Potential efficacy of gut microbiome dysbiosis on alleviation the progress of osteoarthritis in mice

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Research article

Keywords: osteoarthritis, antibiotic, microbiome, inflammation factors, osteoporosis

Posted Date: May 19th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-28047/v1>

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Abstract

Objective

To evaluate the relationship between the gut microbiota dysbiosis and progression of osteoarthritis (OA)

Design

We used a mice OA model (8 weeks) of destabilization of medial meniscus and the gut microbiome dysbiosis induced by antibiotic treatment (ABT) with ampicillin and neomycin for 8 weeks. The severity of OA was evaluated by micro-CT, X-ray and histology of osteoarthritis Research International (OARSI) score. Microbiome analysis by 16 s DNA qPCR was performed on the feces samples and the serum IL-6, TNF- α , osteocalcin, estrogen, calcium and magnesium ion were measured in enzyme-linked immunosorbent assay (ELISA).

Result

Compare to the normal mice, the bone volume over total volume (BV/TV) ($P < 0.01$ for both subgroup analysis) and the osteoarthritis Research International (OARSI) score ($P < 0.01$ for both subgroup analysis) and in subchondral bone and was decreased significant in ABT treatment of female and male group. ABT decreased the osteophyte score in female mice with standard normal group ($P < 0.05$) and also declined osteophyte maturity score in both female ($P < 0.01$) and male ($P < 0.05$) mice. The level of IL-6 ($P < 0.01$ for both subgroup analysis), TNF- α ($P < 0.01$ for both subgroup) and calcium ($P < 0.01$ for both subgroup analysis) were decreased significant in ABT group than control group while the magnesium ($P < 0.01$ for both subgroup analysis) and estrogen ($P < 0.01$ for both subgroup analysis) were increased significant in ABT female group than that in the control female group. Pearson's correlation analyses demonstrated that subchondral bone BV/TV ($r = -0.6145$, $P = 0.0001$) and OARSI score in medial tibial plateau ($r = -0.5407$, $P = 0.0007$) are negatively correlated with the Firmicutes : Bacteroidetes ratio.

Conclusion

Gut microbiome dysbiosis have a potential efficacy on alleviate the progress of OA.

1. Introduction

Osteoarthritis (OA) is the most common musculoskeletal disease and most notable increase of 35% in total burden and 4% in age-standardized disability-adjusted life-years rates [1, 2] and knee OA accounts for approximately 85% of the burden of osteoarthritis worldwide. For knee OA, strong evidence indicates a variety of moderate to strong risk factors, including menopause, obesity and previous knee injury [3, 4] and associated with metabolic disease, for example high levels of obesity [5].

Gut flora is also a critical determinant of metabolic disease and can be modulated by the combined effects of environmental stimuli and genetic factors [6] and also combine with various conditions

including obesity, diabetes, irritable bowel syndrome, inflammatory bowel disease, depression, and cardiovascular disease[7]. The gastrointestinal tract microbiota profile influence nutraceutical efficacy in a non-blinded randomized clinical trial[8]. The current literature on gut flora and OA suggest that gut microbiome-OA connection sets the stage for discovery of potentially OA of obesity involving strategic manipulation of specific microbial species inhabiting the intestinal space[9]. Current gut flora mechanisms for improved mineral absorption and skeletal health include alterations in gut microbiota composition, production of short-chain fatty acids, altered intestinal pH, biomarker modification, immune system regulation, vitamin D and vitamin K[10, 11].

The effects of gut microbial dysbiosis on musculoskeletal tissue during and after antibiotic treatment in human and animal had attracted the attention of researchers [12, 13]. Several researchers have explored the effect associated with gut microbiota dysbiosis and human life system. Dysbiosis in the composition and abundance of gut microbiota can affect microbiota-gut-brain axis and gut-liver axis [14, 15]. There are two methods of gut microbial dysbiosis, such as antibiotic treatment and germ-free feeding. For the germ-free mice model of destabilized medial meniscus (DMM), these results suggest factors related to the gut microbiota promote the development of OA after joint injury in subchondral bone [16]. Otherwise, Previous studies with intestinal microbiota have also shown that osteoarthritis is characterized by an expansion and/or decrease of bacterial groups in obesity[6]. What's more, imbalances in the gut microbiota, the bacteria that inhabit the intestines, are central to the pathogenesis of obesity[17]. Recent evidence suggests that antibiotic treatment may cause profound alterations of gut epithelium, immune cells, intestinal neural system and long-lasting effects for the host [18]and it might explain why and how antibiotic-caused microbiota changes can impact OA pathogenesis in obesity [19, 20]. The two major inflammatory phenotypes of macrophages are controlled by gut flora through differential consumption of glucose, glutamine, and oxygen. M1 phenotype is triggered by polarization signal from bacterial lipopolysaccharide and Th1 proinflammatory cytokines such as interferon- γ , TNF- α , and IL-1 β , or both, whereas M2 phenotype is triggered by Th2 cytokines such as interleukin-4 and interleukin-13 as well as anti-inflammatory cytokines, IL-6[21]. But for health people, the influence of antibiotics-induced gut microbiota dysbiosis on OA and how the effects are modulated by diet are poorly understood[22].

