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## **NOVEL MODELS OF LATE ONSET, PROGRESSIVE OSTEOARTHRITIS CAUSED BY POINT MUTATIONS IN THE TWO ALPHA CHAINS OF COLLAGEN TYPE I**

### Purpose:

Genetic modification is a useful tool for identifying novel genes associated with osteoarthritis (OA) and we have combined a phenotype-driven screen with ageing to identify mutations resulting in late-onset or age-related disease. As part of the Harwell Ageing Screen mutagenised mice were aged to 18 months and phenotyped at various time points, to allow for detection of late onset disease and to track its progression. This approach has identified 2 novel models of late onset, progressive OA, caused by point mutations in the genes *Col1a2* or *Col1a1*. Defects in the alpha subunits of Collagen I are most notably associated with osteogenesis imperfecta (OI) and Ehlers Danlos syndrome, although there has been an association found between *Col1a2* and OA in a founder population (Snelgrove *et al*, 2005). Our current work seeks to examine the causation between Collagen I defects and OA.

### Methods:

Mutant animals were generated using the mutagen *N*-ethyl-*N*-nitrosourea (ENU). Cohorts were bred from the mutagenised founder mice, and then phenotyped. Initial phenotyping in the musculoskeletal screen included clinical chemistry analysis, grip strength analysis, X-ray and DEXA analysis at multiple time points, and micro-CT and histological analysis at the terminal time point. The mutation was identified using a dense SNP panel and whole genome sequencing. To confirm that our mutants do not have OA secondary to OI we have compared bone mineral density (BMD), size of mouse and bone strength using 3 point bone bending; as bone fragility, abnormal BMD and short stature are common symptoms of OI. To investigate the mechanical properties of the collagen we have performed quasi-static mechanical testing on fascicles dissected from mouse tail tendon.

### Results:

To date we have confirmed the presence of point mutations in two mutant lines with very similar phenotypes. One which creates a new splice acceptor site, causing an extra amino acid to be inserted into Exon 22 of the Collagen type 1 alpha 2 subunit, and the other which creates an early stop in Exon 31 of the alpha 1 subunit (GLN677STOP).

A lack of significant difference between mutant *Col1a2* and wild type animals in BMD, bone strength, and the absence of fractures in these mice, indicate that these animals do not have OI. Histological analysis of the knee joints, confirm the presence of osteophytes and the complete lack of articular cartilage at the load bearing surface of the joint in aged mice.

Mechanical testing of fascicles taken from the tail tendons from mice carrying mutant *Col1a2*, indicate a significant difference in hysteresis between genotypes thus suggesting a difference in the viscoelastic mechanical properties of collagen I in these mice.

### Conclusions:

Mutations in *Col1a2* and *Col1a1* can cause osteoarthritis independent of osteogenesis imperfecta. Whilst OI has been associated with OA before it has always been thought to result from bone damage, particularly in sub-chondral bone. Here we provide a potential alternate explanation.

The current data indicates that the elasticity of the fascicle in our mutant is abnormal. Our working hypothesis is that the collagen I could therefore also cause abnormal elasticity in other tendons, ligaments or fibrocartilage such as the meniscus, causing abnormal loading across the joint and the subsequent development of OA.

We are currently undertaking a more detailed time course with frequent time points, which will enable us to take samples for histological analysis, micro-CT and a gene expression study. The latter will allow us to track disease progression through the expression of key OA-associated genes. Other work underway includes performing transmission electron microscopy to determine the collagen arrangement within the fascicles, and protein studies to determine the level of expression of the mutant protein in collagen I.

Our novel models will be useful tools to investigate the pathogenesis of OA and potentially point to a potential mechanism for the development of this disease in a subset of patients.