosis [35]. In the late stage of GAON, reduced TNF-α levels shifted macrophage polarization to the M2 phenotype, leading to the formation of chronically inflamed fibrovascular tissue, which compromised bone mechanical properties and resulted in joint collapse. Osteoimmune disorder and chronic inflammatory microenvironment may be an important cause of osteonecrosis [36, 37].

Based on our findings, we propose that the interplay between GC, *Lachnospiraceae*, and the inflammatory immune response constitutes a critical 'link' in the pathogenesis of GAON. Evidence suggests that the down-regulation of *Lachnospiraceae* abundance may trigger systemic inflammation. As the primary producer of the anti-inflammatory short-chain fatty acid (SCFA) butyrate in the human gut, *Lachnospiraceae* also contributes to anti-inflammatory effects through the production of di-peptides, equol, reactive sulfur species, and farnesol [38, 39]. To determine if SCFAs play a pivotal role in this pathway, we measured SCFAs levels in patients' fecal samples. Correlation analysis indicated that butyric acid may act as a mediator, with down-regulated *Lachnospiraceae* abundance potentially leading to up-regulated serum inflammatory factors.

To enhance the reliability of our conclusions, we conducted in vivo validation in rats and discovered that the butyric acid content in the feces and serum of rats in the GAON group was significantly lower compared to the Blank group. Furthermore, butyrate supplementation in the NaB group resulted in a significant alleviation of femoral head necrosis compared to the GAON group, indicating that the reduction of butyric acid is an important factor contributing to the progression of GAON, and that butyrate supplementation may be beneficial in mitigating the disease. Our findings, in conjunction with existing

