

Heterochronic gut microbiome transplantation augments the risk of osteoarthritis in mice

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INTRODUCTION: Aging is one of the strongest risk factors for knee osteoarthritis (OA). Previous studies in humans and mice have identified changes in the gut microbiome with aging, although a direct link between these age-associated microbiome changes and knee OA risk has not yet been elucidated. In the current study, we set out to determine whether heterochronic transplantation (young-into-old or old-into-young) of the gut microbiome in mice altered the risk for knee OA development using the destabilization of the medial meniscus (DMM) model.

METHODS: The IACUC of the Oklahoma Medical Research Foundation approved this study. Young (12-week-old, n=7) and old (18-month-old, n=7) adult male and young (n=7) and old (n=6) adult female mice underwent control transplantation via one-time oral gavage of 200uL of sterile vehicle (1:1 mixture of glycerol:PBS). Young male (n=7) and young female (n=6) mice underwent heterochronic transplantation via one-time oral gavage of 200uL diluted cecal content from old male and female mice, respectively. Old male (n=6) and old female (n=5) mice underwent heterochronic transplantation with diluted cecal content from young male and female mice, respectively. Three days later, DMM surgery was performed on a unilateral hind limb. Mice were euthanized 8 weeks later. Knee joints were decalcified, fixed in paraformaldehyde, embedded in paraffin, sectioned, stained with Safranin-O, and OA severity quantified by blinded investigators using the OARSI grading system. Gut microbiome profiling via 16S amplicon deep sequencing is ongoing. Histologic outcomes were calculated using a Student t-test (data were confirmed to be normally distributed), outliers were removed from further analysis (n=1 old female, n=1 young->old male), $P \leq 0.05$ was considered significant.

RESULTS: Female mice had reduced OA histologic severity at both young and old timepoints (young: $P=0.0002$, old: $P=0.003$, **Figure 1**). Both male and female mice had increased severity with age (young male: 7.8 ± 0.7 , summed whole-joint OARSI score, mean \pm SEM, vs. old male: 16 ± 1 , $P=6E-5$; young female: 3.1 ± 0.5 vs. old female: 10 ± 1 , $P=4E-6$). Heterochronic transplantation of old mouse gut microbiota into young recipients did not alter histologic scores, although female mice approached statistical significance (old-into-young M: 8.8 ± 1.0 vs. young M: 7.8 ± 0.7 , $P=0.4$; old-into-young F: 4.6 ± 0.6 vs. young F: 3.1 ± 0.5 , $P=0.08$). However, heterochronic transplantation of young microbiota into old recipients did reduce OA histologic severity in both sexes (young-into-old M: 9.0 ± 1 vs. old M: 16 ± 1 , $P=0.002$; young-into-old F: 8.0 ± 0.7 vs. old F: 10 ± 1 , $P=0.02$).

DISCUSSION: Previous reports have indicated that the gut microbiome changes with age. Our laboratory has previously shown that aged B6 mice have gut microbiome and cartilage microbial DNA patterns that differ from young B6 mice and share some overlaps with obese mice and microbiome alterations associated with OA. In the present study, we have generated evidence that age-associated gut microbiome changes may directly influence the risk of OA in mice following the commonly-used DMM induction technique. Interestingly, neither young male nor young female mice exhibited 'worsening' of OA risk when aged microbiota was transplanted, but old male and female mice both exhibited improvement in histologic score following transplantation with young mouse microbiota. This suggests that the aged microbiome milieu may be more amenable to microbiome therapy, and/or that microbiome-linked processes (likely gut microbiome-interface innate immunological processes) may play a stronger role in aged animals compared to young animals.

SIGNIFICANCE/CLINICAL RELEVANCE: (1-2 sentences): This study suggests that gut microbiome changes associated with age may play a direct role in OA risk and may be modifiable via gut microbiome interventions to restore a 'youthful' microbiome milieu.

Figure 1: Heterochronic gut microbiome transplantation histologic outcomes

