Introduction: Inherited gene mutations can be predisposing factors for osteoarthritis (OA) and intervertebral disc (IVD) degeneration, particularly those for type II, IX and XI collagens [1]. Mice homozygous for inactivated Col9a1 gene prematurely develop evidence of OA and IVD degeneration [2–4]. Histological analysis of 6 month mutant knee joints shows irregular cartilage surfaces, erosion, and softening particularly on medial tibial plateau [4], and analyses of the spine show reduced cellularity and increased endplate mineralization at 3 months [3]. While these changes bear similarities to human pathology, it is unknown whether these mice experience functional or symptomatic changes that parallel those documented for human disease. This study’s objective was to compare gait and pain sensitivity of type IX collagen knockout mice in late adulthood (12 months) to wild-type controls to determine if functional and pain parameters can serve as useful biomarkers of pathology.

Materials and Methods: Knockout (KO, n=10) and wildtype (WT, n=10) gait was measured in a 6 cm x 36 cm arena with transparent floor. A mirror placed below the floor at a 45 degree angle allowed for sagittal and transverse views. Subjects were allowed for 5 - 15 minutes (unprompted) and were then encouraged to move by brushing the subject with a cotton swab (prompted). Gait was recorded via a high-speed digital video camera (200 fps); approximately 3 unprompted and 2 prompted trials were recorded per subject. Nose, tail, and paw pixel coordinates were digitized from high-speed videos. Velocity, stride length, step width, and duty factor (percentage of a stride a limb is in ground contact) were then calculated. Mechanical and thermal withdrawal thresholds were used as pain measures. First, von Frey filaments (Stoelting, 70 - 4000 mg force) were applied to a subject’s left, then right, hind paws in four trials, with applications separated by 1 minute. Frequency of paw withdrawal was determined for each filament, and the mechanical force where the likelihood of withdrawal equals 50% was determined through sigmoidal fits. To investigate thermal sensitivity, hot plate/tail flick tests were conducted. Individuals were placed on a 52.0 +/- 0.2 degree hot plate; paw flick latency was recorded. Then, a subject was placed with its mid-portion tail beneath a radiant light source. Heat was applied via the light; tail withdrawal latency was recorded. Thermal tests were repeated 6 times over 2 hours.

Gait was compared via generalized linear models designed to account for known variations due to subject size, weight, or velocity. Pain sensitivity was compared via analysis of variance.

Results: Mean KO velocity was 18% lower than WTs in unprompted trials (p < 0.0004), and at lower velocities, KOs selected higher duty factors (p < 0.001) and shorter stride lengths (p < 0.02, Fig. 1). KOs also selected wider step widths in unprompted trials (11%, p < 0.0001). KO-WT differences were less substantial when subjects were prompted; however, KOs still tended to use higher duty factors at lower velocities. In pain sensitivity, KOs showed increased mechanical sensitivity (von Frey test), represented by a 36% drop in the 50% mechanical withdrawal threshold (Fig. 2). No KO-WT differences were observed in thermal sensitivity testing.

Discussion: These data indicate gait changes in type IX collagen KO mice may be related to joint degeneration. Differences were particularly evident at velocities below 25 cm/sec. These velocities were commonly observed when mice freely explored without external stimuli prompting locomotion, and in these trials, KOs used shorter strides and higher duty factors. This gait difference may be associated with the increased mechanical sensitivity indicated in von Frey tests, such that subjects are responsive to pain generated during his or her locomotion, ground contact, and joint loading. It is not currently known if gait changes relate to generalized pain, or if specific changes are linked to knee or IVD degeneration, in this model. Future work will correlate the extent of knee and IVD pathologies to gait and pain parameters in order to further evaluate these measures as potential biomarkers.


Acknowledgements: Support was provided by NIH AR047442, AR050245 and T32EB001630 (KDA).