The role of medial meniscus changes in C57BL/6/J mouse with aging and in Senescence-Accelerated Mice Prone 8 (SAMP8) as a spontaneous osteoarthritis model

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INTRODUCTION: In the course of primary osteoarthritis (OA) development, increased attention has been directed towards understanding the significance of meniscus degeneration and extrusion. Human clinical studies suggest extrusion as an early indicator of OA. Interestingly, histological studies in mice have indicated that meniscus degeneration initiates prior to cartilage degeneration1. While meniscus extrusion has been extensively studied in cases of root tears and OA, limited knowledge is available concerning its occurrence before OA onset in healthy individuals. Furthermore, the assessment of meniscus extrusion and tissue degeneration during the OA development process in natural aging and senescence accelerated mice remains largely unexplored. This study aimed to detect the occurrence of meniscus extrusion in relation to the chronological progression of cartilage, subchondral, and meniscus degeneration using biplane histological knee sections from two distinct mouse models. We hypothesized that meniscus extrusion and histological degeneration could be detected prior to cartilage damage, providing supporting evidence for extrusion as an early clinical sign of OA onset.

METHODS: Histological knee sections were obtained from two mouse models: natural aging C57BL/6 (B6), and senescence accelerated SAMP8 (P8) in control to senescence resistant SAMRI (R1) mice models. All animal experiments were performed according to protocols (A14-138-2, A17-30-2) approved by the Hiroshima University Animal Care and Use Committee. Both coronal and sagittal knee sections were used to fully describe medial meniscus anterior, medial and posterior extrusion in relation to surrounding tissue degeneration. Samples were stained for collagen II, X and Safranin-O for histological analysis scoring, to evaluate cartilage, bone, and posterior extrusion. Sagittal sections were scored based on histological grade previously developed and validated by our group3. Meniscus extrusion was measured using oARSI scoring. Extra-osseous bone changes were noted in anterior extrusion, as evaluated using OARSI scoring. Cartilage degeneration scores. Meniscus posterior extrusion significantly increased, in parallel with meniscus degeneration, posterior menisco-tibial ligament at 11 weeks, and cartilage degeneration at 14 weeks. Meniscus extrusion significantly increased only at 22 weeks when severe OA was present. Switching to the sagittal plane in B6 mice, knees were evaluated at 3, 12, and 22 months of age (n=10/group). (Fig. 1C) Posterior meniscus extrusion increased significantly, in parallel with meniscus degeneration, posterior menisco-tibial ligament degeneration scores and overall meniscus length. (Fig. 1D) However, osteoarthritis did not manifest at these observed time points, as indicated by low tissue degeneration scores. Meniscus posterior extrusion significantly increased only at 22 weeks when severe OA was present. Switching to the sagittal plane in B6 mice, knees were evaluated at 3, 12, and 22 months of age (n=10/group). 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RESULTS SECTION: B6 mice in the coronal plane were evaluated at 3, 12, and 22 months (n=10/group). While meniscus degeneration showed a noticeable increase across time points, it’s noteworthy that meniscus extrusion only became evident at 22 months. (Fig. 1A) At this juncture, knee OA was apparent, as evidenced by significant increases in OARSI and subchondral bone scores. (Fig. 1B) Examining the coronal plane in the P8/R1 model mice were compared at 6, 11, 14, and 23 weeks of age (n=10/group). In the spontaneous OA group, subchondral bone changes were evident from 6 weeks, followed by meniscus degeneration at 11 weeks, and cartilage degeneration at 14 weeks. Meniscus extrusion significantly increased only at 22 weeks when severe OA was present. Switching to the sagittal plane in B6 mice, knees were evaluated at 3, 12, and 22 months of age (n=10/group). (Fig. 1C) Posterior meniscus extrusion increased significantly, in parallel with meniscus degeneration, posterior menisco-tibial ligament degeneration scores and overall meniscus length. (Fig. 1D) However, osteoarthritis did not manifest at these observed time points, as indicated by low tissue degeneration scores. Meniscus posterior extrusion demonstrated a good correlation with cartilage, subchondral bone, meniscus, and ligament scores (r = 0.72, 0.67, 0.78, 0.86, respectively; p<0.05). In contrast, anterior meniscus extrusion showed no significant differences between time points and had poor correlation with other scores. Finally, in the sagittal plane of the P8/R1 group, a pilot study was conducted at 6 and 14 weeks to ascertain if extrusion would be evident at the early stages of OA (n=4/group, n=8/group respectively). At 14 weeks, there was no presence of posterior extrusion or ligament degeneration. However, notable increases were observed in meniscus and subchondral bone scores.

DISCUSSION: Findings in the coronal plane suggest that the medial meniscus extrusion is likely a consequence of knee OA, rather than being evident before the manifestation of OA. Coronal plane extrusion was present in both natural aging and spontaneous OA models, occurring alongside apparent degenerative changes. Remarkably, the absence of notable increase in anterior extrusion, the presence of coronal extrusion at later time points, and the initial rise of posterior extrusion follow a distinct progression from posterior to anterior. This pattern can potentially be attributed to the posterior joint being subjected to increased load due to knee flexion of 120-150 degrees during the gait cycle of mice. In the sagittal group of naturally aging mice, posterior extrusion significantly increased with age. Meniscus and posterior ligament degeneration scores demonstrated a substantial correlation with the extrusion ratio, both showing significant increases. The absence of posterior extrusion or ligament degeneration in 14-week-old P8 mice with early OA changes suggests that posterior extrusion results from natural degeneration and posterior joint load. This proposition is supported by the significant increase in sagittal B6 mice meniscus radial length over the observed time points. Increase in meniscus size has previously been reported to be a consequence of degeneration, likely resulting from the separation of micro-fibrils2. These findings point towards a natural degenerative extrusion path, without involving root tear, knee trauma, or being part of the genetic OA pathway.

SIGNIFICANCE/CLINICAL RELEVANCE: This study provides quantitative evidence to suggest a natural degenerative extrusion path with meniscus extrusion and menisco-tibial ligament degeneration occurring during aging without OA or meniscus trauma in mice.


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