We hypothesized that antibiotic depletion gut flora which could reduce a source of microbial products that mean suppress innate immune response to restrain chronic low-grade proinflammatory state and postpone the development of OA. We explore the associations between gut flora, estrogen, systemic inflammation profiles and joint damage in antibiotics-induced dysbiosis mice with the purpose of better understand the development of OA.

2. Materials And Methods

Experimental animals and study design

Mice used in this study were 8 weeks and purchased from Department of Laboratory Animal Science of Peking University Health Science Center. The experiment and protocol were approved by The Peking

University Third Hospital Committee on Ethics in the Care and Use of Laboratory Animals(No. LA2019209). Total of thirty-six C57BL/6N mice (Male and female matching) were housed in a standard animal facility under controlled temperature (22°C) and photoperiod (12 h light and 12 h dark) and had free access to fresh water and food. The mice were first treated with knee surgery, and interventions measure were given one week after the surgery. Animals were allocated to antibiotics-induced group (ABT) (ampicillin(1.0 g/L) and neomycin (0.5 g/L) or the standard control group (drinking fresh water) lasted for 8 weeks [23].

Induction Of Destabilization Of The Medial Meniscus (DMM) Surgery

Methods and results description follow Animal Research Reporting in Vivo Experiments guidelines and DMM was induced by transecting the medial meniscotibial ligament in the right knee as described by Dr. Glassion[24, 25]. We performed the DMM procedure on one knee in all mice and used the unoperated left leg as a control instead of performing sham surgery in a separate group of animals which in pervious studies[16]. Mice subjected to DMM/sham were assessed for weight, fat percent in 16 weeks after surgery. Dissected knees were fixed, micro-CT scanned and then histology. Blood and stool samples were collected.

Blood Collection And Serum IL-6,TNF- α ,OCN And Estrogen Measurements

Blood was collected in coagulation-promoting tubes and centrifuged at 3000 rpm for 15 min and plasma were collected and stored in -80°C. Plasma IL-6,TNF- α ,OCN and estrogen(Meimian Biotechnology ,Yancheng, Jiangsu, China) were performed using commercial enzyme-linked immunosorbent assay (ELISA) kits. the level of Calcium ion and magnesium ion were quantification by Methyl thymol blue microplate method(Shanghai yuanye Bio-Technology Co., Ltd)

Radiological Anslysis

Dissected knees were fixed overnight(4% paraformaldehyde). The specimens were scanned in a sample holder with PBS and scanned using a soft X-ray apparatus with a small-animal high-resolution collimator (Faxitron® LX-60 Cabinet radiography system, US) and μ -CT(Inveon, Siemens, Erlangen, Germany) at a spatial resolution of 55 kVp, 145 mA, 300 ms integration time, and a 6-mm isotropic voxel edge. A region of interest contain the medial tibial plateau which defined as the region of the tibia superior to the growth plate, posterior to the insertion of the anterior cruciate ligament (ACL), and medial to the midline of the intercondylar notch. We analysis the subchondral bone sclerosis and remodeling in this region with a direct-model morphometric measure of bone volume (BV) over total volume (TV), trabecular

thickness(Tb.Th), trabecular number(Tb.N), and trabecular spacing(Tb.Sp) in the medial tibial subchondral trabecular bone which has been describe in pervious studies[26]. Peripheral osteophytes were not analysis in μ -CT.

Histology Assessment

Fixed knees were decalcified in 10% EDTA for 14 days and embedded in paraffin and frontal sections(5 μ m). The Safranin-O/Fast Green staining and HE were subjected and the images were taken a stereomicroscope (SMZ745T, Nikon, Tokyo, Japan). For OA grading, we used the OARSI staining histopathology score for evaluating the OA severity[27]. Osteophyte size and osteophyte maturity were scored on coded digital images of the same location of the anterior-medial tibia in each animal[28]. All histological scores were found by an evaluator (ZYG) blinded to the group assignment and we reported the OARSI score either medial femoral condyle, medial tibial plateau, lateral femoral condyle using ImageJ and the results were reported as average.

Microbiome Analysis At

At age 16 weeks, the control and ABT mice were killed and caeca sample were collected and frozen at -80°C .Fecal sample were isolated by the Qiagen QIAamp DNA Mini Kit, as directed by the manufacturer (Qiagen, Hilden, Germany) and total concentration of DNA were measure by NanoDrop (ThermoFisher, Waltham,USA). The total bacterial load and specificity bacterial load were tested by quantitative PCR(qPCR) in previous studies[29–31]. The universal primer and specificity bacterial perimer include α -proteobacteria, γ -proteobacteria, Bacteroidetes, Firmicutes, actinobacteria (Aokedingsheng Technologies,Beijing,China) were upload in supplement table1. The qPCR protocol was completed on ABT StepOne (Applied Biosystems) and qPCRs contained 2Fast SYBR Green Master Mix (Applied Biosystems), 0.25 mmol/L primers, and 150 mg/mL DNA template. Samples underwent an initial denaturing step at 95°C for 5minutes, followed by 30 cycles of 95°C for 30 seconds, 60°C for 40 seconds, and 72°C for 30 seconds, and ending with a final elongation step of 72°C for 5 minutes.

