Investigating the Role of DRD4 in the Development of Post-Traumatic Osteoarthritis

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INTRODUCTION: Osteoarthritis (OA) is a leading cause of disability and post-traumatic OA (PTOA) commonly follows ACL rupture, yet there are no disease-modifying therapies. Aromoline was discovered to increase type II collagen expression, likely through the dopamine receptor D4 (DRD4). DRD4 is an inhibitory G-protein that may interact with TGF-β signaling. We hypothesized that loss of DRD4 would exacerbate PTOA, worsening gait and pain behaviors, subchondral remodeling, and cartilage catabolism, when compared with wild-type (WT) littermate controls.

METHODS: **Ethics**: All procedures approved by UCF IACUC. **Animals**: B6.129P2-Drd4^{tm1Dkg}/J DRD4 knockout (KO) and WT littermates (target N=60 total; per genotype: 6 female sham, 9 female injured, 6 male sham, 9 male injured). **Injury model**: Non-invasive tibial compression overload to induce ACL rupture under isoflurane (**Fig. 1A&B**); buprenorphine for analgesia. Sham mice receive anesthesia/analgesia without loading. DigiGait treadmill analysis (e.g., stride length, paw angle) and Von Frey mechanical allodynia quantify locomotor function and nociception. **Gait and pain**: Baseline (10- and 11-weeks) and post-injury at 2 days, 2-, 4-, and 8-weeks. Digigait videos were pre-processed with AI assisted image analysis to help segment the feet from Naired legs and exclude the nose and tail. **Molecular imaging**: In vivo MMP activity at day 5 and week 8 using MMPSense750 with IVIS Spectrum; quantitative signal analysis across groups [6]. **Structural imaging**: At 8 weeks post injury, mice were euthanized and ex vivo μCT of harvested knees taken to quantify osteophyte burden and subchondral trabecular metrics (bone volume fraction, trabecular number/thickness/spacing; **Fig. 1C&D**) followed by histological assessment. **Statistics**: Three-way ANOVA with Tukey post-hoc (α=0.05) performed using GraphPad Prism.

RESULTS: There was a significant decrease in bone volume fraction in the injured medial compartment vs. the uninjured medial compartment for both wild type and knockout animals, but only in the knockout mice did we see a decrease in the lateral compartment (**Fig. 1E**). This decrease was further supported by histology showing less cartilage loss and osteophyte formation in the wildtype (**Fig. 1F**) vs. the knockout (**Fig. 1G**). Von Frey analysis showed that knockout females have more sensitive feet than knockout males prior to injury at 11-weeks of age. There was also a significant difference between the sensitivity of the injured leg and uninjured leg 2 days post-injury. The significant difference in allodynia that is present at 2-days post injury is not present at 2-weeks post injury and all later time points. Joint width analysis showed that there was a significant difference between the injured and uninjured legs at 8-weeks post-injury. There was also a difference between males and females 8-weeks post injury. Analysis of stride length showed that there was a significant decrease in stride length after injury. In terms of paw angle, there was an interaction between knockout and sex at 8 weeks post injury. In vivo MMP analysis showed a significant increase in activity in the injured leg vs. the non-injured leg at 5 days post injury. There was no significant difference by 8-weeks, in neither case was there an obvious effect of knockout or sex.

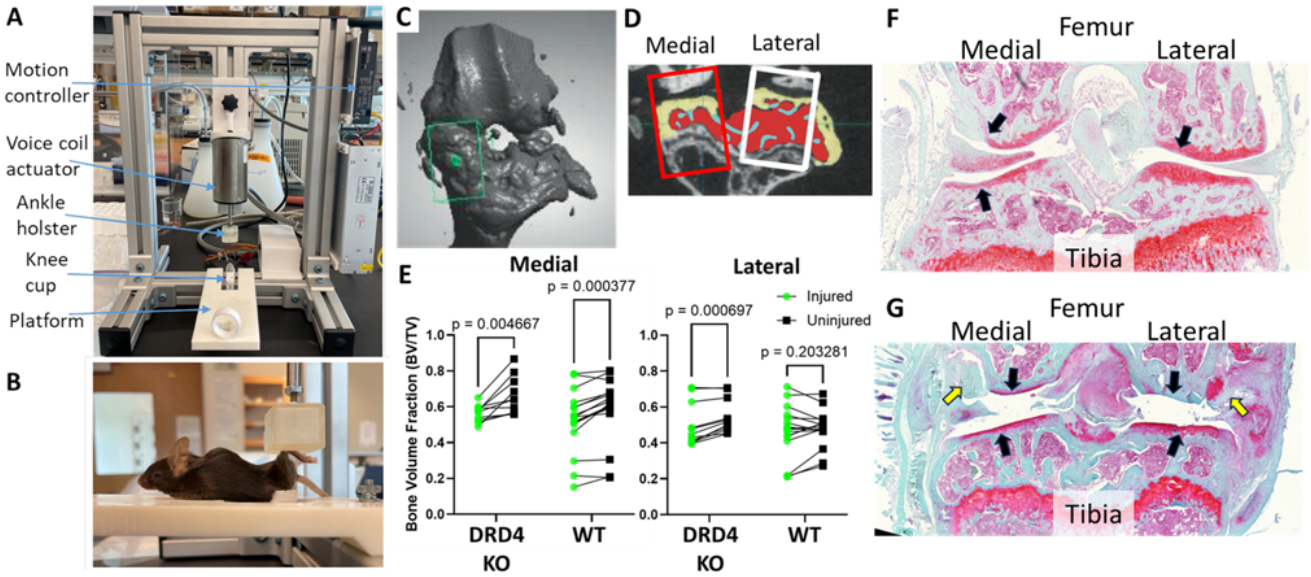

Figure 1 – Non-invasive knee injury device, μCT and histology assessment
A) Non-invasive knee injury device. B) Mouse positioned in the device. C) μCT reconstruction of the injured joint. D) μCT processing identifying the medial and lateral condyles of the tibia. E) Bone volume fraction analysis of the DRD4 knockout and wildtype mouse, no difference was seen between male and female mice so this shows both combined, 8 weeks post injury n ≥ 10. F) Safranin-O histology of the injured joint from a wild-type female, showing both the femur and tibia, meniscal horns protruding into the joint and loss of glycosaminoglycan indicated by the black arrows. G) shows histology in the knockout female with more pronounced cartilage loss and fibrillation in both medial and lateral compartments supporting the μCT data. Yellow arrows indicating osteophyte formation.

DISCUSSION: This study aimed to determine whether DRD4 knockout accelerates functional decline, bone remodeling, and cartilage catabolism after ACL rupture. Limitations include reliance on one injury model and terminal μCT at 8 weeks; however, inclusion of both sexes and multimodal outcomes (gait, pain, μCT, and MMP imaging) strengthens translational relevance. It was clearly evident across multiple metrics that the ACL tear caused PTOA-like changes. This model produces quite a severe change in the mouse and more significant differences might be seen in a less severe model. Despite modest differences between genotypes, the results suggest that DRD4 contributes meaningfully to joint homeostasis and may represent a viable therapeutic target.

SIGNIFICANCE/CLINICAL RELEVANCE: Establishing DRD4 as a modulator of PTOA could reveal a novel, druggable pathway to slow cartilage loss and preserve function after ACL injury, thereby informing disease-modifying strategies for younger, active patients.

ACKNOWLEDGEMENTS: Supported by the University of Central Florida College of Medicine.