A Novel Knee Joint Laxity Measurement Device in Mice

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INTRODUCTION: Anterior cruciate ligament reconstruction (ACLR) is a common procedure in orthopedic surgery to treat torn ACLs. Unfortunately, ACL graft failures are reported in up to 34.2% of athletes[1], and post-traumatic osteoarthritis (PTOA) is a common sequela of ACL injuries, with an incidence of up to 50% at 10-20 years post-injury[2,3]. Mouse models are important in evaluating factors impacting ACLR outcomes, including PTOA. Common evaluative methods used to study healing outcomes after ACLR include gait analysis, biomechanical testing, and histological assessments. However, common clinical diagnostic exams to assess ACL laxity (i.e., Lachman, pivot-shift, and anterior drawer tests) are not easily performed in small animal models[4]. There is currently no reproducible method to quantitatively assess mouse joint stability *in vivo*. A standardized method to quantitatively assess translations and rotations in the mouse knee would allow for the correlation of joint function with measures of tissue structure and composition. It will also allow for comparison of knee laxity over time after ACLR and tracking of the progression of PTOA. The aim of this study was to develop a system and determine a quantification method to reproducibly quantify knee joint laxity in mouse knees.

METHODS: Design & Manufacturing: Fusion360 software was used to design & 3D print a custom device to apply a consistent anterior force to the tibia with the femur immobilized, analogous to an anterior drawer test, followed by high-resolution radiography (Faxitron). The device consists of (1) a holder securing the mouse in a supine position, stabilizing the hip, femur, and foot with the knee flexed at 90°, (2) a platform securing the holder inside the Faxitron, (3) a pulling mechanism consisting of a clamp around the proximal tibia and a thread hooked onto the clamp connecting a 10g weight via a pulley system (**Fig 1**). Device reproducibility & validation experiment: Cadaveric 14 to 16-week-old C57BL6 male mice (n=6) with intact ACLs were used. The experiment consisted of 2 parts: (1) Testing on the right knee with 0g & 10g of anterior tibial force, repeating each test 3 times per mouse per weight; (2) Rupturing the ACL in the right knee with a previously established device allowing consistent closed rupture of the ACL[5], followed by repeating part (1) with this ruptured ACL. X-ray images were taken before and after each loading session. 10g was chosen as it is approximately ½ of the mouse's body weight. Quantification of laxity: Measurement of anterior tibial translation (ATT) was used to determine knee joint laxity. Fusion360 was used to quantify ATT by measuring the position of the tibia relative to the femur using 3 measurement methods (**Fig 2**), one devised by our lab and the other two based on previous studies (Kotelsky et al.[6] and Iwata et al.[7]). ATT represents the net translation (i.e., the measurement with 10g minus the measurement with 0g) and was normalized for tibial width. The reproducibility of each approach was quantified by looking at the standard deviation within the measurements for each mouse (**Fig 3**). Statistical analysis: Statistical differences were analyzed using one/two-way ANOVA with post-hoc Tukey.

RESULTS: The device was able to detect a statistically significant increase in normalized ATT (represented as a % of tibial width) in the ruptured ACL group compared to the intact ACL group using all 3 measurement methods (**Fig 2**). The mean differences between intact ACL vs. ruptured ACL for methods 1, 2, and 3 were -10.55% (p=.0026, CI [-17.23, -3.87]), -9.85% (p=.004, CI [-16.46, -3.24]), and -9.63% (p=.0032, CI [-15.91, -3.36]) respectively with increase in knee laxity after ACL rupture. Measurement method 3, referencing both the lateral and medial femoral condyles[7], consistently has the lowest mean variation of 1.683±0.668%, 1.550±0.814%, 2.783±2.584%, and 4.633±2.946% for the intact 0g, intact 10g, ruptured 0g, and ruptured 10g groups respectively (**Fig 3**). However, there were no statistically significant differences between the mean variations using the different measurement methods.