Statistical analysis

All measurements are presented as the mean $\pm$ standard deviation (SD) and a *P*-value of ≤ 0.05 was considered statistically significant. The data were analyzed using GraphPad Prism 8.02(La Jolla California USA) and one-way ANOVA followed by Tukey's multiple.

3. Result

1.Flora analysis following dysbiosis induced by ABT treatment in mice

Antibiotics and control-treatment was administered via drinking water to adult sex-matched C57BL/6T mice with DMM surgery (Fig. 1A). In order to dysbiosis the intestinal microbiota, 8-week-old C57BL/7 mice were treated by broad-spectrum antibiotics through 16 s DNA qPCR analysis (Fig. 1B-F). ABT decreased total load of colonic bacterial in female and male ($P < 0.001$ for both analysis, Fig. 1B) mice than control group. These are significant difference in control-female, ABT-female, control-male and ABT-female in subgroup analysis (Fig. 1C). Female antibiotic and control-treated mice demonstrated significant increases in α -proteobacteria ($P < 0.05$ for both analysis, Fig. 1F) and bacteroidetes ($P < 0.01$ for both analysis, Fig. 1F) and significant decrease γ -proteobacteria ($P < 0.01$, Fig. 1G). Male antibiotic versus control-treated mice showed a significant decrease in Firmicutes ($P < 0.01$, Fig. 1G).

2. Antibiotic-induced dysbiosis causes decrease subchondral bone sclerosis, osteophyte formation and improve cartilage damage via gut barrier dysfunction

To understand the effect of ABT on the subchondral bone sclerosis, the subchondral bone sclerosis also has been analysis after DMM surgery by micro-CT scan and analysis. The Fig. 2A-B obtained from the preliminary analysis of micro-CT of the joint in DMM. The result show that ABT inhibit subchondral bone sclerosis in BV/TV in both female and male mice ($P < 0.05$ for both subgroup analysis, Fig. 2E) and also found that ABT treatment have significant difference between male and female mice ($P < 0.01$, Fig. 2E). Micro-CT demonstrated that female ABT significant increased the subchondral trabecular thickness ($P < 0.01$ for both subgroup analysis, Fig. 2F) and significant decreased trabecular number ($P < 0.01$ for both subgroup analysis, Fig. 2C) and trabecular separation ($P < 0.01$ for both subgroup analysis, Fig. 2D). To the contrary, male ABT significant decreased trabecular separation ($P < 0.01$ for both subgroup analysis, Fig. 2D).

Figure 3 shows osteophyte formation in knee joint via a X-ray apparatus and HE staining. Obvious osteophytes, uneven joint surfaces, and narrowed joint spaces has been observed in radiological images and osteophyte formation were indicated by black bar in HE staining images (Fig. 3A-3B). ABT treatment has decreased the osteophyte score in female mice after DMM surgery ($P < 0.05$, Fig. 3C) and also declined osteophyte maturity score in both female ($P < 0.01$, Fig. 3D) and male ($P < 0.05$, Fig. 3D) mice. ABT treatment have significant difference between male and female mice in osteophyte score. ($P < 0.01$, Fig. 3E)

An overview of cartilage damage in joint via Safranin-O/Fast Green staining and the OARSI score has used to analysis the medial femoral condyle, medial tibial plateau, lateral femoral condyle in Fig. 4. ABT treatment has decreased the OARSI score in medial tibial plateau ($P < 0.01$ for both subgroup analysis, Fig. 4C) and medial femoral condyle ($P < 0.01$ for both subgroup analysis, Fig. 4C) in both male and female mice. ABT treatment also improve in lateral femoral condyl ($P < 0.01$ for both subgroup analysis, Fig. 4E) in male mice and there is no difference significant in LFC in female mice. ABT treatment have significant difference between male and female mice in lateral femoral condyl ($P < 0.01$, Fig. 4E)

