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Microbiome-derived bile acids link elevated antigenic response and bone erosion in rheumatoid arthritis

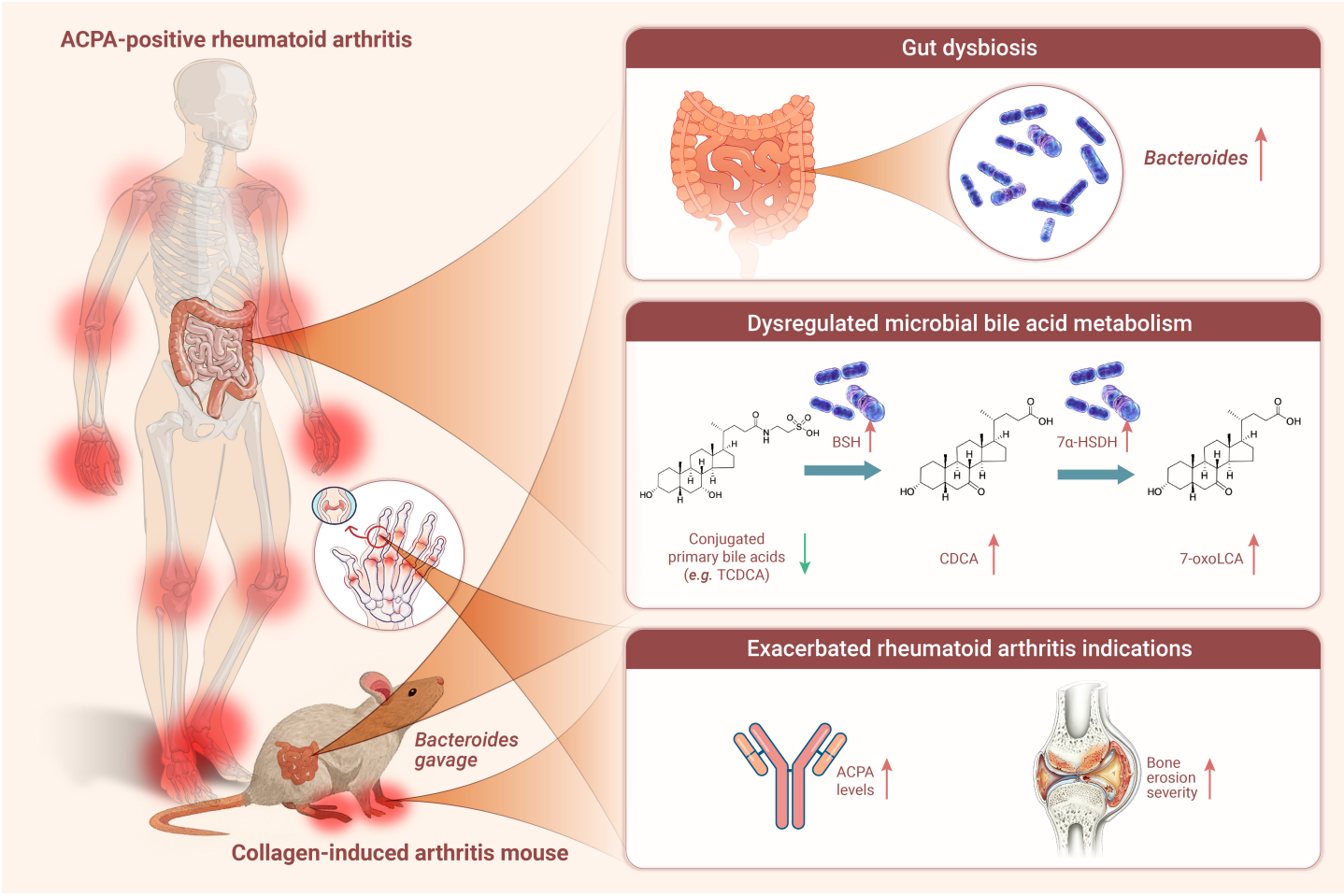
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GRAPHICAL ABSTRACT



PUBLIC SUMMARY

- *Bacteroides* enrichment characterizes the gut microbiome of rheumatoid arthritis (RA) patients.
- Gut microbiome alterations are associated with microbial and circulating metabolic shifts.
- Altered bile acid metabolism links the gut microbiome to immune responses and bone erosion in RA.
- *Bacteroides* enrichment remodels bile acid metabolism and promotes inflammation and bone erosion.

REPORT Basic Medicine

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Microbiome-derived bile acids link elevated antigenic response and bone erosion in rheumatoid arthritis

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Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by chronic inflammation and progressive bone erosion. It has been widely recognized that gut microbial dysbiosis is an important contributor to the pathogenesis of RA. Yet, the metabolites that mediate the impacts of the gut microbiome on RA are less well understood. This study investigates the gut microbiota-bile acid axis as a critical contributor to RA pathogenesis. Analysis of fecal and serum samples from a discovery set revealed profound yet diverse gut dysbiosis and metabolic perturbations in RA patients. In those *Bacteroides*-dominated RA patients, *Bacteroides* species expressing bile salt hydrolase (BSH) and 7α-hydroxysteroid dehydrogenase (HSDH) were elevated, leading to increased secondary bile acid production. This gut microbiota-bile acid axis was strongly correlated with elevated anti-citrullinated peptide antibody (ACPA) levels and enhanced bone erosion severity in this subgroup of RA patients. The observations were also supported by the patients from a validation set in which ACPA-positive patients had more abundant bacteria expressing BSH and 7α-HSDH, together with dysregulated microbial bile acid metabolism and more severe bone erosion as compared with ACPA-negative ones. *Bacteroides* treatment in collagen-induced arthritis mice significantly aggravated arthritis severity and bone erosion, increased pro-inflammatory cytokine levels, and disturbed microbial bile acid metabolism in a manner that disrupted FXR-FGF15 signaling, mirroring the findings in human cohorts. These results provide insights into the role of gut dysbiosis in RA in a manifestation-specific manner, as well as the functions of bile acids in this gut-joint axis, which may be a potential intervention target for controlling RA conditions.

INTRODUCTION

Rheumatoid arthritis (RA) is a systemic autoimmune disease causing intensive pain, inflammation, and joint destruction, which could lead to deformity, unsteadiness and disability.[1] RA is a clinically and immunologically heterogeneous disease, classically stratified into anti-citrullinated peptide antibody (ACPA)-positive and rheumatoid factor (RF)-positive subtypes.[2] ACPA and RF often precede clinical manifestations of RA, and their presence is influenced by distinct genetic and environmental risk factors.[1,2] Notably, while both antibodies are used diagnostically, ACPA is more specific to RA and has stronger associations with disease severity, particularly bone erosion.[3]

Accumulating evidence indicates that the gut microbiome serves as an important environmental factor related to the pathology of this disease.[4-8] Alterations in the gut microbiota were observed in RA patients and partially normalized after therapy.[4] It was reported that microbial dysbiosis contributed to the development of joint inflammation in genetically arthritis-prone SKG mice.[9] Particularly, human leukocyte antigen alleles influence the predisposition to RA through interaction with the intestinal microbiome.[5,10] The gut microbiota can interact with the immune system to modulate the balance between Th17 and Treg cells, and the production of pro-inflammatory cytokines such as IL-17A and TNF-α.[11] Alterations in microbial composition have been associated with disease activity, autoantibody status, other autoimmune markers, and the extent of bone erosion in RA patients.[11-13] Previous microbiome studies in RA mainly focused on describing taxonomic differences between RA patients and healthy controls.[4,14-16] However, these studies were largely limited to compositional analyses, without linking microbial shifts to metabolic consequences. Moreover, most cohorts contained heterogeneous RA populations, making it difficult to identify phenotype-specific microbial signatures.

Emerging evidence suggests that the gut microbiome and its products can affect immune homeostasis and host susceptibility to immune-mediated diseases and disorders.[17] Although the mechanisms have not yet been systematically studied, the alterations in the microbiota in RA likely result in changes in bacterial products that cross the intestinal barrier and act as crucial signals or priming stimuli for the innate immune system.[11] The fecal metabolome provides a functional readout of microbial activities that mediate host-microbe interactions.[18] Besides, the human gut microbiota produces dozens of metabolites that can be transferred into the bloodstream, where they can have systemic effects on the host.[19,20] Thus, fecal and serum metabolomics are useful tools to investigate the metabolic changes in response to endogenous or exogenous stimuli and the crosstalk between the intestinal microbiota and the host. For instance, immunomodulators such as short-chain fatty acids derived from microbial fermentation of dietary fibers in the intestine have been revealed to protect against inflammation-induced bone loss and inhibit osteoclast differentiation and bone resorption.[6] Nevertheless, investigations have rarely focused on fecal metabolomics in RA and illustrated the role of microbiome-derived metabolites.

