The anatomical distribution of ochronotic pigment in alkaptonuric mice reveals that ageing and mechanical loading make collagen susceptible to chemical attack.

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Musculoskeletal disease is often related to tissue damage from mechanical loading or “wear and tear”. Currently there are no good experimental models predicting early tissues changes in response to mechanical damage. Alkaptonuria (AKU) is an ultra-rare disease where there is failure to metabolise homogentisic acid (HGA), which is characteristically elevated in the blood, despite urinary excretion. Over time HGA is deposited as ochronotic pigment in connective tissues leading to musculoskeletal disorders including tendon ruptures, heart valve stenosis and severe osteoarthritis. Here, the anatomical distribution of ochronotic pigment was investigated in AKU mice to determine potential factors initiating ochronosis.

Formalin-fixed, paraffin sections from AKU mice (*Hgd tm1a -/-*) were stained with Schmorl’s stain to detect ochronotic pigmentation. Within large weight bearing joints (knee, hip, shoulder, elbow), pigmentation was identified within the calcified articular cartilage (CAC). In the knee joint, pigmentation was also observed in the fibrocartilaginous calcified meniscus. Quantification of pigmented chondrons in the CAC in representative knee joint sections of mice aged 7-52 weeks shows that pigmentation initiates at 9 weeks and progresses linearly with age. In the vertebral column, pigmentation was found in the calcified vertebral endplates, but not in bone nor disc (non-calcified fibrocartilage). A striking observation was the significantly greater number (mean ±SEM; n=5 sections) of pigmented chondrons in the endplates of the L2 intervertebral disc (IVD)(48.4 ±4.5) compared with an IVD from the base of the tail (2.2 ±0.6)(p=0.002), reflecting greater loading in the lumbar region. In addition, clusters of pigmented chondrons were observed in areas of greater tensile stress, such as the insertions of tendons and ligaments, including the Achilles tendon on the calcaneus and the cruciates on the tibia/femur. No pigmentation was observed in the eye, ear, liver, kidney nor notably the heart valves.

Anatomically, the initial sites of ochronosis are in tissues subjected to the most mechanical loading, such as CAC in weight-bearing joints. Interestingly, calcified tissue appears more susceptible to early pigmentation. In addition, greater stress appears to lead to increased pigmentation; in effect HGA behaves like an endogenous marker of repetitive load-induced matrix damage.

Ethics statement: All animals were house and maintained under UK Home Office guidelines.