3. ABT decreased the inflammation and osteoblasts

In order to determine whether inflammation play a role in injury-induced OA development, circulating TNF- α ($P < 0.01$, Fig. 5B) and IL-6 ($P < 0.01$, Fig. 5C) levels were decreased significant in antibiotic-treatment verse control-treated subgroup in both male and female mice. As a marker of bone formation, we measured decrease levels in serum osteocalcin (OCN) in antibiotic mice compared with control-treated mice in both male and female mice ($P < 0.01$, Fig. 5A). To investigate the gut flora mechanisms for mineral absorption in ABT mice, we measured serum calcium (Ca^{2+}) and magnesium (Mg^{2+}) level. Serum calcium has been decreased significant in ABT mice with control-treated mice ($P < 0.01$, Fig. 5D) and we also found the serum magnesium has been increased significant in ABT female mice compared with control female mice and no significant differences were seen in male mice ($P < 0.01$, Fig. 5E). To understanding whether the gender can affect the development of OA, we also found that antibiotic also has increased the level of serum estrogen compared with control group in female mice ($P < 0.01$, Fig. 5F). Pearson's correlation analyses demonstrated that subchondral bone BV/TV ($r = 0.6115$, $P = 0.0000$, Fig. 5G) and OARSI score in medial tibial plateau ($r = 0.5861$, $P = 0.0002$, Fig. 5H) are positively correlated with the Firmicutes : Bacteroidetes ratio.

4. Discussion

In this study, we evaluated the effect of antibiotic on the intestinal microbial composition and the relationship between the gut microbiota dysbiosis and progression of osteoarthritis (OA). The concentration of serum OCN, TNF- α and IL-6, calcium was significantly decrease with a marked change in gut Flora in 8 weeks of oval antibiotic administration in OA. In contrast, the concentration of serum magnesium and estrogen were increased in antibiotic induced female mice. The results shown that antibiotic change intestinal bacterial and imply that antibiotic decrease the OA development, which may be caused by restrain its inflammatory reaction

Antibiotics alter the composition and functions of the microbiota and produce long-lasting effects for host[18], such as education of the immune system[32, 33], colonization resistance[34, 35] and allergic and metabolic syndromes[36]. In the intestinal, the gut microbiota catalyzes the conversion of drugs into absorbable, hydroxylation, and reduction[37]. We choose ampicillin and neomycin with less absorption in the intestine as an intervention. Another experiment of interfere with the intestinal flora is observe changes in osteoarthritis using germ-free mice. Ulici et.al found that GF mice will suppress the gut microbiota and decrease of development of OA after joint injury[16].

There are many methods to measure the severity of OA, such as OARSI, the Articular Cartilage Structure score, Safranin-O stains score and radiological test. Histopathology is currently the gold standard for assessing of OA in animal models[38]. This semi-quantitative scoring system proposed in this study was relatively easy and reproducibility to apply for both experienced and novices scores[39]. The micro-CT is another powerful technique utilized to study 3D structures reconstructed. Botter suggest that osteoarthritis in the murine knee using collagenase injections has been show significant changes in subchondral bone architecture could be detected and quantified in 3D with micro-CT analysis[40]. Soft X-ray apparatus also the important method to evaluate the severity of OA[41].

In early stages of OA in humans, elevated bone remodeling, subchondral bone loss and articular cartilage regression was observed, and was considered as a determinant of OA progression[42], subchondral sclerosis may be observed only during more advanced stages of OA[43]. In our studies, we found that antibiotic-induced dysbiosis for 8 weeks contribute more advanced stages of OA. There are three potential mechanisms by which the gut microbiota can influence bone tissues: 1)regulation of nutrient absorption at the gut epithelium, 2)regulation of the mucosal and systemic inflammation and immune system, 3)translocation of microbial contents across the gut endothelial barrier[44]. The overproduction of cytokines and growth factors is the important mediator in the pathophysiology of OA[45]. In OA pathogenesis particularly in synovial inflammation, activated chondrocytes and synovial fibroblasts (TNF- α and IL-6) have been identified as vital players[46]. Zhao et.al found that both spontaneous and surgically induced OA models indicated that TNF- α led to an accelerated OA-like phenotype[47]. TNF- α control the degeneration of articular cartilage matrix, which makes them prime targets for therapeutic strategies[48]. Latourte et.al found that IL-6 induces chondrocyte catabolism mainly via Stat3 signalling, a pathway activated in cartilage from joint subjected to DMM[49]. Intra-articular injections of IL-6 reproduced OA-like cartilage lesions in mice, and IL-6 knockout (KO) mice develop less severe posttraumatic OA lesions[50]. Lu J, et al investigated that correlation between the amount of intestinal flora and the number of CD4 + T lymphocytes and the levels of TNF- α and IL-6[51]. In our study, we found that antibiotics reduce the inflammatory response throughout the body after inhibiting the intestinal flora. This also provides new evidence for the intestinal flora-joint axis in osteoarthritis.