Herein our study aimed to investigate RA-associated biomarkers and discover the association between metabolome perturbation and microbial dysbiosis under a rheumatic state. We combined microbial profiling and metabolomic profiling of fecal and serum samples from RA subjects and healthy control (HC) individuals in a 94-member discovery set. One of our major observations was that RA-elevated *Bacteroides* enriched secondary bile acid metabolism and showed positive links with the antigenic response in RA. In another independent validation set, targeted quantification of bile acids and shotgun metagenomic sequencing demonstrated the associations

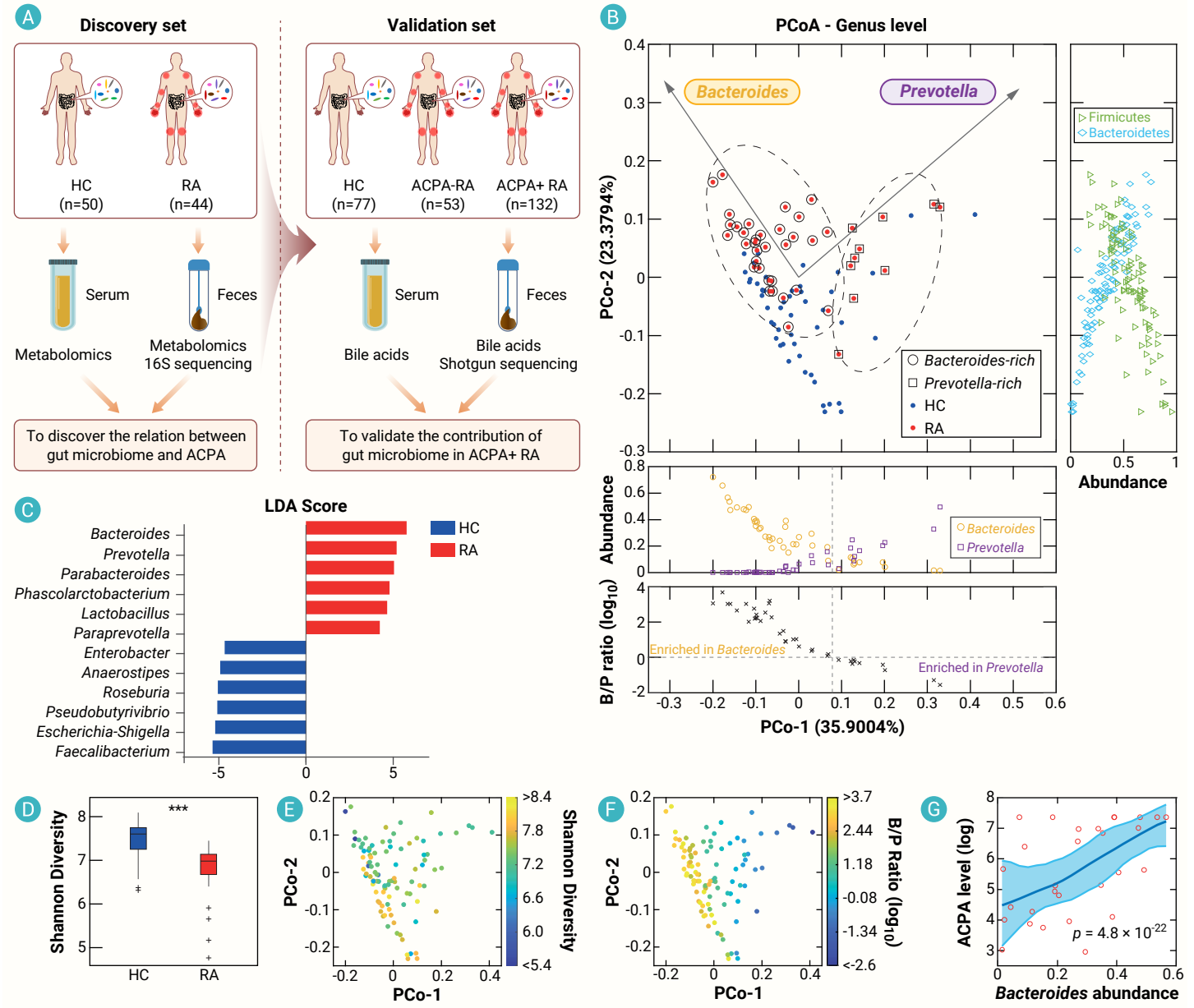


Figure 1. Gut microbial alterations between rheumatoid arthritis (RA) and healthy control (HC) groups (A) Overview shows the study setting. ACPA, anti-citrullinated peptide antibody. (B) Principal Coordinates Analysis (PCoA) at the genus level based on the distance method of Jensen-Shannon Divergence. Each point represents one sample. Scatter plots of the relative abundances of the phyla Firmicutes and Bacteroidetes (PCo2) and the genera *Bacteroides* and *Prevotella* (PCo1) in each sample are shown across the corresponding principal component. (C) Linear discriminant analysis (LDA) scores of differentially abundant genera by LDA Effect Size. (D) Shannon index of the two groups estimated with the number of OTUs in each sample that gradually changes over the ordination of samples in Jensen-Shannon distance-transformed space. Median and the first and third quartiles are represented by solid lines (Mann-Whitney test, ***$p < 0.001$). (E) Shannon diversity index of each sample. (F) *Bacteroides*/*Prevotella* ratio (B/P ratio) in each sample over the ordination of samples in Jensen-Shannon distance-transformed space. (G) The correlation between *Bacteroides* abundance and anti-citrullinated peptide antibody (ACPA) level determined using the robust LOWESS (locally weighted scatterplot smoothing) method. The shaded area indicates the range of the 94% confidence interval (from the 3rd to the 97th percentiles) through bootstrap resamplings.

among the gut microbiome, secondary bile acids, antigenic response, and bone erosion in RA, validating the previous findings. The association was further validated in the collagen-induced arthritis (CIA) mouse model with *Bacteroides* treatment. Our study identified the role of bile acid metabolism in host-microbiome interactions of RA patients, especially in those whose gut microbiotas were dominated by the *Bacteroides* genus, making our findings a useful resource for the study of potential intervention targets for RA.

MATERIALS AND METHODS

Clinical sample collection

In the study, we collected serum and/or stool samples from subjects in two independent cohorts (50 healthy subjects and 44 RA patients in the discovery set; 77 healthy subjects, 53 ACPA- RA patients, and 132 ACPA+ RA patients in the validation set) recruited from Guanghua Hospital, Shanghai, China (Figure 1A, Table S1). Healthy volunteers were enrolled during routine physical examination. We recruited individuals with RA aged between 18 and 75 years who were diagnosed with RA with a disease duration of at least 6 months and met the American College of Rheumatology (ACR) criteria and the selection criteria. Given that the gut microbiome and metabolites vary with many different factors, including diet, diseases and medication, subjects were selected according to the following criteria to balance the baseline of the two groups. Individuals with different habits (*e.g.*, a complete vegetarian diet, alcohol or yogurt consumption) that may influence gut microbiome were excluded. In addition, individuals who suffered from other diseases, such as kidney, liver, lung, cardiovascular, hematologic diseases, other autoimmune diseases, mental diseases, and metabolic diseases, were excluded, especially individuals who were experiencing gastrointestinal diseases. Women who were pregnant, breastfeeding, or planning to be pregnant were excluded.

Furthermore, individuals continuously receiving medications, especially disease-modifying antirheumatic drugs, nonsteroidal anti-inflammatory drugs, corticosteroids, or painkillers for over six months, and individuals who received the aforementioned medications within one month were excluded (Table S2). Furthermore, individuals who had received probiotics or antibiotics within two months were excluded. Clinical information of subjects was collected upon their first visit to the hospital according to standard procedures. Serum and fecal samples were quenched within 30 min after collection. All samples were stored at -80 °C until analysis. Informed consent was obtained from all participants or their legal representatives before sample collection. Questionnaires including diet information, dietary habits, smoker/nonsmoker status and basic treatment were administered. Dietary information was collected based on self-reported food consumption frequencies and dietary habits, including breakfast frequency, special dietary patterns (low-fat, low-sodium, and high-fiber diets), consumption of staple foods (rice and cereals), potatoes, meat and eggs, vegetables, fruits, legumes, dairy products, fermented foods, beverages (water, tea, and coffee), soup, and alcohol intake, as well as nutrient-derived variables (total fat and alcohol intake). The study was approved by the Ethics Committee of Guanghua Hospital, Shanghai, China (2023-K-42). Informed consent was obtained from all participants or their legal representatives.

Collagen-induced arthritis in mice

DBA/1 mice (male, 7-8 weeks old) were purchased from GemPharmatech. *B. ovatus* (BNCC359491) and *B. thetaiotaomicron* (BNCC354380) were purchased from BNCC (BeNa Culture Collection, China). *B. dorei* (DSM17855) was commercially obtained from DSMZ (Germany). The bacteria were administered to the mice by oral gavage at a dose of 2.5×10^9 cfu/100 μL, 5 times per week. Arthritis was induced by intradermal injection of 100 μL of complete Freund's adjuvant containing 100 μg of chick type II collagen at the base of the tail on the first day and a booster immunization on day 21. Beginning on day 28, the mice were assessed for disease severity twice per week. The severity of arthritis was assessed by grading the four limbs of CIA mice according to previous criteria.[21] All protocols used in the mouse study received approval from the Committees of Animal Ethics and Experimental Safety of the Hong Kong Baptist University (REC/23-24/0240).

Metabolic profiling of human samples

Fecal metabolites were extracted via sonication from freeze-dried feces with chilled water and methanol. Serum metabolites were extracted from 50 μL of serum through deproteinization with 200 μL of methanol containing internal standards. After centrifugation, the supernatants were collected and lyophilized. The residue was redissolved for the subsequent global metabolomics analysis using an ultra-high liquid chromatograph-mass spectrometer and gas chromatography-mass spectrometry according to a previously reported method.[22] Quality control (QC) samples were prepared by pooling an equal volume of each sample together to monitor the repeatability and stability of LC-MS analysis. The samples were analyzed randomly. MS data were processed according to a previously reported method.[23] The metabolic features were annotated following Metabolomics Standards Initiative (MSI) guidelines[24] by comparing accurate mass, retention time, MS/MS pattern and isotope pattern of metabolites in QC samples with those of authentic standards or databases, *e.g.*, METLIN (www.metlin.scripps.edu), HMDB (www.hmdb.ca/), mzCloud (www.mzcloud.org), MassBank of North America (mona.fiehnlab.ucdavis.edu/), *etc.* Enrichment analysis was performed through MetaboAnalyst 6.0 (www.metaboanalyst.ca/). In consideration of human and microbial metabolomes, enrichment analysis was done using a pathway library built based on those of *Homo sapiens* and bacteria from the KEGG database.