DISCUSSION: This study successfully developed a device to quantify anterior tibial translation in cadaveric mice. The device was able to detect significant differences between intact and ruptured ACLs. However, the model presented with some limitations: (1) varied rotation in the femur due to differences in hip mobility and body placement of the mouse when lying supine, (2) difficulty in reproducing a 90° angle in the knee. Adjustments have been made in the device design to address these limitations such as incorporating an adjustable bumper underneath the hip to adjust for the femoral rotation of each mouse and using a clear resin to print the foot holder to help adjust the angle of the knee. Further validation of the device will be done using live animals under brief anesthesia. After assessing the reproducibility and sensitivity of the device with live mice, the device will be used to evaluate the impact of timing (immediate versus delayed) of ACLR on knee stability in a mouse ACL rupture model. The system can also be used to assess the progression of PTOA after ACLR given the association between reduced knee stability and the progression of PTOA[8].

SIGNIFICANCE/CLINICAL RELEVANCE: Establishing a reproducible method to quantify knee joint laxity in mouse models provides an objective measurement to study outcomes after ACLR, and may thus provide valuable insights into improvements in the treatment & management of ACL injuries.

REFERENCES: [1]Costa et al. *J Exp Orthop*, 2022; [2]Cinque et al. *Am J Sports Med*, 2018; [3]Dare & Rodeo. *Curr Rheumatol Rep*, 2014; [4]Cardona-Ramirez et al. *J Orthop Res*, 2022; [5]Croen et al. *Am J Sports Med*, 2023; [6]Kotelsky et al. *Osteoarthr Cartil Open*, 2022; [7]Iwata et al. *Knee Surg Sports Traumatol Arthrosc*, 2007; [8]Friel & Chu. *Clin Sports Med*, 2013.

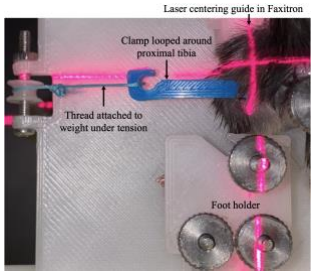

Figure 1: Photograph of the device zoomed at the right leg.

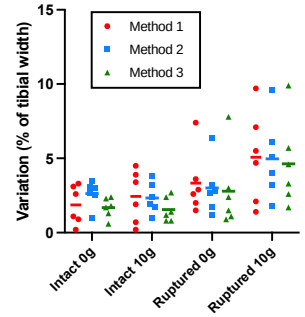

Figure 3: Variation, represented by SD, within replicate measurements per mouse in each ACL and weight group. Horizontal lines represent the mean. Method 3 has the lowest mean SD across all groups.

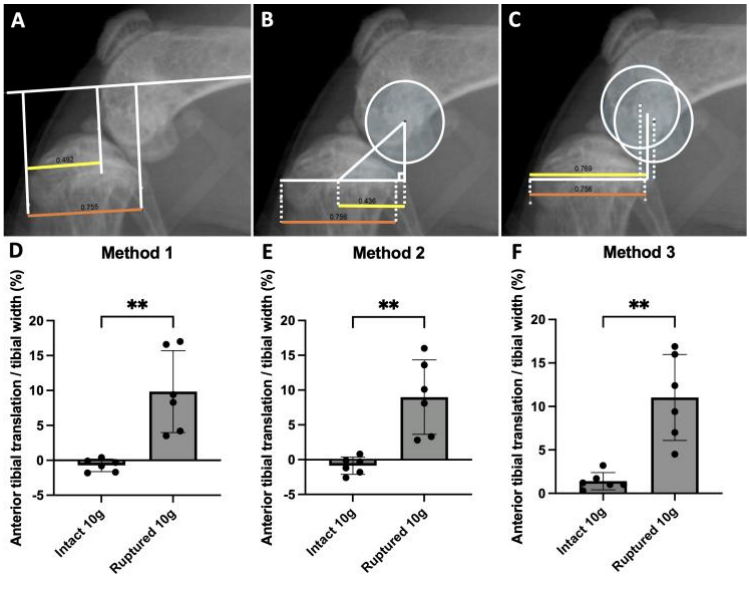

Figure 2: Normalized ATT for intact ACL & ruptured ACL. Three measurement methods were used for ATT **(A-C)**. Graphs of each method showing ATT normalized by tibial width for each ACL group **(D-F)**. Data represented as means ± SD. **P<0.01.