The role of the intestinal flora in the absorption of substances is very important, such as the absorption of inorganic and energy substances in the host. Scholz-Ahrens et.al studied that probiotic can increase the level of calcium prevented the decrease in bone metabolism in ovariectomized rats and the main mechanism of this process is increased intestinal absorptive bones and increased bowel microbial mass and reduced turnover of calcium in bones[52]. Whisner et.al studies the prebiotic increases calcium absorption, which may be mediated by the gut microbiota[53]. In our studies, we investigated that antibiotic decrease the total load of gut microbiota and it also mean that interaction mechanisms between intestinal flora and minerals[54]. Antibiotics perturbed gut microbial composition. Community diversity and richness were reduced, and the phyla Firmicutes/Bacteroidetes ratio was decreased by antibiotics[55]. This process leads to impaired gut barrier integrity and release of LPS from intestinal gram-negative bacteria for example Bacteroidetes into the bloodstream[56]. Therefore, we found that the decrease of community diversity was increase the subchondral bone sclerosis and cartilage damage.

Gender was also reported specifically influence subchondral bone. Men are reported to have a higher prevalence of OA than women before the age of 50 years, but after this age the prevalence is higher in women, which coincides with menopause[57]. The estrogen replacement therapy exhibit a reduced risk of hip and knee OA in postmenopausal women[58]. Son et.al found that up of estrogen abrogates experimental OA via the estrogen-related receptor family of orphan nuclear receptor[59]. A lack of estrogens increases subchondral bone remodeling, and this bone remodeling has been suggested to ultimately induce osteoarthritis changes[60]. In general, acute loss of estrogens increases the levels of reactive oxygen species and activates nuclear factor- κ B and pro-inflammatory cytokine production,

indicating their predominant anti-inflammatory properties [61]. In our previous studies, we found that deficiency of estrogen can accelerate the development of OA which induced by ovariectomy[62]. What's more, antibiotic-induced dysbiosis also increased the level of estrogen which may play positive role in OA.

The role of osteocalcin in bone remodeling, specific expression and regulation in osteoblasts which is important biomarker in osteoporosis and level of reduced Osteocalcin expression were maintained in human osteoarthritic chondrocytes and represented a suitable biomarker of osteoarthritic chondrocyte activation[63]. Wagatsuma et.al found that diversity of gut microbiota affecting serum level of osteocalcin in patient[64]. It means that the occurrence of osteoporosis causes uneven cartilage stress, which further aggravates joints and cartilage damage which make knee osteoarthritis more serious[57]. At the same time, the exacerbation of osteoarthritis reduces the activity of mice and reduces stress stimuli which further aggravated osteoporosis[58]. In our studies, we found that antibiotic treatment of gut flora reduced the level of OCN which may contribute to development of OA and osteoporosis.

In addition, the effect of gut flora is also considered important factors for mice with sexual maturity[65]. However, the osteoarthritis after osteoporosis is also common in the clinical.

5. Conclusion

In summary, by inducing the intestinal microbiota dysbiosis that lead to improvement of OA, these results can help in leading to strategies for new treatments to prevent the symptoms and long-term sequelae of OA. In addition, this study contributes to our understanding of gut flora- joint axis in aspect of OA and provide the relationship between inflammation response, gender and absorption of substances. Further studies of the differences in the transcriptional response of antibiotic treatment should help elucidate the mechanisms of OA and osteoporosis by ABT underlying the contribution of the development of OA.

Abbreviations

OA
osteoarthritis
ABT
antibiotic treatment
ELISA
enzyme-linked immunosorbent assay
BV/TV
bone volume over total volume
OARSI
the osteoarthritis Research International score
DMM
destabilized medial meniscus
LPS

lipopolysaccharide
ROI
region of interest
ACL
anterior cruciate ligament
Tb.Th
trabecular thickness
Tb.N
trabecular number
Tb.Sp
trabecular spacing
qPCR
quantitative PCR
OCN
osteocalcin
IL-6
interleukin- 6
TNF- α
tumor necrosis factor- α

Declarations

Authors' contributions

Conception and design:CLS,ZYG

Acquisiton, analysis and interpretation of the data: ZYG,TTS,WZ,JLJ, CGZ.

Drafting and writing: ZYG

Final approval of article: CLS, ZYG, TTS, WZ, JLJ, CGZ.

Competing interests

Authors have no conflict of interest to disclose.

Acknowledgements

We would like to thanks to Peking University Third Hospital Central Laboratory for providing pathological support.

Ethics approval and consent to participate

This study has received approval by Peking University Animal Ethics Committee (permission number: LA2019209)

Funding

This work was partly supported by the National Natural Science Foundation of China (Grants no. 81874010, 81672133, 11872076).