Targeted analysis of bile acids

Methanol containing internal standards (cholic acid-d_4, deoxycholic acid-d_4, glycocholic acid-d_4, lithocholic acid-d_4) was added to serum, and then vortexed to extract the metabolites. Freeze-dried feces were homogenized in a mixture of chilled methanol/water (4:1, v/v) and internal standards. After centrifugation, the supernatants were lyophilized. The residues were dissolved for subsequent analysis.

Fifty-four bile acids (Table S3) in serum and fecal samples were quantified by Ultimate 3000 RSLC coupled to QuantivaTM triple-quadrupole MS (Thermo Scientific, USA). An ACQUITY UPLC BEH C18 column (100 mm × 2.1 mm, 1.7 μm, Waters, USA) was applied for metabolite separation at 45 °C. The mobile phases were water and acetonitrile, both containing 0.01% formic acid. The flow rate was 0.35 mL/min. The LC gradient program was as follows: 0-0.5 min, 25 % B; 14 min, 40 % B; 25 min, 67.5 % B; 25.5-28.5 min, 100 % B. The MS was equipped with a heated electrospray ionization source in negative ion mode. The spray voltage was 2.6 kV. The pressure of the sheath and auxiliary gas were set at 30 and 10 arb, respectively. The ion transfer tube temperature and vaporizer temperature were 300 °C. The CID gas was set at 2 mTorr. Data acquisition was performed using the selected reaction monitoring mode. Raw data were processed using TraceFinder software (version 4.1, Thermo Scientific, USA). Metabolite concentrations were quantified based on calibration curves generated from corresponding authentic standards. Calibration curves were established by plotting the peak area ratio of each analyte to the internal standard against the nominal concentration of the standard using a weighted ($1/X^2$) linear regression model. The intra-assay coefficients of variation (CVs) for all metabolites were below 20.0%, as evaluated using quality control samples injected every 20 samples throughout the analytical sequence.

Radiological evaluation by modified Sharp's method

The radiological evaluation of the hands was done by two qualified doctors. The readers of the radiographs were blind to the order of the radiographs and the clinical information of RA patients. The scores of bone erosion and narrowing/(sub)luxation were estimated according to the modified Sharp/van der Heijde score (SHS) method.[25]

Micro-computed tomography

Micro-computed tomography (micro-CT) imaging and analysis were performed on a vivaCT 40 system (Scanco Medical, Switzerland). Imaging was carried out at a voxel size of 17.5 μm, a voltage of 70 kV with a current of 114 μA, and an integration time of 200 ms. The volume of interest in the tibial proximal metaphysis started from 0.25 mm distal to the growth plate.

Transcriptome sequencing

Total RNA was extracted from mouse liver and bone tissues. For bone RNA extraction, the diaphyses of the right tibia and femur were isolated after removing all surrounding soft tissues, including muscles, tendons, ligaments, and periosteum. Bone samples were rinsed with phosphate-buffered saline and cut into small fragments before RNA extraction.[26] Poly(A)+ mRNA was enriched from total RNA using oligo(dT)-coupled magnetic beads, followed by fragmentation and reverse transcription using random hexamer primers. Double-stranded cDNA was synthesized, end-repaired, A-tailed, ligated to sequencing adapters, PCR-amplified, and circularized to construct sequencing libraries. Qualified libraries were sequenced on the DNBSEQ platform (BGI, Shenzhen, China). Raw sequencing reads were subjected to quality control using SOAPnuke by removing adapter-contaminated reads, reads containing >1% ambiguous bases (N), and low-quality reads. Clean reads were aligned to the mouse reference genome using HISAT2, and gene expression levels were quantified.

Statistical analysis

Statistical analysis was performed using R (v3.6) and SPSS. Data distribution was tested by using the Shapiro-Wilk test. β-diversity analyses were performed using Bray-Curtis distances, which are widely used and appropriate for relative abundance data. The significance of differential gut microbiome and metabolome was examined by a two-tailed Wilcoxon rank-sum test between HC and RA groups. Differential abundance of the gut microbiome and metabolites in ACPA+ RA, ACPA- RA and HC individuals was assessed by one-way ANOVA. Adjusted *p* values were calculated by the Benjamini-Hochberg correction for multiple tests. Associations between the clinical index and the gut microbiome as well as metabolomes were assessed by boosted additive generalized linear models after adjustment for age, sex, and BMI using MaAsLin2 package. To evaluate whether RA-associated microbial alterations were independent of potential confounding factors,

including age, sex, BMI, and dietary patterns, PERMANOVA was performed based on the microbiome distance matrix. Dietary variables reflecting fiber, fat, and protein intake were combined by principal component analysis and the first principal component (Diet_PC1) was included as a composite dietary pattern indicator.

RESULTS

Gut microbiome dysbiosis in response to RA

We investigated the microbial compositions of fecal samples from a discovery set consisting of 44 RA patients and 50 healthy subjects by using 16S rRNA amplicon sequencing (Figures 1A & S1A-B, Table S1). PERMANOVA and canonical correspondence analysis showed that the gut microbiome of RA and HC groups separated along the vector for RA status, confirming that microbial variations were associated mainly with RA status rather than with other covariates (age, sex, BMI and diet) (Table S4, Figure S1C). The principal coordinates analysis (PCoA) at the genus level was applied to examine the principal driving factors of microbial variance (Figure 1B). The plot of PCoA revealed a significant dissimilarity of microbial composition between the healthy control (HC) and RA groups (PERMANOVA, $p < 0.001$), mainly explained by PCo-2. The PCo-2 dimension was significantly associated with the abundances of the phyla Firmicutes and Bacteroidetes, which explained about 23.4% of sample variations. We observed that RA individuals had more Bacteroidetes ($p < 0.001$) and fewer Firmicutes ($p < 0.01$) than control individuals (Figures S1A & D), which was consistent with the previous reports.[27,28] The first principal coordinate (PCo-1, explaining 35.9% variation), however, was strongly correlated with the abundances of two Bacteroidetes genera, *Prevotella* and *Bacteroides* (Figure 1B), which have been defined as two major indicators of human gut enterotypes.[29] As reported in the previous studies,[30] *Bacteroides*-dominated and *Prevotella*-dominated constitutions were found to be relatively non-overlapping (Figure 1B). *Bacteroides* and *Prevotella* predominated in the gut microbiome of two subgroups of RA subjects, respectively, contributing more variation among samples than the difference between the RA and control individuals. Previous studies have identified both *Prevotella*-rich and *Bacteroides*-rich microbial composition in fecal samples from the RA patients.[8,9,31] In addition, linear discriminant analysis (LDA) confirmed that *Bacteroides* and *Prevotella* were the top two genera that significantly contributed to the distinction between RA and HC (Figure 1C). In addition to *Bacteroides* and *Prevotella*, genera *Parabacteroides, Phascolarctobacterium*, *Lactobacillus,* and *Paraprevotella* were enriched in RA patients, whereas *Faecalibacterium*, *Escherichia-Shigella*, *Pseudobutyrivibrio*, *Roseburia, Anaerostipes*, and *Enterobacter* were found to be less abundant in RA (Figure 1C). Among these, *Pseudobutyrivibrio* was previously reported to be negatively associated with psoriatic arthritis.[32] The control-enriched genera *Roseburia* and *Faecalibacterium* were producers of butyrate, a metabolite involved in the regulation of immune and bone homeostasis.[6,33,34]

Differences in the taxonomic composition of the two groups led to varying community properties, such as richness and evenness. RA individuals presented lower alpha diversities (Shannon index, ACE index, Chao index, Fisher index, and Simpson index, $p < 0.0001$, Figures 1D & S1E) than control individuals, suggesting that bacterial richness and evenness were lower in RA patients than in control individuals. The reduction in microbial richness was found in both *Bacteroides*- and *Prevotella*-dominated RA patients (Figure 1E). In contrast, the *Bacteroides*/*Prevotella* ratio changed in the subjects, independent of the disease conditions (Figure 1F). In ACPA-seropositive RA patients, we found that *Bacteroides* positively correlated with serum ACPA (Figure 1G). Together, these findings revealed that the gut microbiome of RA individuals showed great dissimilarity to healthy individuals but the gut microbial changes in RA were strongly correlated with the RA manifestations, suggesting potential roles of microbial shifts in this disease.

Fecal and serum metabolic shifts related to the gut microbiome

Because gut lumen metabolites and circulating metabolomes represent a distinctive avenue for probing microbiome-host interaction, we performed nontargeted metabolomics on both fecal and serum samples from 94 subjects in the discovery set to examine alterations of microbiota-associated metabolites in response to RA. A total of 467 and 292 compounds were identified with the Metabolomics Standards Initiative level 1 or 2 identification[24] in fecal and serum metabolic profiling, respectively (Tables S5-6). Out of them, 164 compounds overlapped in the two sample types (Figure S2A). The metabolic profiling of feces and serum distinguished RA individuals from the HC group, as illustrated in the partial least squares discriminant analysis (PLS-DA) score plot (Figures 2A-B), suggesting metabolic changes occurred in RA. To investigate the extent to which the gut microbiome was related to fecal and circulating metabolites in the host, we evaluated the inherent correlations between microbiome and metabolome in the two-way orthogonal partial least squares (O2PLS) model. Both metabolomes in fecal and serum samples showed significant correlations with the gut microbiome (Figures S2B-C), thereby supporting the notion that microbial and circulating metabolomes provide a functional readout of microbial activities and disease.

