Abnormal Tibial Torsion May Cause An Earliest Detectable Deformity in STR/OrtCrlj Osteoarthritis Mouse Model

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**Introduction:** Morbidity caused by osteoarthritis of the knee joint (knee OA) enormously expands medical expense for the treatment. Physicians and surgeons as well as researchers are anxious to develop treatments for OA patients at early stages of knee OA. Reported model animals develop detached anterior cruciate ligament or excised menisci, which mainly reflects post-traumatic late stages of OA. Lack of choice is apparent among the available models to be used for testing the efficacy of potential drugs, which target the early stages. A few reported models, which may be suitable for studying early stages, are those of spontaneous knee OA in guinea pigs, C57BL/6j and STR/ori mice. Our histological observation as well as soft x-ray and micro-three dimensional computed tomography (3DCT) profiles supported that early changes typically observed among OA patients including the internal tibial torsion are reproducible in STR/ OrtCrlj mice. Abnormality was detectable even in the 10- to 20-week-old animals. We believe that the internal tibial torsion reflects mechanisms through which osteoarthritis develops in STR/OrtCrlj mice.

**Materials and Methods:** STR/OrtCrlj mice were obtained from Charles River Laboratories Japan, Inc. (Yokohama, Japan) and analyzed at either 5 weeks (n=2), 10 weeks (n=18), 15 weeks (n=2), 20 weeks (n=18), 25 weeks (n=2), 26 weeks (n=2), 35 weeks (n=2), or 45 weeks (n=2) of age. The mice were euthanized by intraperitoneal injection of sodium pentobarbital, and bilateral lower limbs were excised from each hip joint and cleaned. The excised limbs were immediately reduced with 4% paraformaldehyde in phosphate buffer solution (Wako Pure Chemical Industries, Ltd., Tokyo, Japan) at 4°C for 48 hours. Samples were analyzed by Soft X-ray system (SOFTEX-CMB4; SOFTEX Corporation, Kanagawa, Japan). The tibiae were analyzed by high-resolution micro-CT system (MCT-100CB; Hitachi Medico Technology Corporation, Kashiwa-shi, Japan). The histological studies, dissected bilateral lower limbs were further decalcified in 3M EDTA for 2 to 3 weeks. Slides were prepared with 4-μm-thick sections, embedded in paraffin, and stained with classical hematoxylin/eosin or safranine O.

**Results:** Abnormality of STR/OrtCrlj knee joint was evaluated on soft X-ray radiograph of the lateral view. Joint space narrowing, subchondral bone sclerosis and osteophyte formation were noted in the several knees of 25 week-old mice. Except for one out of 24 knees in 12 animals of 35-week and 2 out of 4 knees in two 45-week-old mice, all the animals showed subchondral bone sclerotic changes in patella and subluxation of tibiofemoral joint. Histological abnormality of femorotibial joints was common at 25 weeks, while it was found only sporadically at 20 weeks. There was a greater difference in cartilage degeneration between 26 and over 35 weeks. No CBA, C57BL/6j or ICR control mice, both males and females, showed any such abnormal changes in the knee joint up to 45 weeks. Histological characteristics of patellofemoral joint tissue were analyzed with the intention to detect earlier abnormality in the articular cartilage. Alterations of cell density and gradient and the decreased staining with Safranin O were ubiquitous observed in the patellar groove of femoral cartilage. These alterations were already seen in patellofemoral joint cartilage of all the knees of 10-week-old mice. Most of them, however, showed the decreased intensity by Safranin O staining only in the cartilage of femoral side but not in the patella side. At 20 weeks, disorders in gradient and density of cells were seen in most of the mice. The loss of Safranin O staining was moderate to severe. The articular surface was quite irregular and the craft was frequently seen. Dislocation of patellae was observed in 1 of 18 10-week-old and 4 of 18 20-week-old mice. The dislocated patellae showed markedly abnormal histology.

In 20-week-old STR/OrtCrlj mice with age-matched C57BL/6j mice as a control, radiographic and micro-CT observations pointed out internal rotation of tibiae. Although the extent of such deformity was variable, the mice with more significant twist had dislocated patella. These findings in STR/OrtCrlj mice were detectable from 10 weeks of age. Morphological measurements of tibial torsion by micro-CT 3D analysis showed that typical STR/OrtCrlj tibiae at 35 weeks were twisted by 10 to 27 degrees when the two axes, the proximal posterior condyle and bimalleolar axes are superimposed [2].

**Discussion:** Previous reports suggested that the histological changes preceded the morphological changes on soft X-ray radiograms. The earliest histological change detected in STR/ori knee joint was microscopic alteration in the insertion of anterior cruciate ligament. These reports of histological changes, detected with various dyes such as Azure, Alcian-blue, HE or Toluidine blue, cannot be compared to each other to assess the degree of cartilage matrix degradation. The histological changes in patellofemoral and femorotibial joint cartilage in dislocated patella cases have been reported. Schunke et al. described that the osteoarthritic changes first appeared on the medial tibial plateau and that as the disease progressed, there was a loss of cartilage which resulted in a pronounced instability of the knee and a varus deformity [1]. This was followed by medial patellar subluxation. The authors concluded that cartilage degeneration in the medial tibial condyle develops spontaneously due to an unknown pathogenesis and this event is responsible for secondary patellar subluxation. In this study, we identified the area of the earliest reproducible histological change at 10 weeks, in the cartilage tissue of STR/OrtCrlj knee joints. This is the first report of abnormal tibial torsion in the STR/OrtCrlj mice. Varus, valgus, and other axial deformities are well known in osteoarthritis of the knee joint. Torsional abnormalities are considered as a possible cause by some authors but by others, as a result. Particularly, Turner reported that the knee OA patients with patellofemoral instability had greater than normal external tibial torsion, whereas patients with panarticular osteoarthritis of the knee had reduced external torsion or true internal torsion. In addition, Yagi et al. reported tibial torsional deformities in association with medial-type osteoarthritic knees [2]. Eckhoff et al. introduced a technique by 3DCT reconstruction to assess rotation of the tibia from the structural landmarks they selected [3]. Our results by their measurement method revealed tibial torsional deformity in STR/OrtCrlj lower limbs. We believe that the internal rotational tibial torsion in STR/OrtCrlj cause patellofemoral joint instability and osteoarthritic knees.​


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