Availability of data and materials

No application

Consent for publication

No application

References

1. DALYs GBD, Collaborators H. Global, regional, and national disability-adjusted life-years (DALYs) for 315 diseases and injuries and healthy life expectancy (HALE), 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet*. 2016;388(10053):1603–58.
2. Dequeker J, Luyten FP. The history of osteoarthritis-osteoarthrosis. *Ann Rheum Dis*. 2008;67(1):5–10.
3. Hunter DJ, Bierma-Zeinstra S. *Osteoarthritis* *Lancet*. 2019;393(10182):1745–59.
4. Silverwood V, et al. Current evidence on risk factors for knee osteoarthritis in older adults: a systematic review and meta-analysis. *Osteoarthritis Cartilage*. 2015;23(4):507–15.
5. Berenbaum F, et al. Modern-day environmental factors in the pathogenesis of osteoarthritis. *Nat Rev Rheumatol*. 2018;14(11):674–81.
6. Liu Y, et al. Gut microbiota and obesity-associated osteoarthritis. *Osteoarthritis Cartilage*. 2019;27(9):1257–65.
7. Hills RD Jr, et al., *Gut Microbiome: Profound Implications for Diet and Disease*. *Nutrients*, 2019. 11(7).
8. Coulson S, et al. Green-lipped mussel extract (*Perna canaliculus*) and glucosamine sulphate in patients with knee osteoarthritis: therapeutic efficacy and effects on gastrointestinal microbiota profiles. *Inflammopharmacology*. 2013;21(1):79–90.
9. Schott EM, et al. Targeting the gut microbiome to treat the osteoarthritis of obesity. *JCI insight*. 2018;3(8):e95997.

10. Whisner CM, Castillo LF. Prebiotics, Bone and Mineral Metabolism. *Calcif Tissue Int*. 2018;102(4):443–79.
11. Ghareghani M, et al. Latitude, Vitamin D, Melatonin, and Gut Microbiota Act in Concert to Initiate Multiple Sclerosis: A New Mechanistic Pathway. *Front Immunol*. 2018;9:2484.
12. Perez-Cobas AE, et al. Gut microbiota disturbance during antibiotic therapy: a multi-omic approach. *Gut*. 2013;62(11):1591–601.
13. Jin S, et al. Low-dose penicillin exposure in early life decreases Th17 and the susceptibility to DSS colitis in mice through gut microbiota modification. *Sci Rep*. 2017;7:43662.
14. Sun M-F, Shen Y-Q. Dysbiosis of gut microbiota and microbial metabolites in Parkinson's Disease. *Ageing Res Rev*. 2018;45:53–61.
15. Kim S, Jazwinski SM. The Gut Microbiota and Healthy Aging: A Mini-Review. *Gerontology*. 2018;64(6):513–20.
16. Ulici V, et al. Osteoarthritis induced by destabilization of the medial meniscus is reduced in germ-free mice. *Osteoarthritis Cartilage*. 2018;26(8):1098–109.
17. Seganfredo FB, et al. Weight-loss interventions and gut microbiota changes in overweight and obese patients: a systematic review. *Obes Rev*. 2017;18(8):832–51.
18. Becattini S, Taur Y, Pamer EG. Antibiotic-Induced Changes in the Intestinal Microbiota and Disease. *Trends Mol Med*. 2016;22(6):458–78.
19. Morgun A, et al. Uncovering effects of antibiotics on the host and microbiota using transkingdom gene networks. *Gut*. 2015;64(11):1732–43.
20. Pickard JM, et al. Gut microbiota: Role in pathogen colonization, immune responses, and inflammatory disease. *Immunol Rev*. 2017;279(1):70–89.
21. Belizario JE, Faintuch J, Garay-Malpartida M. Gut Microbiome Dysbiosis and Immunometabolism: New Frontiers for Treatment of Metabolic Diseases. *Mediators Inflamm*. 2018;2018:2037838.
22. Weaver CM. Diet, gut microbiome, and bone health. *Curr Osteoporos Rep*. 2015;13(2):125–30.
23. Vijay-Kumar M, et al. Metabolic syndrome and altered gut microbiota in mice lacking Toll-like receptor 5. *Science*. 2010;328(5975):228–31.
24. Glasson SS, Blanchet TJ, Morris EA. The surgical destabilization of the medial meniscus (DMM) model of osteoarthritis in the 129/SvEv mouse. *Osteoarthritis Cartilage*. 2007;15(9):1061–9.
25. Glasson SS, et al. Deletion of active ADAMTS5 prevents cartilage degradation in a murine model of osteoarthritis. *Nature*. 2005;434(7033):644–8.
26. Longobardi L, et al. Role of the C-C chemokine receptor-2 in a murine model of injury-induced osteoarthritis. *Osteoarthritis Cartilage*. 2017;25(6):914–25.
27. Glasson SS, et al. The OARSI histopathology initiative - recommendations for histological assessments of osteoarthritis in the mouse. *Osteoarthritis cartilage*. 2010;18 **Suppl 3**:S17–23.
28. Little CB, et al. Matrix metalloproteinase 13-deficient mice are resistant to osteoarthritic cartilage erosion but not chondrocyte hypertrophy or osteophyte development. *Arthritis Rheum*.

