

Murine Post Traumatic Osteoarthritis Alters the Functional Output of the Gut Microbiome

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Purpose: Osteoarthritis (OA) is the leading cause of physical disability globally. One initiator of OA is trauma to the joint, which is commonly referred to as post-traumatic osteoarthritis (PTOA). With 35% percent of patients developing PTOA within 10 years of injury, the need to both understand the disease process and develop therapeutic interventions is vital. One potential factor associated with or contributing to the disease process is the gut microbiome. The gut microbiome has been characterized in other types of OA, such as the OA of obesity; however, very little is known about the gut microbiome's relationship to the PTOA process. Recent investigations into the composition of the gut microbiome during OA in humans has resulted in conflicting reports, spanning from significant shifts in the composition of the gut microbiome to instances where no changes were observed to community structure as a result of disease. Thus, we seek to determine in a preclinical model if any specific changes occur in the gut microbiome during PTOA progression. We employed a multiomic approach, in a mouse model of PTOA, to explore the metagenome, metatranscriptome, and metabolome of the gut microbiome during disease progression. In this investigation, we hypothesize that PTOA is associated with shifts in the composition of the gut microbiome that contribute pathologically to disease progression.

Methods: PTOA was induced via bilateral destabilization of the medial meniscus (DMM) in 18-week-old male C57BL/6 mice consuming a standard chow diet. An uninjured cohort of animals progressed through the experimental timeline simultaneously to serve as age-matched controls. Fecal samples were collected weekly, and mice were sacrificed 24 weeks after PTOA was induced. At the time of sacrifice, cecal and fecal content was collected for 16S sequencing, metagenomic and metatranscriptomic pathway analysis, and metabolomic characterization. Colon tissue was harvested for RNA-Seq analysis. Joint tissue was isolated for histological examination and microcomputed tomography (μ CT). All experiential procedures were performed under an approved protocol from the University of Colorado IACUC.

Results: As expected, mice receiving the DMM surgery developed PTOA in a reproducible manner that resembles OA progression in human PTOA. Injured animals had a significant decrease in cartilage area on both the femur and tibia, with μ CT further confirming OA progression, as indicated by the increase in mineralized meniscus volume in the injured cohort. To our surprise, injured animals that developed PTOA showed little change in the composition of the gut microbiome by 16S characterization (Fig 1A). This was evidenced by the lack of statistically significant differences in the abundance of any community members at the time of harvest by 16S characterization. Species characterization by shotgun metagenomic analysis revealed elevations of *Lachnospiraceae* and *Anaeroplasm* in the gut microbiome of animals with PTOA and a reduction in the abundance of *Bifidobacterium pseudolongum* in the gut microbiome of injured animals. Although metagenomic data revealed modest changes in the composition of the gut microbiome, it did reveal extensive pathway changes. In fact, at the metagenomic level, 71 pathways associated with *Biosynthesis*, 21 pathways associated with *Degradation/Assimilation and Utilization*, and 14 pathways associated with the *Generation of Precursor Metabolites and Energy* were altered in the cecal contents of animals with PTOA (Fig 1B). Of note, the pathway for 2-oxobutanoate degradation II was overrepresented in the microbiome of uninjured mice compared to animals with PTOA. This pathway is associated with the production of propionate. Meta-transcriptomics of the cecal contents provided insight into active pathways, with SqueezeMeta uncovering a total of 8 KEGG orthology groups that were differentially represented between uninjured controls and injured animals, 5 of which were downregulated in the injured animals and 3 of which were activated in the injured animals (Fig 1C). In addition to Kegg orthology groups, 125 significant Clusters of Ortholog Groups (COGs) and 30 Protein Families (PFAMs) were identified, with numerous pathways indicating increases in immune system avoidance in injured animals. To further understand the functional alterations in the gut microbiome, we performed metabolomics on the fecal and cecal contents. In total, 26 metabolites were altered at the time of harvest, with short-chain fatty acids (SCFA), butyrate, and propionate decreasing in the gut microbiome of injured animals (Fig 1D). Finally, to understand the impact of these functional changes on the host, we performed RNA-Seq on colon tissue, which revealed changes in energy synthesis and fatty acid pathways.

Discussion: Despite the large global burden of PTOA, all current treatments remain palliative. It is known that the gut microbiome is a novel treatment target for OA; however, very little is known about the role of the gut microbiome in the development of PTOA. Here we verify findings seen in human studies that PTOA does not alter community composition. But we demonstrate, for the first time, that the gut microbiome possesses significant functional alteration during PTOA progression, including previously unrecognized impacts on SCFA production and immune system avoidance, in addition to 26 metabolites that are differentially produced by the gut microbiome of animals with PTOA. Interestingly, loss of butyric acid in the PTOA mouse cohort (Fig 1D) aligns with findings that butyrate supplementation can mitigate joint degeneration in murine PTOA.

Significance: These findings provide the first evidence that, while murine PTOA does not shift the composition of the gut microbiome, it has a major influence on gene expression and functional output of the gut microbiome. Deeper investigations into the implications of these functional changes on PTOA will help us further our understanding of the gut-joint axis, possibly providing novel therapeutic targets to treat PTOA.

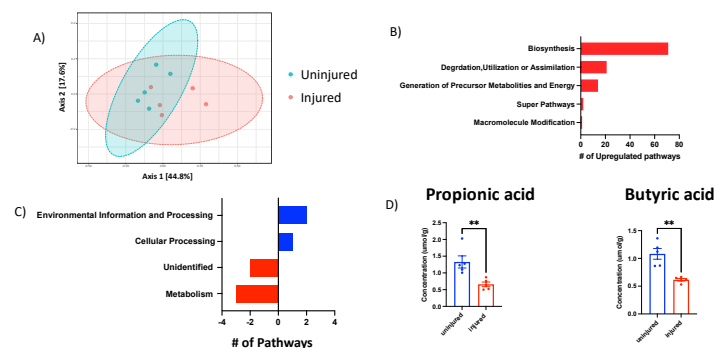


Figure 1: PTOA has little effect on the composition of the gut microbiome but significantly alters its functional output.

The PCA plot demonstrates that the gut microbiome composition is not altered between injured and uninjured animals (A). Shotgun metagenomics reveals 106 pathways that are altered in abundance in mice with PTOA (B). Metatranscriptomics indicates 8 KEGG orthology groups are altered in animals with PTOA (C). SCFAs propionic acid and butyric acid are decreased in animals with PTOA (D).