

Validation of Non-Invasive Mouse Model of Posterior Cruciate Ligament Injury with Manual Procedure Applying Posterior Force to the Tibia.

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INTRODUCTION: Injuries around the knee are a risk factor for post-traumatic osteoarthritis (PTOA), with ligament injuries being important contributors [1]. Preclinical, non-invasive (closed injury, externally induced without an incision) mouse models to induce PTOA are widely used, and the anterior cruciate ligament (ACL) injury model have been used in most studies [2]. Posterior cruciate ligament (PCL) injury likewise increases PTOA risk [3], but animal models reproducing PCL injury remain scarce. To our knowledge, reports of a non-invasive mouse PCL model are limited to conference abstracts, lacking detailed procedures and systematic, multimodal reproducibility testing; thus, no standardized model exists [4]. Our objective was to standardize a non-invasive mouse PCL injury model by refining the procedure and validating its reproducibility.

METHODS: Study design: This study was approved by IACUC. A total of 27 C57BL/6 mice (16 weeks old, male) were used. Only males were selected to avoid the influence of female hormones such as relaxin on ligament laxity. The manual procedure was applied to the right knee of each mouse, and the contralateral left knee served as the control without injury induction. Of these, 19 mice underwent in vivo biomechanical testing with load measurement and were subsequently assigned to macroscopic inspection (n = 6), phosphotungstic acid (PTA)-enhanced micro-CT (n = 6), or histology (n = 7) in order to confirm PCL injury. The remaining 8 mice without load measurement were evaluated by ex vivo radiography (gravity sag view) to assess posterior tibial translation (PTT). The overall study flow is summarized in Fig. 1. **Manual procedure:** Mice were placed in a supine position under general anesthesia on a custom-made table (Fig. 2A, B). The right knee was flexed at 90° and secured in the fixture (Fig. 2C). A posteriorly directed force was applied to the tibia resulting in PTT (Fig. 2D). A distinct “pop” was detected at the time of injury, and instability was immediately assessed by the posterior drawer test (PDT). **In vivo biomechanical test:** A load cell was applied to the hind limb, and a posteriorly directed force was delivered to the tibia while recording the loading curve. The peak load was defined as the posterior tibial force at PCL injury. **Macroscopic inspection:** After biomechanical testing, the knee joint capsule was incised in the allocated mice. The PCL was inspected macroscopically from both the anterior and posterior aspects to evaluate fiber continuity. **PTA-enhanced micro-CT:** Hind limbs were harvested after biomechanical testing, dehydrated, and stained with 0.7% PTA in 90% methanol for 5–6 weeks according to a previously described protocol [5]. After staining, the samples were scanned using micro-CT, and the PCL substance was evaluated for continuity. **Histology:** Paraffin-embedded sagittal sections (5 μm) of the knee joint were prepared and stained with hematoxylin and eosin (H&E). Sections were evaluated for fiber continuity of the PCL substance. **Ex vivo radiography:** Hind limbs were positioned laterally as shown in Fig. 6A, with the knee flexed and the distal segment left unsupported to permit gravity-induced posterior sag of the tibia. Lateral radiographs were then obtained in the gravity sag view (Fig. 6B), and PTT was defined as the perpendicular distance between the solid and dotted reference lines. **Statistical analysis:** Data are presented as mean ± SD (range). For PTT, within-animal comparisons between the manual and contralateral control sides were performed using a two-tailed paired t test (α = 0.05) in EZR (version 1.68).

RESULTS: All 27 knees exhibited a “pop” sound and an immediate positive posterior drawer test (100%), and no periarticular fractures were detected on radiographs or micro-CT. In 19 mice with load measurement, PCL injury with PTT occurred at 21.69 ± 3.10 N (range, 17.73–26.55) and was confirmed by at least one modality: macroscopic findings (discontinuous fibers at the substance; Fig. 3A, C), PTA-enhanced micro-CT (discontinuity with reduced staining contrast compared with the control side; Fig. 4A, B), or histology (discontinuity with loss of organized fiber alignment; Fig. 5A, C). No concomitant ACL injury was identified in these mice. Gravity sag view radiographs (n = 8) showed greater PTT on the manual side than the control side: 0.35 ± 0.04 mm (range, 0.29–0.40) vs 0.10 ± 0.06 mm (range, 0.02–0.18); p < 0.001.