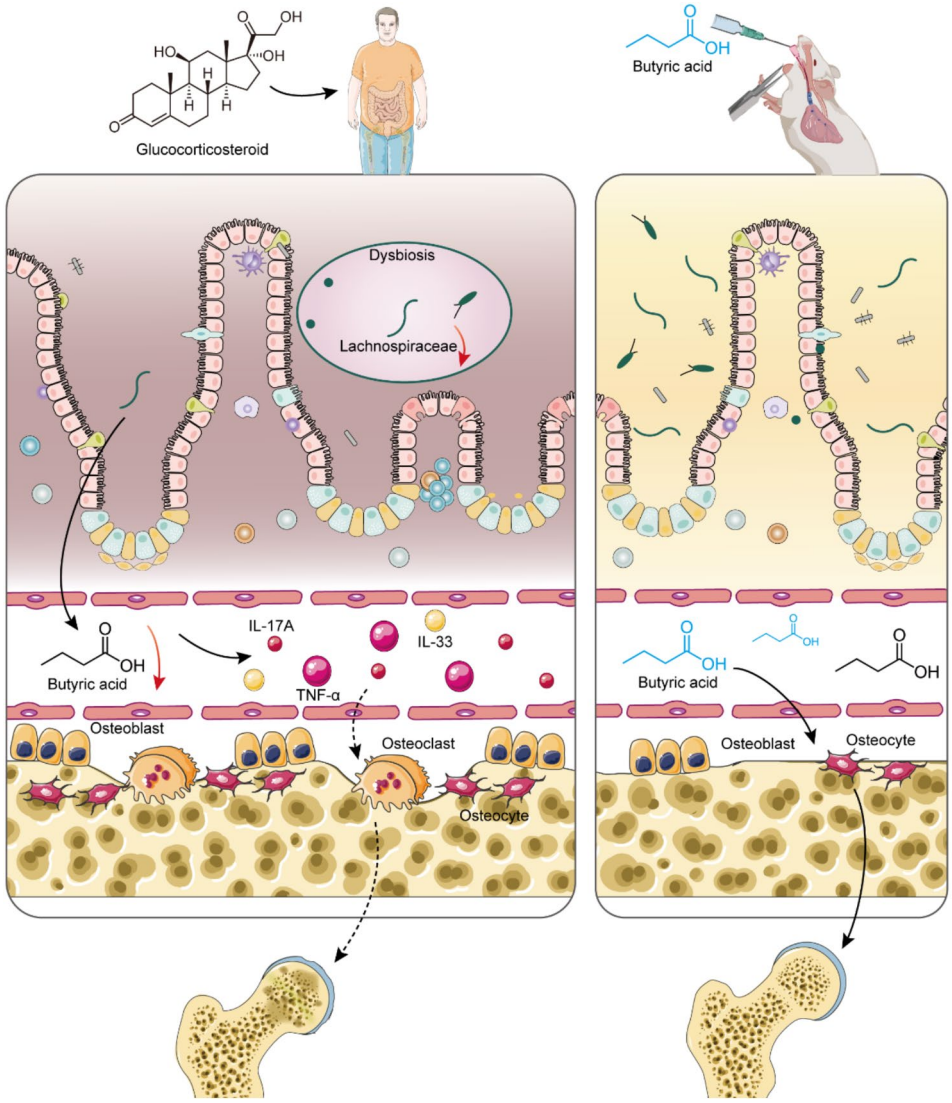

Fig. 11 Proposed model showing the interplay between glucocorticoids, *Lachnospiraceae*, and inflammation in GAON development. GC users experience gut microbiota dysbiosis, particularly with reduced *Lachnospiraceae*, resulting in butyric acid deficiency and enhanced inflammatory response, as indicated by elevated levels of IL-17 A, IL-33, and TNF-α. This chronic inflammation may disrupt the balance between osteogenesis and osteoblastogenesis, lead to osteoblast apoptosis and accelerated osteonecrosis. Solid lines represent established links, while dashed lines suggest new connections inferred from our study

literature, suggest that the osteoprotective role of butyric acid in GAON warrants attention. While the beneficial effects of butyric acid in the pathological mechanisms of rheumatoid arthritis (RA) have been documented [12, 40], its role in GAON, particularly among GC users who are also at risk of GAON, has been less explored. Butyrate treatment can suppress proinflammatory cytokines and osteoclast differentiation, prevent bone destruction and promote bone restoration [40]. In this study, butyric acid was identified as a crucial link in the pathway from GC-induced down-regulation of *Lachnospiraceae* abundance to the subsequent up-regulation of inflammation, leading to GAON. However, further research is needed to confirm the integrity of this pathway and the pivotal role of butyric acid.

It is widely acknowledged that treating GAON is challenging, and the emphasis in clinical practice should be on preemptive interventions before GAON manifests. The prevailing pathophysiological theories of GAON focus on blood supply disruptions and lipid metabolism disorders, with inflammation typically seen as a secondary consequence that arises post-onset, causing pain and bone marrow edema [5]. Our research diverges from this mainstream perspective by suggesting that gut microbiome imbalance and heightened inflammatory responses may be key instigators of GAON. Furthermore, animal studies suggest that butyrate supplementation may hold

significant potential in GAON prevention. Given the complexity of such diseases, a comprehensive approach is essential, and we are confident that our findings could offer valuable insights into both the etiology and clinical management strategies for GAON.

In this research, we initially employed bidirectional MR analysis to theoretically explore the causal connections between 211 gut microbiota taxa and GAON, effectively narrowing our focus and conserving considerable manpower and financial resources. Following this, RNA-sequencing was performed on GAON and normal bone samples to make credible predictions about the potential mechanisms through which the gut microbiota might trigger GAON. We then proceeded to collect fecal and serum samples from participants for clinical validation, examining the link between GCs usage and alterations in the gut microbiota, along with the incidence of GAON, as well as the potential intermediary role of SCFAs. Lastly, in vivo rat experiments were conducted to substantiate the significant bridging function of butyrate, between GC-induced shifts in the gut microbiota and the onset of GAON. Our conclusions were corroborated through a multifaceted approach encompassing genetic studies, observational clinical research, and animal experiment.

Of course, this study inevitably has some limitations. Firstly, due to the limitation of the sample size, the IVs of the gut microbiota obtained are limited, which in turn restricts the statistical power of the MR analysis and may overlook other biologically relevant taxa. Secondly, although we included patients who had been using GCs for over three months with a history of more than 2 g of prednisolone or equivalent hormone use, we did not impose restrictions on the underlying diseases of the included GC users, nor did we conduct subgroup analyses on different types of GCs, dosages, and baseline levels of inflammatory mediators under GC use. Many patients use large amounts of GCs due to inflammatory or autoimmune diseases. GCs can exert anti-inflammatory effects, but they also lead to gut microbiota dysbiosis. Patients may have poorly controlled inflammatory responses or inflammatory translocation after gut microbiota dysbiosis, or an inflammatory response cascade with the underlying disease, which can induce GAON. For instance, the incidence of GAON in patients with Systemic Lupus Erythematosus (SLE) is higher than in patients with other underlying diseases using GCs [41], possibly because serum inflammatory levels in SLE patients are relatively more challenging to stabilize. Furthermore, we only investigated the levels of anti-inflammatory cytokines IL-17 A, IL-33, and TNF-α in the patients' serum, and did not employ metabolomics or proteomics methods to screen for changes in all pro-inflammatory and anti-inflammatory factors in the serum, to fully discuss the extensive link between the inflammatory response following the downregulation of *Lachnospiraceae*-Butyrate levels due to GC use and the occurrence of GAON. Finally, in verifying the protective effect of butyrate on bones, we utilized rat experiments as a proxy for human studies, which may introduce species-specific biases. In the future, we need to conduct a broader analysis of the microbiota and larger-scale clinical trials to verify the therapeutic potential of butyrate supplements.