Next, we examined the overall pathway changes of fecal and serum metabolites using MetaboAnalyst.[35] The results of altered pathways revealed distinct metabolic shifts between fecal and serum samples (Figure 2C). The top altered microbial pathways in feces and serum included bile acid biosynthesis, tryptophan metabolism, and aminoacyl-tRNA biosynthesis. Interestingly, accumulating evidence has implicated that bile acids and tryptophan catabolites produced by the gut microbiome are crucial mediators in the cross-talk between the host and the gut microbiome.[36,37] To further investigate the role of microbial bile acid metabolism in the disease, we performed targeted quantification using LC-MS/MS to get a thorough profile of bile acids (Figures 2D-F). No difference in the total fecal and serum bile acids was observed between the RA and HC groups (Figure 2D). Nevertheless, the constitution of the fecal bile acid pool differed between the groups. Patients with RA had lower proportions of conjugated primary bile acids and conjugated secondary bile acids but a higher percentage of unconjugated secondary bile acids in feces (Figures 2E & G). Reduced levels of serum fibroblast growth factor-19 (FGF-19) (Figure 2H), a regulator of bile acid metabolism, suggested evidence of impaired farnesoid X receptor (FXR)-mediated signaling in RA.[38]

The observed metabolic alterations in RA patients evidenced that different microbial communities have varying metabolic properties, aligning with the previous report that the microbiome is one of the strongest determinants of circulating metabolomes.[39] The dominant *Bacteroides* is well known to be involved in microbial bile acid metabolism.[37] Bile acids were highlighted in both fecal and serum metabolome (Figure 2C, Tables S5-6), inspiring our subsequent study on bile acids. The bacterial genera expressing bile salt hydrolase (BSH), which is involved in the deconjugation of glyco- or tauro-conjugated bile acids, included *Bacteroides*, *Clostridium*, *Bifidobacterium* and *Lactobacillus*.[26,37,40] After deconjugation, the unconjugated bile acids were modified via 7α-dehydroxylation catalyzed by multiple enzymes to produce deoxycholic acid (DCA) and lithocholic acid (LCA), or by hydroxysteroid dehydrogenase (HSDH) to produce iso-, allo- and oxo-bile acids.[37,40] We found an increase in the abundance of the bacterial genera expressing BSH and 7α-HSDH[26,37,40] (Figure 2I), leading to the reduction of fecal conjugated bile acids, such as glycocholic acid (GCA), glycochenodeoxycholic acid (GCDCA), glycohyocholic acid (GHCA), taurodeoxycholic acid (TDCA) and taurolithocholic acid (TLCA) (Figure 2J). Decreased circulating LCA (Figure 2K) suggested downregulated 7α-dehydroxylation in the microbiome of RA patients. In brief, these results indicated that the altered gut microbiota, especially the augmentation of *Bacteroides* in RA individuals, was associated with bile acids in feces and host circulation.

Gut microbiome and microbiome-derived bile acids associated with clinical indices

To ascertain the associations between clinical indices and microbial alterations, we performed boosted additive generalized linear models. After adjusting the data by age, sex and BMI, we found that *Bacteroides* positively correlated with ACPA, but negatively associated with the levels of RF (IgM and IgG) as well as erythrocyte sedimentation rate (ESR), a marker of inflammation in the body (Figure 3A). On the contrary, RA-enriched *Prevotella* was positively associated with RF IgM, RF IgG and ESR but not with ACPA. These results suggested that *Bacteroides*- and *Prevotella*-rich gut microbial composition may be associated with distinct antigenic responses of ACPA and RF in the host. Intriguingly, we also found that the genera expressing

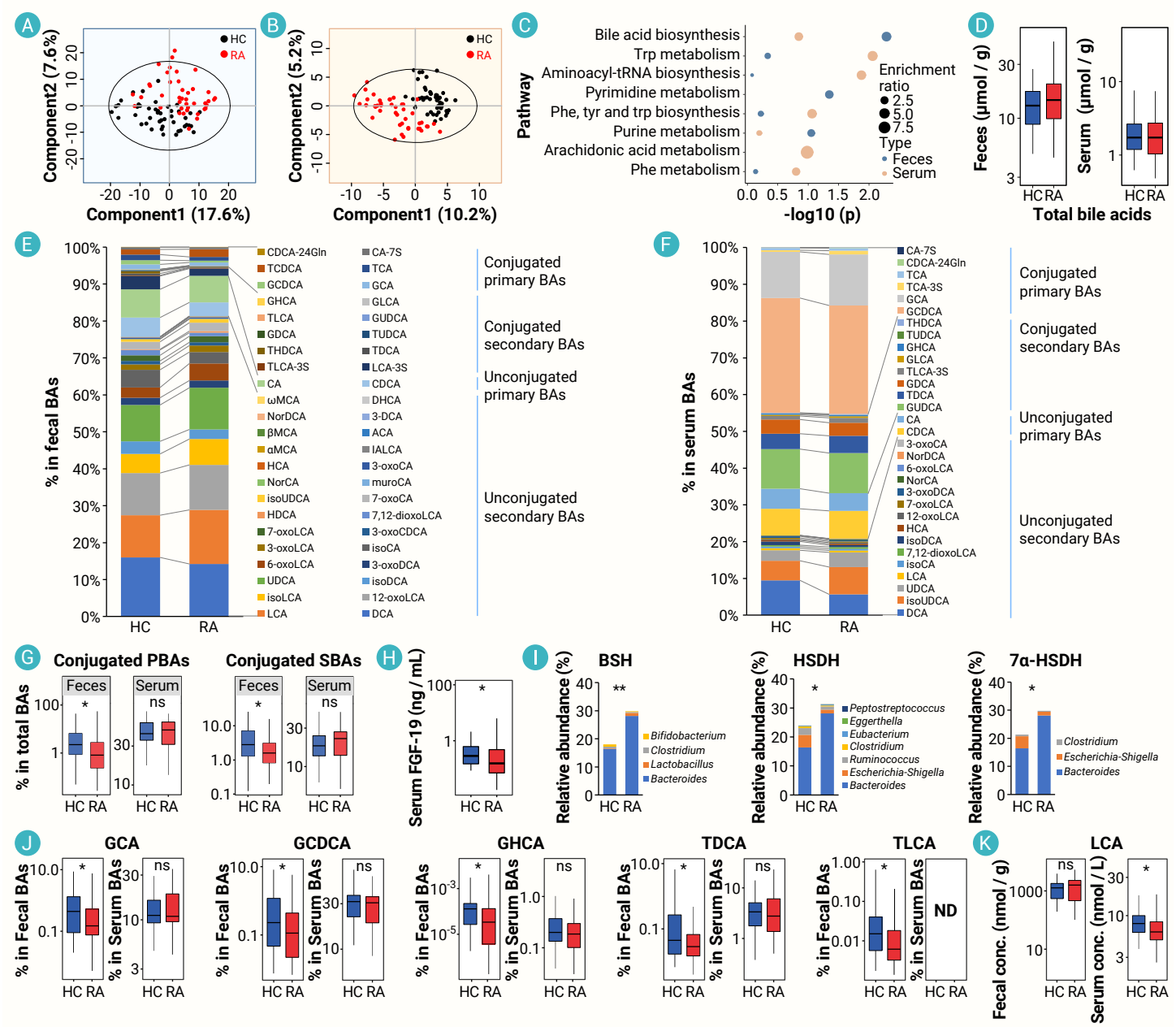


Figure 2. Metabolic shifts relate to the gut microbiome (A-B) PLS-DA plots of fecal (A) and serum (B) metabolomes. (C) Enrichment analysis reveals perturbation of metabolic pathways in feces and serum in response to RA. Trp, tryptophan; Phe, phenylalanine; Tyr, tyrosine. (D) Total bile acid content in fecal and serum samples. (E-F) Components of bile acids in feces (E) and serum (F). (G) Proportion of conjugated primary bile acids and conjugated secondary bile acids. (H) Serum concentration of FGF-19. (I) Average abundance of genera expressing specific enzymes involved in bile acid synthesis. (J) Percentage distribution of bile acids in feces and serum. (K) Fecal and serum concentrations of LCA. * $p < 0.05$, ** $p < 0.01$, ns not significant. BA, bile acid; 12-oxoLCA, 12-ketolithocholic acid; 3-DCA, 3-deoxycholic acid; 3-oxoCA, 3-dehydrocholic acid; 3-oxoCDCA, dehydrochenodeoxycholic acid; 3-oxoDCA, 3-oxodeoxycholic acid; 3-oxoLCA, dehydrolithocholic acid; 6-oxoLCA, 6-ketolithocholic acid; 7,12-dioxoLCA, 7,12-diketolithocholic acid; 7-oxoCA, 7-ketodeoxycholic acid; 7-oxoLCA, 7-ketolithocholic acid; ACA, allocholic acid; CA, cholic acid; CA-7S, cholic acid 7-sulfate; CDCA, chenodeoxycholic acid; CDCA-24Gln, chenodeoxycholic acid 24-acyl-β-d-glucuronide; DCA, deoxycholic acid; DHCA, dehydrocholic acid; GCA, glycocholic acid; GCDCA, glycochenodeoxycholic acid; GDCA, glycodeoxycholic acid; GHCA, glycohyocholic acid; GLCA, glycolithocholic acid; GUDCA, glycoursodeoxycholic acid; HCA, hyocholic acid; HDCA, hyodeoxycholic acid; IALCA, alloisolithocholic acid; isoCA, isocholic acid; isoDCA, isodeoxycholic acid; isoLCA, isolithocholic acid; isoUDCA, isoursodeoxycholic acid; LCA, lithocholic acid; LCA-3S, lithocholic acid-3-sulfate; muroCA, murocholic acid; NorCA, norcholic acid; NorDCA, 23-nordeoxycholic acid; TCA, taurocholic acid; TCA-3S, taurocholic acid 3-sulfate; TCDCA, taurochenodeoxycholic acid; TDCA, taurodeoxycholic acid; THDCA, taurohyodeoxycholic acid; TLCA, taurolithocholic acid; TLCA-3S, taurolithocholic acid-3-sulfate; TUDCA, tauroursodeoxycholic acid; UDCA, ursodeoxycholic acid; αMCA, α-muricholic acid; βMCA, β-muricholic acid; ωMCA, ω-muricholic acid; BSH, bile salt hydrolase; HSDH, hydroxysteroid dehydrogenase.

