Methods: The right tibia of 8 week-old CBA mice were loaded 3 times a week for 2 weeks as described previously. Ankle joints were dissected, wax embedded and sectioned (sagittal, 9 μm). Toluidine blue stained sections at regular interval across the ankle joint were scored for articular cartilage (AC) lesions (grade 0-4; 0=normal; 1=loss of stain; 2=lesion in uncalled cartilage; 3=lesion in calcified cartilage; 4=exposed subchondral bone). Maximum scores across the tibia-talus, talus-calcaneum and tibio-fibula joints were used and were compared to non-loaded control joints.

Conclusions: Col2-pdEGFP mice are a suitable model to isolate by FACS a mixed population of proliferative and (pre)hypertrophic chondrocytes and to study growth plate chondrocyte biology in postnatal mice older than 4 weeks old.

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MOUSE ANKLE JOINTS ARE, UNLIKE KNEES, RESISTANT TO LOAD-INDUCED LESION FORMATION

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Purpose: Incidence of osteoarthritis (OA) in human ankle joints has been reported to be much lower than in the knee. In addition, ankle cartilage has been shown to be relatively resistant to damage from mechanical and biochemical insults compared to the knee joint. The mechanisms involved in this protective role of ankle cartilage are, however, still unknown. Development of an animal model for studying the effects of mechanical loading in the ankle joint would be very valuable in helping to define those mechanisms. Herein, we exploited a mouse model used for arthritis and ankle joint loading in a mouse, in which localised load-induced femoral cartilage lesions are formed, to determine whether it may also serve as a model to study this relative protection of ankle joints.

Methods: Standard culturing conditions induced dedifferentiation of the pd2EGFP-chondrocytes as indicated by the 20-fold increase in col1 by T14. The latter does not occur under serum free culturing conditions.

Conclusions: Col2-pdEGFP mice are a suitable model to isolate by FACS a mixed population of proliferative and (pre)hypertrophic chondrocytes and to study growth plate chondrocyte biology in postnatal mice older than 4 weeks old.

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IDENTIFICATION OF JOINT OCHRONUSIS IN A MOUSE MODEL OF ALKAPTONURIA

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Purpose: Alkaptonuria (AKU) is a rare genetic disease resulting from deficiency of the enzyme homogentisate 1,2-dioxygenase (HGD). It is characterized by elevated circulation of homogentisic acid (HGA), a metabolite that is deposited as a dark polymer, termed ochronotic pigment, in connective tissues particularly the articular cartilages of the weight-bearing joints. Ochronosis leads to a severe, early onset osteoarthropathy, which currently has no known treatment. One of the limitations in studying ochronosis is the lack of experimental models. Mice deficient in HGD exist but do not normally develop ochronosis, despite elevated circulating and urinary HGA. We recently detected deposition of ochronotic pigment in the kidneys of mice, heterozygous for the AKU mutation and homozygous for the AKU+/- FAH-/- genotype. These mice produce a normally drinking anti-oxidant that inhibits HGA polymerisation and protects joint cartilage from nitisinone for 11 months and sacrificed for study. Animals were dissected and joint tissues and organs processed for routine histology. Tissues were stained with Schmorl’s reagent which is a sensitive stain for ochronotic pigment.

Results: Macroscopic investigation of the renal system revealed pigmented nodules in the kidney. Microscopy confirmed these to be ochronotic pigment. Initial inspection of the joints did not show gross pigmentation or evidence of joint degeneration. However, careful histological analysis revealed the presence of ochronotic pigment in the articular cartilages of the knee joints. Chondrocytes in the calcified cartilage of the distal femur, proximal tibia and fibula all displayed pigment in the pericellular and territorial matrix. Pigmentation was not seen in the bone matrix, or other cartilage zones. Staining with Schmorl’s reagent confirmed the presence of ochronotic pigment in these regions, consistent with findings in human AKU tissues.

Conclusions: Here we describe the first observations of ochronosis in joint tissues of mice. The pattern of initial pigmentation appears to be remarkably similar to that in human AKU, indicating that this will be an excellent model to investigate the initiation and progression of ochronosis. It had previously been thought that animals with the AKU mutation did not display any of the phenotype observed in humans. Several theories have been advanced for the lack of ochronosis, including insufficient life span; failure to reach threshold circulating levels of HGA and/or the protective effects of ascorbic acid, which in mice is an endogenously produced anti-oxidant that inhibits HGA polymerisation and protects joint matrix components. The AKU+/- FAH-/- mice are normally maintained on nitisinone and some mice can adapt to long term withdrawal, undergoing spontaneous, clonal loss of heterozygosity for the AKU mutation. The subsequent increase in HGA coupled with deterioration of renal function appears to trigger pigment deposition in the joints of the mice. We believe these mice will provide an excellent model to investigate ochronosis and to develop therapeutic strategies for prevention of joint destruction in AKU. This model should also be useful to investigate pathogenesis of generalized osteoarthropathy.