Conclusion

As shown in Fig. 11, glucocorticoid-induced downregulation of *Lachnospiraceae* and butyric acid levels, along with an upregulation of inflammatory response, may contribute to the development of GAON. Butyric acid is an important mediator in the *Lachnospiraceae*-inflammatory pathway and a valuable bone-protective factor. Therefore, monitoring the abundance of *Lachnospiraceae*, levels of butyric acid, and serum inflammatory markers during GC treatment may be of great significance for the early screening and warning of GAON. Concurrently, butyrate supplementation may represent a potential method for the prophylaxis and treatment of GAON.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12967-024-05813-4.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Acknowledgements

The authors extend heartfelt thanks to all the participants and researchers involved in this study, whose contributions and efforts that have enabled this research to proceed smoothly and yield results.

Author contributions

Conceptualization and initial manuscript drafting: Mingbin Guo. Methodological design: Shuai He. Sample collection and data organization: Wei Song, Jianbin Mai, Xinwei Yuan, Yixuan Huang, and Hongzhong Xi. Project administration: Guangquan Sun and Yugen Chen. Supervision and funding acquisition: Bin Du and Xin Liu. All authors have read and approved the final manuscript.

Funding

This research was supported by Jiangsu Administration of Traditional Chinese Medicine [grant number MS202220], National Natural Science Foundation of China [grant number 82074471 and 81804117] and Jiangsu Provincial Medical Key Discipline (Laboratory)Cultivation Unit [grant number JSDW202252].

Data availability

All data relevant to the study are included in the article or available as online supplemental information. The GWAS datasets associated with this study are available from the MiBioGen Consortium (https://mibiogen.gcc.rug.nl) and FinnGen Research (https://r9.finngen.fi/).

Declarations

Ethics approval and consent to participate

Sample collection was approved by the Ethics Committee at Jiangsu Provincial Hospital of Traditional Chinese Medicine (No. 2023NL-037-01). All volunteers voluntarily donated their own discarded femoral head specimens, stools, blood and signed an informed consent form. All animal experiments were approved by the Animal Ethics Committee of Nanjing University of Chinese Medicine (202306A059), and were conducted according to the National Research Council Guide for Care and Use of Laboratory Animals.

Competing interests

No potential conflict of interest was reported by the author(s).

Received: 28 March 2024 / Accepted: 29 October 2024
Published online: 11 November 2024