BSH and HSDH[38] showed positive correlations with ACPA (Figure 3B). Consistently, we observed that ACPA was associated with increased fecal hyodeoxycholic acid (HDCA), serum taurohyodeoxycholic acid (THDCA) and glycoursodeoxycholic acid (GUDCA) (Figure 3C). Conversely, ACPA negatively correlated with 12-ketolithocholic acid (12-oxoLCA), 3-dehydrocholic acid (3-oxoCA), dehydrochenodeoxycholic acid (3-oxoCDCA), isodeoxycholic acid (isoDCA), isolithocholic acid (isoLCA), DCA, LCA, 23-nordeoxycholic acid (NorDCA) and glycolithocholic acid (GLCA), indicating that bile acids may be potential molecules linking gut microbiota and ACPA in RA patients. Moreover, norcholic acid (NorCA), which is an FXR antagonist,[41] positively correlated with clinical indicators, including RA duration, C-reactive protein (CRP), disease activity score 28 (DAS28), ESR, RF IgM, RF IgG and RF IgA. Fecal GCA is negatively associated with DAS28(3). These findings highlight that the microbiome and its derived bile acids underlay the clinical characteristics of RA patients.

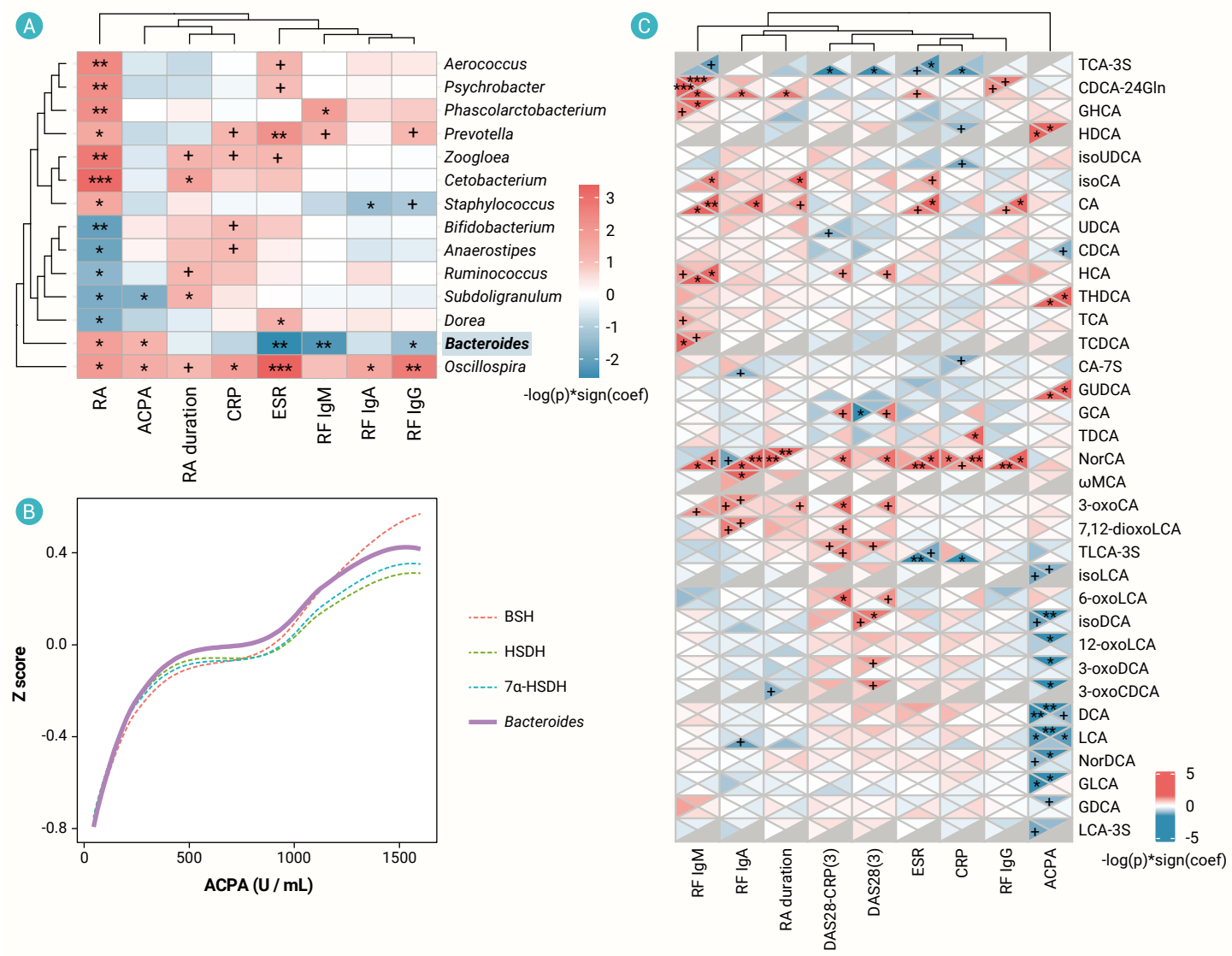


Figure 3. Gut microbiome and microbial bile acid shifts reflect antigenic response in RA patients (A) Correlations between RA-associated genera and clinical indices calculated by boosted additive generalized linear models after adjustment for age, sex, and BMI. (B) Loess curves showed the correlation between ACPA and the genera involved in bile acid metabolism. (C) Heatmap showed that the dysregulation of bile acid metabolism related to RA phenotypes. In every rectangular lattice, the up and down triangles denoted the association between clinical indices and bile acid concentrations in fecal and serum samples, respectively. The left and right triangles represented the association between clinical indices and the percentages of bile acids after adjustment for age, sex, and BMI in fecal and serum samples, respectively. In (A) and (C), $^{+}p < 0.1$, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. Red and blue colors denoted positive and negative associations, respectively. The grey color represented that bile acids were not detected in samples or excluded according to the quality control. ACPA, anti-citrullinated peptide antibody; BSH, bile salt hydrolase; CRP, C-reactive protein; RF, rheumatoid factor; DAS, disease activity score; ESR, erythrocyte sedimentation rate; HSDH, hydroxysteroid dehydrogenase.

Microbial bile acids contributed to elevated ACPA expression and bone erosion in RA

To confirm the robustness and reproducibility of the association between the gut microbiome and ACPA in RA, we validated the correlation in another independent cohort: 132 $ACPA^{+}$ RA patients, 53 $ACPA^{-}$ RA patients, and 77 HC controls. We performed shotgun metagenomic sequencing and targeted bile acid profiling of fecal and/or serum samples from this validation set (Figure 1A, Table S1). $ACPA^{+}$ RA patients exhibited lower α diversities compared to both HC and $ACPA^{-}$ RA groups (Figures 4A & S3A), indicating reduced bacterial richness in this group. Additionally, β diversity of the gut microbiome was higher in the $ACPA^{+}$ RA patients, suggesting a more heterogeneous microbial community structure than in the $ACPA^{-}$ RA and HC groups (Figure 4B). At the genus level, key taxa identified in the discovery set showed consistent directional changes in the validation cohort. Specifically, *Bacteroides*, *Lactobacillus,* and *Parabacteroides* were enriched in the $ACPA^{+}$ RA group, whereas butyrate-producing genera, including *Roseburia*, were depleted (Figure 4C). The species-level resolution afforded by the shotgun metagenomic data provided further insights, revealing significant enrichment of *Bacteroides* species, *B. dorei*, *B. ovatus*, and *B. thetaiotaomicron*, in the $ACPA^{+}$ RA individuals (Figures 4D-E). Notably, these species harbored genes encoding BSH enzymes,[26,37,40] and increased abundance of these genes might lead to the reduced levels of glyco-conjugated or tauro-conjugated bile acids, including GCDCA, TDCA and TLCA (Figures 4F & S4). In addition, *B. thetaiotaomicron* expressed genes encoding 7α-HSDH, the enzyme that converts chenodeoxycholic acid (CDCA) to 7-ketolithocholic acid (7-oxoLCA).[42]

Compared to the other two groups, $ACPA^{+}$ RA patients exhibited elevated levels of CDCA, 7-oxoLCA, ursodeoxycholic acid (UDCA), tauroursodeoxycholic acid (TUDCA) and GUDCA, along with reduced amounts of 3-oxoDCA, 3-oxoLCA, 3-oxoCDCA, LCA, DCA, isoDCA, isoLCA and 12-oxoLCA (Figures 4F & S4). To investigate the metabolic capacity of these microbial communities, we introduced deuterated GCDCA-D_4 into fecal protein extracts and quantified the resulting metabolic alterations. The results indicated that the gut microbiome in $ACPA^{+}$ RA patients was prone to modify CDCA through 7α-epimerization rather than 7α-dehydroxylation or 3α- dehydrogenation (Figure 4G). Correspondingly, we observed a significant decrease in the expression of the genes encoding enzymes required for 7α-dehydroxylation in the $ACPA^{+}$ RA group (Figure S3B), resulting in reduced production of downstream bile acids from CA, CDCA, and UDCA (Figure 4F). Furthermore, serum FGF-19

