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A *Col2a1* Mutation Leads to Increased Degradation of Type II Collagen in Articular Cartilage: A Murine Model for Osteoarthritis

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Normal articular cartilage is characterized by a resilient collagen and proteoglycan matrix interdispersed with chondrocytes. Collagen type II is the most abundant collagen found in the matrix and is responsible for providing tensile strength to the tissue. The highly conserved *Col2a1* gene is responsible for coding all type II collagen α -helix chains.¹

The process of enzymatic MMP cleavage is a natural part of collagen recycling, but has been observed to be upregulated in articular cartilage of osteoarthritis (OA) patients. It is hypothesized that further denaturation (unwinding) of enzymatically cleaved collagen takes place following enzymatic cleavage by MMP. Because increased degraded type II collagen has been observed in human OA patients through use of immunohistochemistry, there is a need for an animal model to study and use to develop treatments.^{2,3}

C3H mice with disproportionate micromelia (*Dmm*) carry a radiation-induced mutation in the region of the *Col2a1* gene that codes the C-propeptide of type II collagen. The purpose of this study was to test the utility of the *Dmm* mouse as an animal model to study the pathogenesis of OA. *Dmm* is the only known homozygous *Col2a1* C-propeptide mutation animal model.⁴ The type II collagen specific antibody COL2-3/4m was used to immunolocalize degraded type II collagen in *Dmm* mice and verify their utility as an animal model for studying degraded type II collagen associated with OA.⁵

Knee joints from 3, 6, 9, and 12 month-old control (+/+) and heterozygous (D/+) animals were used in the present study. The knee joints were fixed in 4% formaldehyde and then decalcified in formic acid decalcifier. Next, the joints were sectioned coronally at 6 μ m using a cryostat and mounted on slides. After preparative blocking steps the sections were incubated overnight at 4° C with COL2-3/4m primary antibody. To visualize the staining the sections were then incubated with a secondary antibody conjugated with a fluorescing chromophore (FITC) and observed and photographed under a fluorescent microscope. After photographing, pixels stained at the superficial zone of the articular cartilage were quantified using the Adobe Photoshop 7.0 magic wand function. A nonparametric analysis of variance using ranks of the quantified values was performed using SAS software.

Dmm heterozygotes showed more staining for degraded collagen than wild-type mice of the same age. Differences between mutant and control degraded collagen were significant ($P < 0.05$) in six-, nine-, and twelve-month animals. The three-month animals showed no significant differences between wild type and mutant degraded collagen ($P = 0.34$) and staining did not differ significantly between age groups. The quantified

values demonstrate that the *Dmm* heterozygotes develop heightened levels of degraded collagen near six months of age and that the levels remain heightened at approximately the same level until at least twelve months.

The present study suggests the utility of *Dmm* heterozygotes as an appropriate model for the study of degraded type II collagen associated with genetically predisposed osteoarthritis. The data presented herein will be submitted for publication in *Osteoarthritis and Cartilage*. Future studies using *Dmm* mice might include developing and testing therapeutic interventions targeted at reducing collagenase activity, regulating interleukin levels related to collagen degradation, or preparing gene therapy treatments for type II collagen C-propeptide defects.⁶

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6. Acknowledgements: I thank Dr. A. Robin Poole of the Joint Diseases Laboratory, Shriners Hospital for Children in Montreal, Canada for generously providing the COL2-3/4m antibody and Dr. Dennis Eggett of the Center for Statistical Consultation and Collaborative Research at Brigham Young University for performing the statistical analyses.

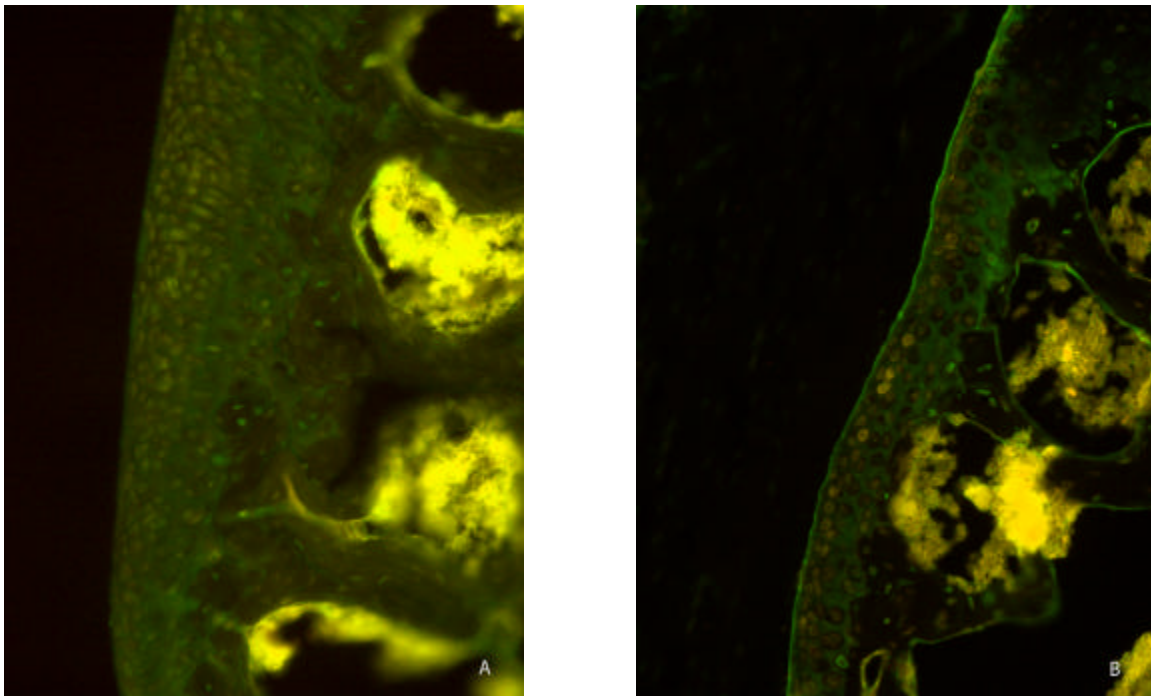


Figure – Comparison of wild-type (A) and mutant (B) articular cartilage stained with COL2-3/4m primary antibody and FITC conjugated secondary antibody. Tissue was photographed under a fluorescent microscope.