- 2009;60(12):3723–33.
29. Schmittgen TD, Livak KJ. *Analyzing real-time PCR data by the comparative CT method*. 3(6): p. 1101–1108.
  30. Hathaway-Schrader JD, et al., *Antibiotic Perturbation of Gut Microbiota Dysregulates Osteoimmune Cross Talk in Postpubertal Skeletal Development*. 2019.
  31. Gregoris TBD, et al., *Improvement of phylum- and class-specific primers for real-time PCR quantification of bacterial taxa*. 2011. 86(3): p. 351–356.
  32. Fonseca DMd, et al. Microbiota-Dependent Sequelae of Acute Infection Compromise Tissue-Specific Immunity. *Cell*. 2015;163(2):354–66.
  33. Horai R, et al. Microbiota-Dependent Activation of an Autoreactive T Cell Receptor Provokes Autoimmunity in an Immunologically Privileged Site. *Immunity*. 2015;43(2):343–53.
  34. Ubeda C, et al. Vancomycin-resistant Enterococcus domination of intestinal microbiota is enabled by antibiotic treatment in mice and precedes bloodstream invasion in humans. *J Clin Investig*. 2010;120(12):4332–41.
  35. Lewis BB, et al. Loss of Microbiota-Mediated Colonization Resistance to *Clostridium difficile* Infection With Oral Vancomycin Compared With Metronidazole. *The Journal of infectious diseases*. 2015;212(10):1656–65.
  36. Cox LM, et al. Altering the intestinal microbiota during a critical developmental window has lasting metabolic consequences. *Cell*. 2014;158(4):705–21.
  37. Kim D-H. *Gut Microbiota-Mediated Drug-Antibiotic Interactions*. *Drug metabolism and disposition: the biological fate of chemicals*, 2015. 43(10): p. 1581–1589.
  38. Rutgers M, et al. Evaluation of histological scoring systems for tissue-engineered, repaired and osteoarthritic cartilage. *Osteoarthritis cartilage*. 2010;18(1):12–23.
  39. Mobasheri A, et al., *Osteoarthritis Year in Review 2016: biomarkers (biochemical markers)*. *Osteoarthritis and cartilage*, 2017. 25(2): p. 199–208.
  40. Botter SM, et al. Quantification of subchondral bone changes in a murine osteoarthritis model using micro-CT. *Biorheology*. 2006;43(3,4):379–88.
  41. Kamekura S, et al. Osteoarthritis development in novel experimental mouse models induced by knee joint instability. *Osteoarthritis Cartilage*. 2005;13(7):632–41.
  42. Bettica P, et al. Evidence for increased bone resorption in patients with progressive knee osteoarthritis: longitudinal results from the Chingford study. *Arthritis Rheum*. 2002;46(12):3178–84.
  43. Intema F, et al. In early OA, thinning of the subchondral plate is directly related to cartilage damage: results from a canine ACLT-menisectomy model. *Osteoarthritis Cartilage*. 2010;18(5):691–8.
  44. Hernandez CJ, et al. Links Between the Microbiome and Bone. *Journal of bone mineral research: the official journal of the American Society for Bone Mineral Research*. 2016;31(9):1638–46.
  45. Rahmati M, Mobasheri A, Mozafari M. Inflammatory mediators in osteoarthritis: A critical review of the state-of-the-art, current prospects, and future challenges. *Bone*. 2016;85:81–90.

46. Mobasheri A. Intersection of inflammation and herbal medicine in the treatment of osteoarthritis. *Curr Rheumatol Rep*. 2012;14(6):604–16.
47. Zhao Y, et al. Cortistatin binds to TNF- α receptors and protects against osteoarthritis. *EBioMedicine*. 2019;41:556–70.
48. Kapoor M, et al. Role of proinflammatory cytokines in the pathophysiology of osteoarthritis. *Nat Rev Rheumatol*. 2011;7(1):33–42.
49. Latourte A, et al. Systemic inhibition of IL-6/Stat3 signalling protects against experimental osteoarthritis. *Ann Rheum Dis*. 2017;76(4):748–55.
50. Ryu J-H, et al. Interleukin-6 plays an essential role in hypoxia-inducible factor 2 α -induced experimental osteoarthritic cartilage destruction in mice. *Arthritis rheumatism*. 2011;63(9):2732–43.
51. Lu J, et al. Changes in peripheral blood inflammatory factors (TNF- α and IL-6) and intestinal flora in AIDS and HIV-positive individuals. *J Zhejiang Univ Sci B*. 2019;20(10):793–802.
52. Scholz-Ahrens KE, et al., *Effects of probiotics, prebiotics, and synbiotics on mineral metabolism in ovariectomized rats – Impact of bacterial mass, intestinal absorptive area and reduction of bone turn-over*. p. S2352364615300547.
53. Whisner CM, et al. Galacto-oligosaccharides increase calcium absorption and gut bifidobacteria in young girls: a double-blind cross-over trial. *Br J Nutr*. 2013;110(7):1292–303.
54. Hibberd MC, et al. The effects of micronutrient deficiencies on bacterial species from the human gut microbiota. *Science translational medicine*. 2017;9(390):eaal4069.
55. Crouzet L, et al. *Lactobacillus paracasei* CNCM I-3689 reduces vancomycin-resistant *Enterococcus* persistence and promotes *Bacteroidetes* resilience in the gut following antibiotic challenge. *Sci Rep*. 2018;8(1):5098.
56. Baker JM, Al-Nakkash L, Herbst-Kralovetz MM. Estrogen-gut microbiome axis: Physiological and clinical implications. *Maturitas*. 2017;103:45–53.
57. Oliveria SA, et al. Incidence of Symptomatic Hand, Hip, and Knee Osteoarthritis among Patients in a Health Maintenance Organization. *Arthritis Rheum*. 1995;38(8):1134–41.
58. Zhang Y, et al. Estrogen replacement therapy and worsening of radiographic knee osteoarthritis: the Framingham Study. *Arthritis Rheum*. 1998;41(10):1867–73.
59. Son Y-O, et al. Estrogen-related receptor γ causes osteoarthritis by upregulating extracellular matrix-degrading enzymes. *Nature communications*. 2017;8(1):2133–3.
60. Bellido M, et al. Subchondral bone microstructural damage by increased remodelling aggravates experimental osteoarthritis preceded by osteoporosis. *Arthritis research therapy*. 2010;12(4):R152–2.
61. Martín-Millán M, Castañeda S. Estrogens, osteoarthritis and inflammation. *Joint bone spine*. 2013;80(4):368–73.
62. Li S, et al. Vitamin D prevents articular cartilage erosion by regulating collagen II turnover through TGF- β 1 in ovariectomized rats. *Osteoarthritis Cartilage*. 2016;24(2):345–53.