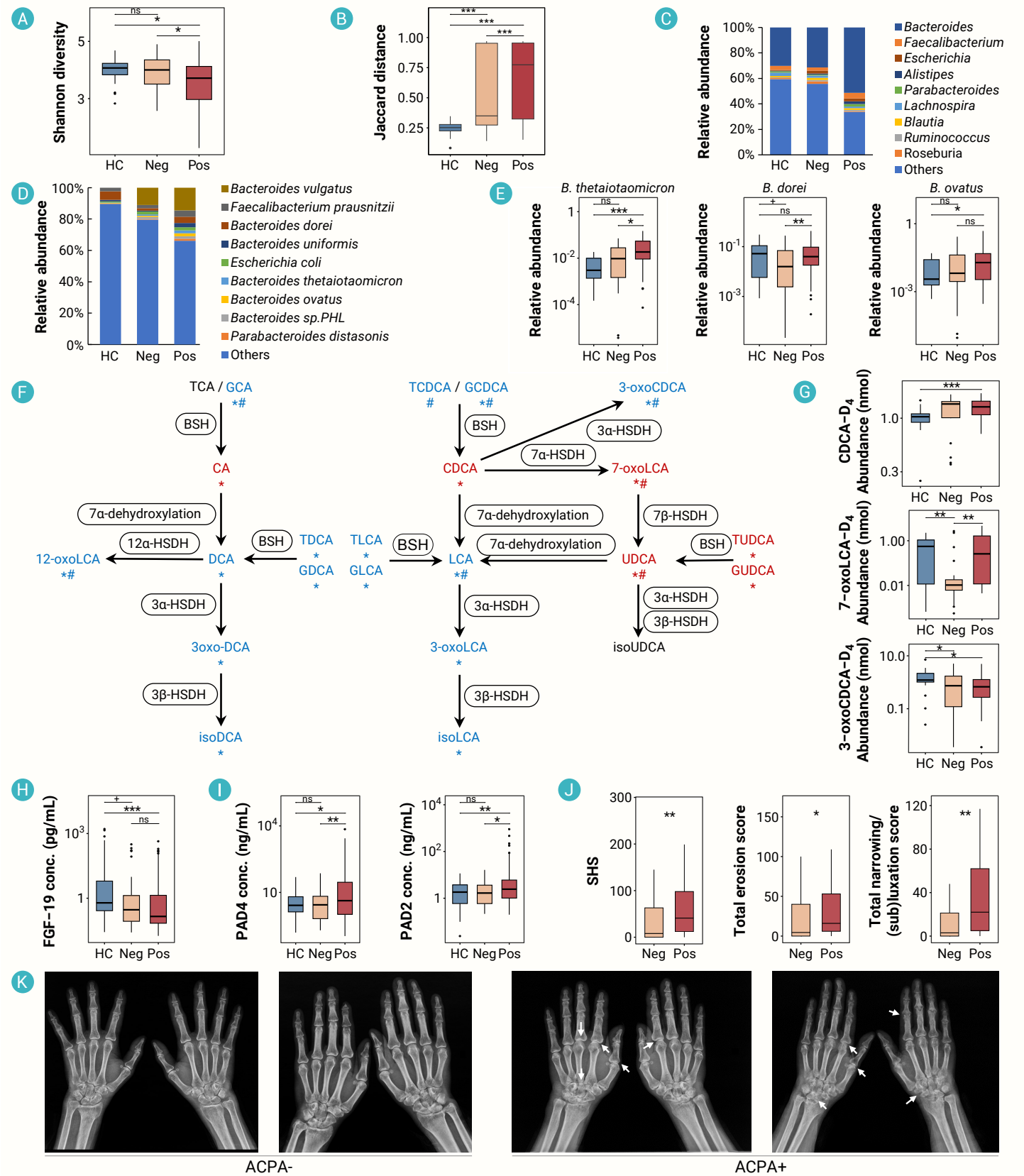


Figure 4. Gut commensal *Bacteroides* and bile acids contribute to elevated ACPA expression and bone erosion in RA (A) Shannon diversity. (B) Beta diversity (Jaccard distance) at the species level. (C-D) The microbiota composition at the genus (C) and species (D) levels. The data are expressed as the relative abundance. (E) Relative abundance of *Bacteroides* species. (F) Microbial bile acid transformations. Red color indicates bile acids increased in ACPA+ RA group, whereas blue color represents bile acids decreased. Comparisons of ACPA+ RA with HC (*) and ACPA- RA (#) were shown. (G) Bile acid metabolism by the fecal protein extracts using GCDCA-D_4 as a starting substrate. (H) Serum concentration of FGF-19. (I) Serum concentration of PAD4 and PAD2. (J) Radiological evaluation of the hands of patients in the ACPA- (n = 36) and ACPA+ (n = 45) RA groups. *p<0.05, **p<0.01, ***p<0.001, ^{ns} not significant. (K) The representative hand X-ray radiographs from patients with a disease duration of 3 years.

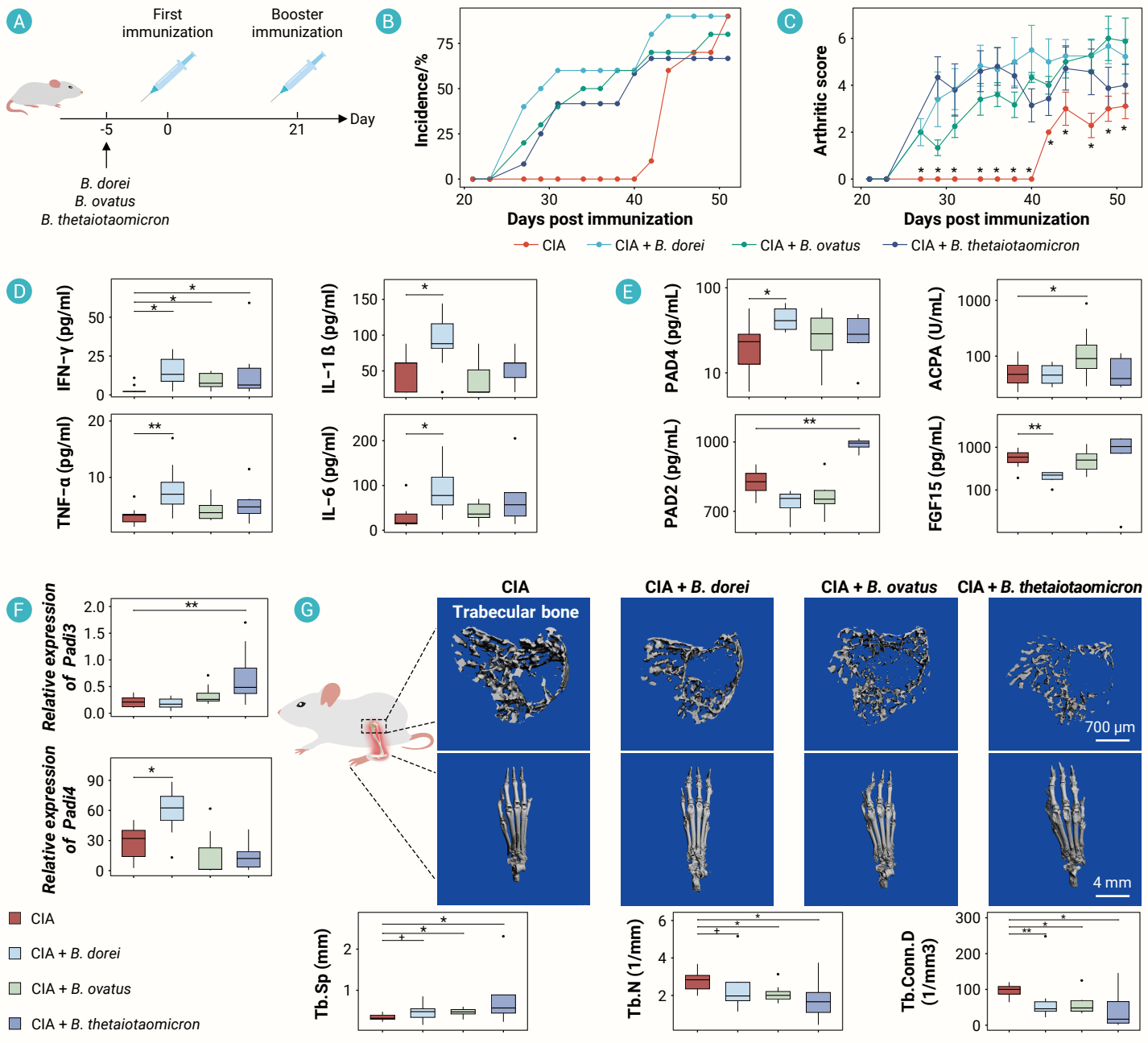


Figure 5. Oral administration of *Bacteroides* species aggravates arthritis in CIA mice (A) Schematic figure of CIA mice with *Bacteroides* species administration. (B) Arthritis incidence in DBA/1 mice. (C) Arthritis scores of DBA/1 mice. (D) Inflammatory cytokine levels in mouse serum. (E) Measurement of PAD4, PAD2, ACPA and FGF-15 concentration in mouse serum. (F) Relative expression levels of citrullination-associated genes in bone. (G) Representative micro-CT images of the trabecular part of the tibias and left hind paws. Micro-CT analysis of the tibia showing trabecular separation (Tb.Sp), trabecular number (Tb.N) and trabecular connectivity density (Tb.Conn.D). $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $^{+}p < 0.1$.

levels in the ACPA^{+} RA group significantly differed from those in the HC group (Figure 4H). These observations collectively suggested that the gut microbiome in ACPA^{+} RA patients showed distinct and dysregulated bile acid metabolism compared to HC and ACPA^{-} RA groups.