DISCUSSION: To establish a non-invasive PCL model, we adapted a manual procedure from published non-invasive ACL protocols [6], inducing PCL injury with posterior knee instability. PCL injury was confirmed by multiple modalities—macroscopic inspection, PTA-enhanced micro-CT, and histology—and instability was quantified as radiographic PTT on gravity sag view, which is more physiologic than an externally applied posterior tibial force in a previous abstract [4]. Compared with a surgical PCL transection model [7], our method more closely mimics the clinical injury pattern while avoiding arthrotomy and specifying load/angle criteria that support standardization. Limitations include incomplete assessment of concomitant meniscus and cartilage injury. In conclusion, our manual loading technique reproducibly induced PCL injury with posterior knee instability, confirmed by multiple modalities and quantified as increased radiographic PTT. ACL integrity was preserved, and no periarticular fractures were detected.

SIGNIFICANCE/CLINICAL RELEVANCE: This study introduces a standardizable, non-invasive mouse PCL injury model that induces posterior knee instability, with demonstrated reproducibility and multiple modalities. The model provides a practical platform for studying PCL-related PTOA mechanisms.

REFERENCES: [1] Muthuri SG et al. Osteoarthritis Cartilage. 2011. [2] Christiansen BA et al. Osteoarthritis Cartilage. 2015. [3] Wang SH et al. PLoS One. 2018. [4] Enomoto S et al. Osteoarthritis Cartilage. 2022. [5] Lesciotto KM et al. Dev Dyn 2020. [6] Okazaki Yet al. Am J Sports Med. 2024. [7] Deng Z et al. Biosci Rep. 2019.

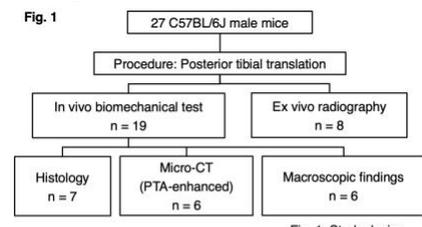


Fig. 1. Study design.

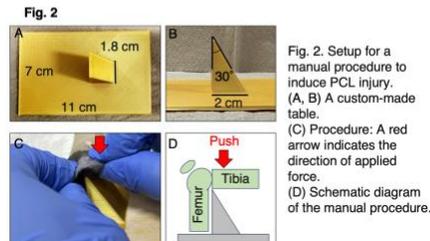


Fig. 2. Setup for a manual procedure to induce PCL injury. (A, B) A custom-made table. (C) Procedure: A red arrow indicates the direction of applied force. (D) Schematic diagram of the manual procedure.

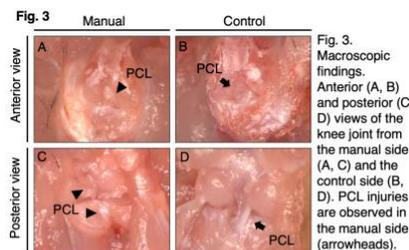


Fig. 3. Macroscopic findings. Anterior (A, B) and posterior (C, D) views of the knee joint from the manual side (A, C) and the control side (B, D). PCL injuries are observed in the manual side (arrowheads).

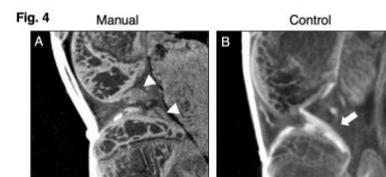


Fig. 4. Representative sagittal images of PTA-enhanced micro-CT. (A) PCL discontinuity with reduced contrast (arrowheads). (B) PCL with continuous, well-enhanced band (arrow).

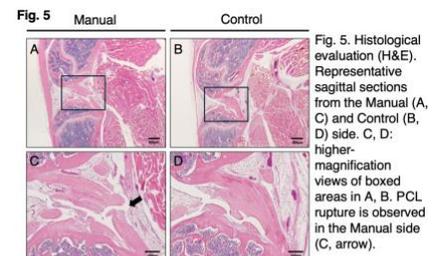


Fig. 5. Histological evaluation (H&E). Representative sagittal sections from the Manual (A, C) and Control (B, D) side. C, D: higher-magnification views of boxed areas in A, B. PCL rupture is observed in the Manual side (C, arrow).

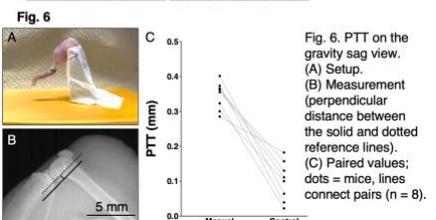


Fig. 6. PTT on the gravity sag view. (A) Setup. (B) Measurement (perpendicular distance between the solid and dotted reference lines). (C) Paired values; dots = mice, lines connect pairs (n = 8).