GPCR Kinase 2 inhibition restores chondrocyte anabolism and ameliorates pain and inflammation in aging-induced osteoarthritis

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Introduction: Knee osteoarthritis (OA) is a major degenerative disease of the articular cartilage that greatly affects patients' quality of life. OA can be primary (age-induced) or secondary (trauma induced), and its pathophysiology is still not completely understood. With no disease modifying treatment (DMOAD), current management options are mainly symptomatic, with the ultimate solution being joint replacement. We have previously shown that the GPCR kinase 2 (GRK2) mediates chondrocyte catabolic signaling and synovial inflammation in post-traumatic OA (PTOA) mice. Furthermore, we repurposed the GRK2 inhibiting antidepressant 'Paroxetine' as a DMOAD where paroxetine-mediated GRK2 inhibition prevented cartilage degeneration and promoted regeneration, along with reduced synovitis (inflammation) in a surgical murine PTOA model (1, 2). Aging knees are characterized by increased cartilage degeneration due to reduced anabolism and increased catabolism, along with increased synovitis. Accordingly, in the current study, we aimed to investigate changes in GRK2 expression and activity in the knee with age and the effectiveness of repurposing Paroxetine (as a GRK2 inhibitor) in the treatment of age-induced knee OA in mice. We ***hypothesize*** that GRK2 expression increases with age, thus, driving catabolic and senescence signaling, and that paroxetine-mediated GRK2 inhibition is effective in the treatment of age-induced OA.

Methods: 20 months old C57B/6 male and female mice were treated with vehicle (PBS) or paroxetine (5 mg/kg/day) by intraperitoneal injection for one month. Mice were then split into two cohorts: Cohort 1- mice were euthanized at the end of treatment; Cohort 2- mice were euthanized 2 weeks after the end of treatment to determine extended drug effects after treatment cessation. 6 months old mice were used as the young control group.

We assessed pain sensitivity at baseline (before treatment; for Cohorts 1 & 2), at the end of treatment (for Cohorts 1 & 2) and 2 weeks after the end of treatment (for Cohort 2). We used acetone test (cold pain sensitivity), von Frey test (mechanical pain sensitivity) and hot plate test (hot pain sensitivity).

After euthanasia, knees were harvested for Micro-CT imaging to determine structural changes in the whole joint and in bone tissues. Knees were then fixed and processed to prepare FFPE sections for histopathological analyses. Histomorphometry of SafO/FastG stained sections using the Osteomeasure system (3) was performed to quantify changes in cartilage degeneration, number of total and anabolic chondrocytes, and synovial thickness. We quantified changes in GRK2 expression and chondrocyte anabolic (Aggrecan) and catabolic markers (MMP13, ADAMTS-5). Furthermore, to identify the molecular pathways regulated by GRK2 in the cartilage and the synovium, we quantified changes in the expression of aging/senescence markers (IGF-1 and SIRT6) using IF.

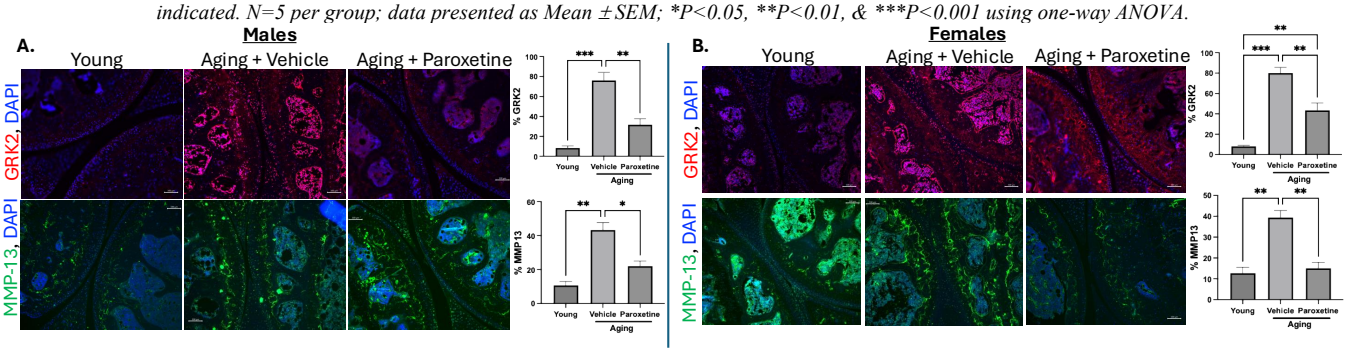
Power and statistical analysis: Studies were performed following ARRIVE guidelines. Power analyses was performed using the G*Power 3.1 software with n=10/group. Statistical analysis was performed using Multiple or one-way ANOVA with Tukey's correction. Data were considered statistically significant if $P < 0.05$ was achieved. Graphed values were expressed as mean ± SEM. Calculations were performed using GraphPad Prism 10.0.

Results: IF staining showed that GRK2 expression was increased in the cartilage and synovium of aging mice in comparison to young controls, where it correlated with decreased chondrocyte anabolism (Aggrecan), increased catabolism (increased MMP-13 but not ADAMTS-5) and increased synovitis. In contrast, aging mice treated with Paroxetine had lower GRK2 expression in their cartilage and synovium in comparison to vehicle treated mice, which was associated with increased chondrocyte anabolism (Aggrecan), reduced catabolism (MMP-13), and reduced synovitis. Paroxetine-mediated anabolic effect was reflected in increased percentage of anabolic chondrocytes, which persisted after 2 weeks of treatment cessation in Cohort 2. Furthermore, aging mice had decreased expression of IGF-1 and SIRT6 in the cartilage and synovium, indicating increased inflammation and senescence, which were normalized by paroxetine treatment, suggesting reduced senescence and inflammation in paroxetine treated mice. Regarding pain sensitivity, Paroxetine treated mice showed reduced sensitivity to both mechanical and thermal pain stimuli after 1 month of treatment; this analgesic effect persisted 2 weeks after the end of treatment in Cohort 2. These effects were obtained in male and female mice (Figure below), suggesting the lack of sexual dimorphism in GRK2-mediated pathogenesis in aging knees.

Discussion: OA greatly affects patients' quality of life, with no disease modifying treatment. Here we identify GRK2 as a major driver of aging-induced OA. Importantly, we repurpose a commonly used antidepressant 'Paroxetine' as a DMOAD that promotes cartilage anabolism and decreases catabolism, therefore promoting cartilage regeneration. In addition, paroxetine exerted potent analgesic and anti-inflammatory effects extending for 2 weeks after treatment cessation. *Future studies:* We will continue our studies to analyze structural changes in the bone and overall joint of aging knees following paroxetine treatment, and to identify direct mechanistic downstream/transcriptional targets of GRK2 and its inhibition by paroxetine.

Significance: OA is a debilitating disease that is projected to increase with the growing aging population, leading to increased disability and further worsens its burden on the healthcare system. As such, a DMOAD is of increasing importance to improve the quality of life of aging patients and to reduce the burden on the healthcare system. With a pathophysiology not completely understood, the development of a DMOAD is hindered. This study shows that GRK2 plays a central role in primary aging-induced OA progression, and that its inhibition could be a novel therapeutic approach. Therefore, we identify GRK2 as a novel therapeutic target that can be regulated using a repurposed FDA-approved drug 'paroxetine', which enables accelerated clinical testing.

Figure. *IF staining for GRK2 and MMP-13 and quantification in the articular cartilage of male (A) and female (B) mice. Respective groups/treatments as indicated. N=5 per group; data presented as Mean ±SEM; *P<0.05, **P<0.01, & ***P<0.001 using one-way ANOVA.*



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