To further explore the immunological implications of these differences, we quantified the concentration of peptidylarginine deiminase (PAD) enzymes that generate the citrullinated proteins, the targets of ACPA.[3] As expected, serum levels of PAD4 and PAD2 were higher in the ACPA^{+} RA group than in the ACPA^{-} RA group (Figure 4I). After reviewing the radiographic data of these patients, we found that ACPA^{+} RA patients had more severe bone erosion and narrowing/(sub)luxation estimated in the light of the modified Sharp/van der Heijde score (SHS) method[25] (Figures 4J-K). Additionally, PAD4 levels positively correlated with the scores of bone erosion and narrowing/(sub)luxation, whereas PAD2 did not show a significant correlation (Figure S5). In summary, these findings suggested a potential causal relationship between the gut microbiome composition, bile acid metabolism, ACPA production, and host phenotypes in RA.

Bacteroides treatment aggravated arthritis severity and bone erosion in mice with collagen-induced arthritis

To evaluate the contribution of *Bacteroides* to RA severity and bone erosion, we employed the CIA mouse model for microbial treatment experiments (Figure 5A). Following oral administration of microbes (*i.e.*, *B. dorei*, *B. ovatus* or *B. thetaiotaomicron*), CIA mice exhibited an accelerated immune response and exacerbated inflammation, as characterized by increased arthritis scores (Figures 5B-C & S6A) and elevated levels of pro-inflammatory cytokines such as IFN-γ, IL-6, IL-1, and TNF-α (Figures 5D & S6B). Hepatic transcriptomic analyses further supported this inflammatory response, revealing increased expression of inflammatory signaling markers, including *Il1b*, *Il6*, *Nfkb1*, and *Stat3* (Figure S6C). Consistent with enhanced

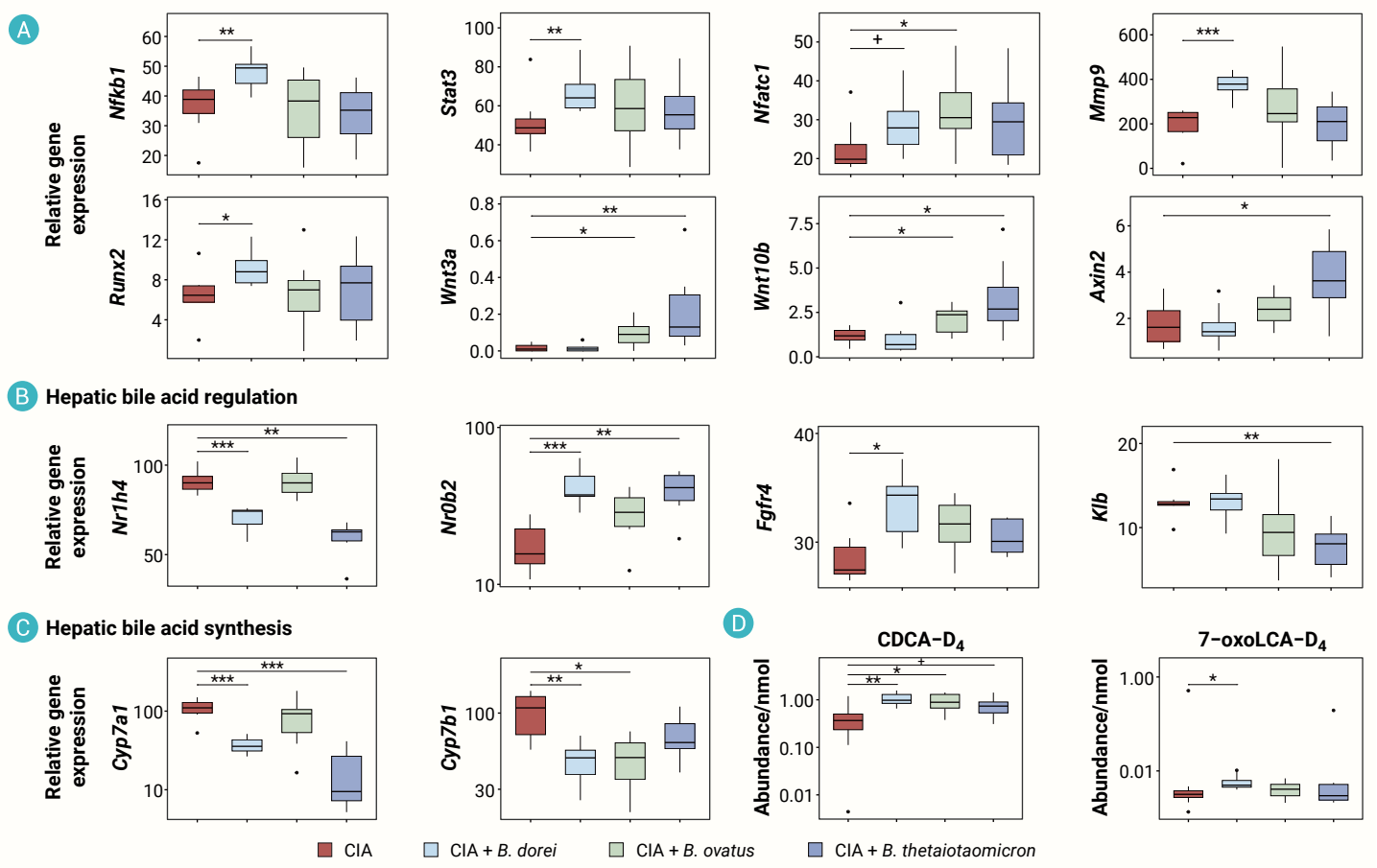


Figure 6. Skeletal and hepatic gene expression in CIA mice administered *Bacteroides* species (A-C) Relative expression levels of the genes functioning in the osteogenic response and inflammatory signaling of the bone (A), hepatic bile acid regulation (B), and hepatic bile acid synthesis (C) were shown. (D) Bile acids produced by mouse fecal protein extracts using GCDCA-D_4 as a starting substrate. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{+}p < 0.1$.

autoimmune activation, *Bacteroides*-treated mice displayed increased serum levels of PAD4, PAD2 and ACPA (Figure 5E), together with increased transcriptional responses of citrullination-associated genes *Padi3* and *Padi4* in bone (Figure 5F). Collectively, these findings support the proposed mechanism by which the *Bacteroides* species may alter immune environments in the host, and subsequently promote arthritis progression through enhanced protein citrullination and antigen-driven immune responses. To further assess the impact of *Bacteroides* on bone phenotype, we performed a micro-CT analysis of tibial bone. The results demonstrated significantly reduced bone mass and more severe bone erosion in *Bacteroides*-treated mice, as evidenced by increased trabecular separation (Tb.Sp) together with decreased trabecular number (Tb.N) and trabecular connectivity density (Tb.Conn.D) (Figure 5G). Increased expression of *Nfkb1* and *Stat3*, together with osteoclast-related genes such as *Nfatc1* and *Mmp9* (Figure 6A), indicated enhanced osteoclastogenic activity. Concurrent upregulation of *Runx2* and the components of the canonical Wnt signaling pathway (e.g., *Wnt3a*, *Wnt10b*, *Axin2*) likely reflected a compensatory osteogenic response that was insufficient to counteract inflammatory bone resorption. These transcriptional alterations were consistent with the aggravated bone erosion observed by micro-CT.

Bacteroides treatments also altered the host bile acid signaling pathway. Administration of *Bacteroides* species disrupted FXR-associated feedback regulation, impaired bile acid synthesis and uptake, and induced compensatory changes in bile acid transport (Figures 6B-C & S6D-E). Specifically, expression of *Nr1h4*, the gene encoding FXR, was reduced following *Bacteroides* administration, whereas the expression of *Nr0b2*, which encodes the downstream mediator SHP, was upregulated. The expression of *Cyp7a1*, the rate-limiting enzyme in classical bile acid synthesis, was reduced. In addition, several genes involved in hepatic bile acid synthesis and cholesterol metabolism, including *Cyp7b1*, *Hmgcr*, *Ldlr*, and *Scap*, were altered, indicating disruption of cholesterol-to-bile acid metabolic flux. These findings suggest enhanced bile acid feedback regulation and suppressed bile acid synthesis in the liver.

Bacteroides administration markedly affected hepatic bile acid transport processes. Expression of the uptake transporters *Slc10a1* and *Slco1a1* was consistently reduced, whereas expression of the efflux transporters, including *Abcc3* and *Abcc4*, was increased, suggesting adaptive remodeling of hepatic bile acid transport. Other transport-related genes, including *Abcb11* and *Abcc2*, exhibited strain-dependent alterations. These changes indicate a compensatory response to disrupted bile acid homeostasis rather than uniform activation of a single transport pathway. Consistent with the reduced serum FGF-19 observed in RA patients, circulating FGF-15, the murine homolog of human FGF-19, was decreased in CIA mice treated with *B. dorei* (Figure 5E). Changed hepatic expression of *Fgfr4* and *Klb* further indicated dysregulation of the FGF-15 signaling axis. Together, these findings indicate that *Bacteroides* treatment disrupts the intestinal-hepatic bile acid regulatory network.

To further characterize microbial bile acid transformation, fecal incubation assays were performed. Administration of *Bacteroides* enhanced microbial conversion of bile acids, resulting in increased production of CDCA and 7-oxoLCA (Figure 6D) and an increased capacity for microbial bile acid metabolism (Figure S7). In contrast, secondary bile acids produced through another pathway, including LCA, 3-oxoLCA, and isoLCA, were reduced (Figure S7).

Collectively, these findings indicate that RA-associated *Bacteroides* species reshape microbial bile acid metabolism, thereby altering bile acid composition and host bile acid signaling. These results reveal that *Bacteroides* enrichment promotes disease progression through coordinated modulation of microbial bile acid transformation and host regulatory pathways, linking gut microbial dysbiosis to inflammatory activation in RA.