References

1. Weinstein RS. Glucocorticoid-induced osteonecrosis. Endocrine. 2012;41(2):183–90. https://doi.org/10.1007/s12020-011-9580-0.
2. Muthu S, Jeyaraman M, Selvaraj P, Jeyaraman N, Potty AG, Gupta A. Dose–response Meta-analysis of Corticosteroid effects in SARS Outbreak: a model for risk stratification and screening strategy for osteonecrosis of femoral Head Post-corticosteroid Therapy for COVID-19. Life (Basel). 2023;13(4):907. https://doi.org/10.3390/life13040907.
3. Weinstein RS. Glucocorticoid-Induced Bone Disease. N Engl J Med. 2011;365(1):62–70. https://doi.org/10.1056/NEJMcp1012926.
4. Sun W, Shi Z, Gao F, Wang B, Li Z. The pathogenesis of multifocal osteonecrosis. Sci Rep. 2016;6(1):29576. https://doi.org/10.1038/srep29576.
5. Chang C, Greenspan A, Gershwin ME. The pathogenesis, diagnosis and clinical manifestations of steroid-induced osteonecrosis. J Autoimmun. 2020;110:102460. https://doi.org/10.1016/j.jaut.2020.102460.
6. Peng J, Yu XJ, Yu LL, et al. The influence of gut microbiome on bone health and related dietary strategies against bone dysfunctions. Food Res Int. 2021;144:110331. https://doi.org/10.1016/j.foodres.2021.110331.
7. Shavandi A, Saeedi P, Gérard P, Jalalvandi E, Cannella D, Bekhit AE. The role of microbiota in tissue repair and regeneration. J Tissue Eng Regen Med. 2020;14(3):539–55. https://doi.org/10.1002/term.3009.
8. Zhou RX, Zhang YW, Cao MM, Liu CH, Rui YF, Li YJ. Linking the relation between gut microbiota and glucocorticoid-induced osteoporosis. J Bone Min Metab. 2023;41(2):145–62. https://doi.org/10.1007/s00774-023-01415-0.
9. Chen CY, Rao SS, Yue T, et al. Glucocorticoid-induced loss of beneficial gut bacterial extracellular vesicles is associated with the pathogenesis of osteonecrosis. Sci Adv. 2022;8(15):eabg8335. https://doi.org/10.1126/sciadv.abg8335.
10. Sjögren K, Engdahl C, Henning P, et al. The gut microbiota regulates bone mass in mice. J Bone Min Res. 2012;27(6):1357–67. https://doi.org/10.1002/jbmr.1588.
11. Zhang J, Lu Y, Wang Y, Ren X, Han J. The impact of the intestinal microbiome on bone health. Intractable Rare Dis Res. 2018;7(3):148–55. https://doi.org/10.5582/irdr.2018.01055.
12. Xu X, Wang M, Wang Z, et al. The bridge of the gut–joint axis: gut microbial metabolites in rheumatoid arthritis. Front Immunol. 2022;13:1007610. https://doi.org/10.3389/fimmu.2022.1007610.
13. Lucas S, Omata Y, Hofmann J, et al. Short-chain fatty acids regulate systemic bone mass and protect from pathological bone loss. Nat Commun. 2018;9:55. https://doi.org/10.1038/s41467-017-02490-4.
14. Yang KL, Mullins BJ, Lejeune A et al. Short Chain Fatty Acids Mitigate Osteoclast-Mediated Arthritic Bone Remodelling. *Arthritis & Rheumatology*. n/a(n/a)(https://doi.org/10.1002/art.42765).
15. Zaiss MM, Jones RM, Schett G, Pacifici R. The gut-bone axis: how bacterial metabolites bridge the distance. J Clin Invest. 129(8):3018–28. https://doi.org/10.1172/JCI128521
16. Emdin CA, Khera AV, Kathiresan S, Mendelian Randomization. JAMA. 2017;318(19):1925–6. https://doi.org/10.1001/jama.2017.17219.
17. Skrivankova VW, Richmond RC, Woolf BAR, et al. Strengthening the reporting of observational studies in epidemiology using mendelian randomisation (STROBE-MR): explanation and elaboration. BMJ. 2021;375:n2233. https://doi.org/10.1136/bmj.n2233.
18. Kurilshikov A, Medina-Gomez C, Bacigalupe R, et al. Large-scale association analyses identify host factors influencing human gut microbiome composition. Nat Genet. 2021;53(2):156–65. https://doi.org/10.1038/s41588-020-00763-1.
19. Kurki MI, Karjalainen J, Palta P, et al. FinnGen provides genetic insights from a well-phenotyped isolated population. Nature. 2023;613(7944):508–18. https://doi.org/10.1038/s41586-022-05473-8.
20. Zhao D, Zhang F, Wang B, et al. Guidelines for clinical diagnosis and treatment of osteonecrosis of the femoral head in adults (2019 version). J Orthop Translation. 2020;21:100–10. https://doi.org/10.1016/j.jot.2019.12.004.
21. Kamat MA, Blackshaw JA, Young R, et al. PhenoScanner V2: an expanded tool for searching human genotype-phenotype associations. Bioinformatics. 2019;35(22):4851–3. https://doi.org/10.1093/bioinformatics/btz469.