63. Tarquini C, et al. Comparison of tissue transglutaminase 2 and bone biological markers osteocalcin, osteopontin and sclerostin expression in human osteoporosis and osteoarthritis. *Amino Acids*. 2017;49(3):683–93.
64. Wagatsuma K, et al. Diversity of Gut Microbiota Affecting Serum Level of Undercarboxylated Osteocalcin in Patients with Crohn's Disease. *Nutrients*. 2019;11(7):1541.
65. Harada N, et al. Role of gut microbiota in sex- and diet-dependent metabolic disorders that lead to early mortality of androgen receptor-deficient male mice. *Am J Physiol Endocrinol Metab*. 2020;318(4):E525–37.

Figures

Figure 1

Flora analysis following antibiotic (ABT) treatment causes dysbiosis in mice. A. Experimental design of the antibiotic treatment (ampicillin(1.0g/L) and neomycin (0.5 g/L) in both male and female group. B. The total load of bacterial in male and female in universal 16S gene analysis. C, F, G: Phylum-level composition in males and females, displayed in pie chart (C) and bar graph (F and G) format. Phylum-level real-time

quantitative PCR analysis was normalized to the universal 16S gene expression. E.The changes in body weight of mice in each group. Unpaired t-test was used. Data are expressed as means $\pm$ standard deviation. n=9 per group (B,C,E,F,G).Con,control group; wks,weeks.

3D and coronal axis in female(A)and male(B)mice. bone volume over total volume (BV/TV), trabecular thickness(Tb.Th), trabecular number(Tb.N), and trabecular spacing(Tb.Sp) were analysis from sections obtained from the anterior/medial margin of the tibia from DMM-operated knees at 8 wks(C,D,E,F). n=9 per group. Con,control group. Data are expressed as means $\pm$ standard deviation.

Figure 3

Antibiotic-induced dysbiosis causes decrease osteophyte formation via gut barrier dysfunction. A and B, anterior subluxation of the tibiae, joint destruction and osteophyte formation were visible in the posterior tibial plateau by lateral X-ray features of the knee joints and HE staining. Osteophyte size(C) and Osteophyte maturity score(D) represent decrease in osteophyte formation and cartilage destruction. Black and red bars indicate osteophyte formation. Data are expressed as means $\pm$ standard deviation. n=9 per group. Con,control group

Figure 4

Antibiotic-induced dysbiosis causes improve cartilage damage via gut barrier dysfunction. A and B, Cartilage damage induced by DMM was measured by OARSI score at the medial tibia plateau in female(A)and male(B)mice. medial femoral condyle (MFC), medial tibial plateau (MTP), lateral femoral condyle (LFC)were analysis from sections obtained from the anterior/medial margin of the tibia from DMM-operated knees at 8 wks(C,D,E). Red bars indicate osteophyte formation. Data are expressed as means $\pm$ standard deviation. n=9 per group. Con,control group

Figure 5

The serum level of OCN(A) ,TNF-α(B),IL-6(C), Ca²⁺(D), Mg²⁺(E) and estrogen(F) were measured by ELISA in control DMM group and ABT DMM mice. Pearson's correlation analyses demonstrated that subchondral bone BV/TV($r = 0.6115$, $P=0.000$,G) and OARSI score in medial tibial plateau($r = 0.5861$, $P=0.002$,H) are positively correlated with the Firmicutes : Bacteroidetes ratio. Data are expressed as means $\pm$ standard deviation. n=9 per group. Con,control group

Supplementary Files

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