DISCUSSION

There is growing interest in the role of microbiome-driven metabolic reprogramming in the pathogenesis of RA.[5,33,43] Our findings reveal that RA status is accompanied by marked microbial dysbiosis, characterized by the high prevalence of *Bacteroides* and *Prevotella*, and a reduction of Firmicutes genera such as *Roseburia* and *Faecalibacterium*. *Bacteroides* and *Prevotella* are both dominant members of the human gut microbiome and have been defined as two major indicators of human gut enterotypes.[29] Previous studies on other cohorts have reported an increased abundance of *Bacteroides* species in RA patients.[4,8,15,44] The overgrowth of *Prevotella* has also been identified as a signature of microbial dysbiosis in new-onset untreated RA patients, which results in enhanced susceptibility to arthritis, increased osteoclast numbers and inflammation-induced bone loss.[6,9,31,45] Previous research has found that direct transformations between *Bacteroides*-rich and *Prevotella*-rich communities within individuals were rare,[46] indicating specific interactions between these taxa and the host phenotypes. Low richness of *Bacteroides*-dominated microflora had reduced functional redundancy, potentially suggesting a decreased resilience to perturbation, in accord with its frequent correlation with dysbiosis.[47] The ecology of *Bacteroides* affects its host by modulating immunity and metabolism in many diseases, such as obesity,[48] polycystic ovary syndrome,[49] lethal inflammatory cardiomyopathy,[50] and familial adenomatous polyposis.[51] For instance, *B. thetaiotaomicron* encodes a cross-reactive β-galactosidase mimetic peptide that activates myosin-specific T cells, triggering autoimmunity with cardiomyopathy.[50] Our findings demonstrate a significant association between increased *Bacteroides* abundance and elevated levels of ACPA, alongside a negative correlation with RF. These results highlight a potential gut microbiota-immune axis that may selectively contribute to ACPA-positive RA pathogenesis.[52,53] RA-associated dysbiosis may not represent a uniform process but encompass different host-microbiome interactions characterized by distinct intestinal ecological states. While the present study focused on the *Bacteroides*-dominated microbiome due to its association with ACPA status and its functional link to bile acid remodeling, the *Prevotella*-dominated subgroup is worth further investigation. Our data showed that *Prevotella* abundance was correlated to RA-related biomarkers such as RF and ESR (Figure 3A), raising the possibility that distinct microbial trajectories contribute to heterogeneous clinical manifestations of RA. However, RF and ESR were anticorrelated with the *Bacteroides* abundance (Figure 3A), highlighting a more complex microbial regulation associated with these clinical features. Because only ten RA patients with *Prevotella*-dominated microbiomes were identified in our discovery cohort (Figure 1B), further characterization of this subgroup was beyond the scope of the present study. Further longitudinal and mechanistic investigations are therefore needed to clarify the role of *Prevotella*-associated microbial states in RA pathogenesis and clinical heterogeneity.

Integration of microbiome and metabolomic profiling revealed a marked reprogramming of microbe-metabolite networks. Many of the annotated metabolites, including amino acids, lipids, and nucleotides, have been previously associated with RA,[54,55] validating our approach. Importantly, we depicted the detailed profile of bile acids in RA patients, discovering alterations in secondary bile acids in RA, such as UDCA, isoDCA and isoLCA, that were not well-characterized in earlier studies. These changes indicate a global remodeling of bile acid metabolic networks, likely reflecting altered microbial enzymatic capacity, including shifts in bile salt biotransformation-related activities. Importantly, these bile acid alterations extend beyond simple abundance changes and have been implicated in immune regulation in RA. These secondary bile acids modulate peripheral regulatory T cells,[56-58] which may contribute to the overactivity of host immune cells. For instance, UDCA has been reported to attenuate the differentiation and activation of Treg cells and finally abates Treg-mediated immunosuppression.[56] Although UDCA has been reported to alleviate osteoarthritis in mice, a previous study reported no significant therapeutic benefit in a collagen-induced arthritis model.[59,60] In addition, isoDCA potentiates the differentiation of peripherally induced Treg cells,[58] and 3-oxoLCA and isoLCA could inhibit the differentiation of Th17 cells.[57] The accumulation of UDCA along with the reduction of isoDCA, isoLCA, and 3-oxoLCA might reflect the dysregulation of peripheral regulatory T-cell modulators in RA patients. Although the present study did not evaluate alterations in immune cells and the involvement of the Treg/Th17 axis requires further validation, these known links suggest a potential mechanism underpinning the *Bacteroides*-associated bile acid alterations in RA pathogenesis. LCA, DCA, isoLCA, and 3-oxoLCA were found to have anti-RA effects.[61] In addition, NorCA is an antagonist of FXR, a bile acid receptor essential for maintaining the intestinal barrier and immunity.[41,62] Intriguingly, these metabolite changes were presumably in line with important microbial biological properties during RA development. The diminishment of conjugated primary bile acids, and 3/12-oxo- or epi-bile acids as well as the augmentation of CA, CDCA and UDCA were in coordination with the accumulation of *Bacteroides* species and downregulation of 7α-dehydroxylation.

Bile acids are not only metabolic end products but also key signaling molecules that regulate host immunity and metabolism. Our data suggest that the changes in the gut microbiome and microbiome-derived metabolites, especially enriched *Bacteroides* and deregulated bile acids, coupled to disrupted FXR-FGF19/15 feedback regulation, presumably contribute to RA progression by modulating host antigenic response and accelerating bone damage. ACPA+ RA patients have more abundant *Bacteroides* species expressing BSH and 7α-HSDH alongside a lower level of genes encoding 7α-dehydroxylase. These changes were associated with the accumulation of UDCA and 7-oxoLCA, and the reduction of 3/12-oxo- or epi-bile acids (*e.g.*, isoLCA, 12-oxoLCA, 3-oxoCDCA). Furthermore, *in vivo Bacteroides* treatment exacerbated the severity of arthritis and bone damage via enhancing secondary bile acid metabolism but reducing isoLCA and 3-oxoLCA levels. Knocking out BSH in these bacteria reduced bile acid deconjugation and diminished their ability to induce colonic RORγ+ Treg cells in germ-free (GF) mice,[58] emphasizing the role of these species in shaping bile acid pools and immune responses. Previous investigations have revealed that patients with osteoporosis had a higher prevalence of *Bacteroides* than healthy subjects.[63,64] Activation of bile acid receptors, FXR, could prevent estrogen - dependent bone loss and enhance osteoblastogenesis in mice.[65] In addition, a previous study has found that supplementation of 3-oxoLCA and isoLCA effectively attenuated the arthritis severity in CIA mice.[61] In addition to bile acid-mediated immune regulation, inflammatory signaling has been implicated in skeletal deterioration, as evidenced by recent findings that TNF signaling promotes age-related bone loss by impairing osteoblast anabolic responses.[66]

Our data support a model in which *Bacteroides* species act as functional drivers of disease-associated metabolic reprogramming rather than passive biomarkers in RA. By reshaping microbial bile acid metabolism, these bacteria alter host bile acid signaling networks and immune homeostasis, ultimately contributing to inflammatory amplification and bone damage. This provides a mechanistic link between gut microbial dysbiosis and systemic autoimmune manifestations in RA. Although our study integrates multi-omics profiling with functional validation in a CIA model, several limitations should be acknowledged. Larger cohorts and longitudinal sampling will be important to further define disease-associated microbial and metabolic changes. Future studies using cocktails of *Bacteroides* species or specific microbe-depleted models, along with receptor-specific interventions targeting FXR or bile acid pathways, will be necessary to establish causality and dissect specific mechanisms underlying bile acid-immune interactions in RA. Furthermore, integrating microbiome and metabolomic profiles with artificial intelligence-based analytical approaches may facilitate the identification of disease-associated molecular signatures and improve patient stratification, supporting the development of precision medicine strategies for RA.[67]

CONCLUSION

By combining 16S amplicon, shotgun metagenomics, and untargeted and targeted metabolomics, we revealed the landscape of gut microbiome and microbial metabolome changes during RA development with high resolution. This is, to our knowledge, the first study to comprehensively demonstrate the role of *Bacteroides* and microbiome-derived bile acids in RA. We highlight a set of previously underexplored secondary bile acids with their potential immunomodulatory effects in RA. These findings provide new insight into the role of the gut microbiome in disease pathogenesis and offer new perspectives for future RA research and potential therapeutic interventions for RA.

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AUTHOR CONTRIBUTIONS

X.S., X.L., and Y.B. contributed equally to this work. X.S. performed the majority of the experiments and data analyses. X.L. conducted the metabolic profiling. Y.B., Q.R., X.W., and H.F. helped collect samples and read the radiographs. L.L. contributed to the metabolic experiments. H.L., X.C., L.T., and Z.Y. assisted with the data analysis. X.H., P.-J.K., and L.-H.T. offered suggestions on the study. X.S. and Z.Y. wrote the manuscript. Z.C., A.L., Z.Y., L.X., and L.T. initiated and supervised the study. All authors contributed to the manuscript and approved the final version.

DECLARATION OF INTERESTS

Aiping Lyu is an Editorial Board member of The Innovation Medicine and was blinded from reviewing or making final decisions on the manuscript. Peer review was handled independently of this member and their research group. The other authors declare no conflicts of interest.

ETHICAL STATEMENT AND PATIENT CONSENT

The study was approved by the Ethics Committee of Guanghua Hospital, Shanghai, China (2023-K-42). Informed consent was obtained from all participants or their legal representatives. The mouse study received approval from the Committees of Animal Ethics and Experimental Safety of the Hong Kong Baptist University (REC/23-24/0240).

DATA AND CODE AVAILABILITY

Data are available from the corresponding author upon reasonable request.

SUPPLEMENTAL INFORMATION

It can be found online at https://doi.org/10.59717/j.xinn-med.2026.100265.

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