22. Slob EAW, Burgess S. A comparison of robust mendelian randomization methods using summary data. Genet Epidemiol. 2020;44(4):313–29. https://doi.org/10.1002/gepi.22295.
23. Floerkemeier T, Budde S, Willbold E, et al. Do biomarkers allow a differentiation between osteonecrosis of the femoral head and osteoarthritis of the hip? - a biochemical, histological and gene expression analysis. Osteoarthritis Cartilage. 2021;29(11):1614–23. https://doi.org/10.1016/j.joca.2021.08.006.
24. Wang Y, Li D, Chen H, Li Z, Feng B, Weng X. Accumulation of Fat not responsible for femoral Head Necrosis, revealed by single-cell RNA sequencing: a preliminary study. Biomolecules. 2023;13(1):171. https://doi.org/10.3390/biom13010171.
25. He H, Li Z, Lu J, et al. Single-cell RNA-seq reveals clonal diversity and prognostic genes of relapsed multiple myeloma. Clin Translational Med. 2022;12(3). https://doi.org/10.1002/ctm2.757.
26. Qiu D, Xia Z, Deng J, Jiao X, Liu L, Li J. Glucorticoid-induced obesity individuals have distinct signatures of the gut microbiome. BioFactors. 2019;45(6):892–901. https://doi.org/10.1002/biof.1565.
27. Wu T, Yang L, Jiang J, et al. Chronic glucocorticoid treatment induced circadian clock disorder leads to lipid metabolism and gut microbiota alterations in rats. Life Sci. 2018;192:173–82. https://doi.org/10.1016/j.lfs.2017.11.049.
28. Schepper JD, Collins F, Rios-Arce ND, et al. Involvement of the gut microbiota and barrier function in glucocorticoid-Induced osteoporosis. J Bone Miner Res. 2020;35(4):801–20. https://doi.org/10.1002/jbmr.3947.
29. Kang HJ, Schepper J, Vidal-Franco M, Parameswaran N, McCabe LR. Lactobacillus reuteri alleviates glucocorticoid induced avascular necrosis of femoral head. FASEB J. 2020;34(S1):1–1. https://doi.org/10.1096/fasebj.2020.34.s1.04882.
30. Lu L, Chen X, Liu Y, Yu X. Gut microbiota and bone metabolism. FASEB J. 2021;35(7):e21740. https://doi.org/10.1096/fj.202100451R.
31. Yu M, Malik Tyagi A, Li JY, et al. PTH induces bone loss via microbial-dependent expansion of intestinal TNF + T cells and Th17 cells. Nat Commun. 2020;11:468. https://doi.org/10.1038/s41467-019-14148-4.
32. Chen X, Zhang Z, Hu Y, et al. Lactulose suppresses osteoclastogenesis and ameliorates Estrogen Deficiency-Induced Bone loss in mice. Aging Dis. 2019;11(3):629–41. https://doi.org/10.14336/AD.2019.0613.
33. Ma J, Guo W, Li Z, Wang B, Li S, Wang P. Hip osteonecrosis is Associated with increased plasma IL-33 level. Mediators Inflamm. 2017;2017:1732638. https://doi.org/10.1155/2017/1732638.
34. Zou D, Zhang K, Yang Y, et al. Th17 and IL-17 exhibit higher levels in osteonecrosis of the femoral head and have a positive correlation with severity of pain. Endokrynol Pol. 2018;69(3):283–90. https://doi.org/10.5603/EP.a2018.0031.
35. Wu X, Xu W, Feng X, et al. TNF-a mediated inflammatory macrophage polarization contributes to the pathogenesis of steroid-induced osteonecrosis in mice. Int J Immunopathol Pharmacol. 2015;28(3):351–61. https://doi.org/10.1177/0394632015593228.
36. Zhang Q, Sun W, Li T, Liu F. Polarization behavior of bone macrophage as Well as Associated Osteoimmunity in Glucocorticoid-Induced osteonecrosis of the femoral head. JIR. 2023;16:879–94. https://doi.org/10.2147/JIR.S401968.
37. Zheng J, Yao Z, Xue L, Wang D, Tan Z. The role of immune cells in modulating chronic inflammation and osteonecrosis. Front Immunol. 2022;13:1064245. https://doi.org/10.3389/fimmu.2022.1064245.
38. Zaplana T, Miele S, Tolonen AC. Lachnospiraceae are emerging industrial biocatalysts and biotherapeutics. Front Bioeng Biotechnol. 2024;11:1324396. https://doi.org/10.3389/fbioe.2023.1324396.
39. Abdugheni R, Wang WZ, Wang YJ, et al. Metabolite profiling of human-originated Lachnospiraceae at the strain level. iMeta. 2022;1(4):e58. https://doi.org/10.1002/imt2.58.

40. He J, Chu Y, Li J, et al. Intestinal butyrate-metabolizing species contribute to autoantibody production and bone erosion in rheumatoid arthritis. Sci Adv. 2022;8(6):eabm1511. https://doi.org/10.1126/sciadv.abm1511.
41. Shigemura T, Nakamura J, Kishida S, et al. Incidence of osteonecrosis associated with corticosteroid therapy among different underlying diseases: prospective MRI study. Rheumatology. 2011;50(11):2023–8. https://doi.org/10.1093/rheumatology/ker277.

Publisher's note