The role of medial meniscus changes in C57BL6/J mouse with aging and in Senescence-Accelerated Mice Prone 8 (SAMP8) as a spontaneous osteoarthritis model

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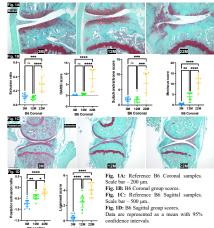
INTRODUCTION: In the course of primary osteoarthritis (OA) development, increased attention has been directed towards understanding the significance of meniscus degeneration and extrusion. Human clinical studies suggest extrusion as an early indicator of OA. Interestingly, histological studies in mice have indicated that meniscus degeneration initiates prior to cartilage degeneration[1]. 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 from two mouse models: natural aging C57BL/6J (B6), and senescence accelerated SAMP8 (P8) in control to senescence resistant SAMR1 (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 tissue scoring. Cartilage degeneration was evaluated using OARSI score. Subchondral bone changes were scored based on histological grade previously developed and validated by our group[2]. Meniscus was assessed based on surface, cell and stain changes using an already developed grade[3]. Extrusion of the meniscus was measured using quantitative ratio using computer software. (Fiji, imagej.net). Extrusion was quantified by the distance to or over the extrusion line (E). The part of meniscus (B) exceeding the extrusion line was divided by the whole meniscus length (A). Meniscus extrusion ratio = B/A, mathematically ranges from -1 to 1. Extrusion line (E) was determined by the end of tibial plateau, while in case of osteophyte excluding it from the bone margin, as recommended by extrusion studies. Histological grade was developed based on cellular and morphological changes to evaluate posterior menisco-tibial ligament in sagittal knee sections. Every available section of the same knee was evaluated and average values were included in the statistics, therefore the statistical unit is the knee of one mouse. Only left knees of male mice were included. Sagittal samples of more than 45 degree of flexion were excluded to avoid false posterior extrusion. Statistical analysis was performed using GraphPad Prism version 10.0.1. B6 model group differences were compared using one-way ANOVA followed by Holm-Sidak correction. If skewness of the data was observed, indicated by Q-Q plot, we used Kruskal-Wallis test with Dunn's correction. P8/R1 mice scores at each timepoint were compared using Mann-Whitney U test followed by Holm-Sidak correction. The relationship between the values were assessed by Spearman's correlation coefficient (r); 95%. The significance level was set at $p < 0.05$.

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 23 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.

Fig. 1A: Reference B6 Coronal samples. Scale bar – 200 μm.
Fig. 1B: B6 Coronal group scores.
Fig. 1C: Reference B6 Sagittal samples. Scale bar – 500 μm..
Fig. 1D: B6 Sagittal group scores.
Data are represented as a mean with 95% confidence intervals.

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-fibrils[4]. 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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