

## The development and structural integrity of articular cartilage in collagen IX, decorin and matrilin-1 knockout mice.

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**INTRODUCTION:** There are several different knockout mice that carry mutations in genes encoding key articular cartilage (AC) molecules. Some of these mutations are lethal and the structure of adult articular cartilage cannot be examined. There are several mutations, however, that do not appear to affect the normal development of the mice but which may reveal information regarding the importance of those proteins in the normal function and development of the tissue. It is shown that collagen IX null mice develop osteoarthritis at a relatively young age and skin fragility is noted in the decorin mutants<sup>1, 2</sup>, while matrilin-1 deficiency shows no obvious physiological changes. In each of these knockouts the development and ultrastructure of the AC appears unaffected<sup>1, 2, and 3</sup>. Optimisation of preparation protocols for light (LM), polarised light (PLM) and high resolution scanning electron (HRSEM) microscopies establish novel ultrastructural features and distinct stages of tissue formation in adult and developing normal (CD1 strain) mouse AC<sup>4</sup>. The investigation into the development and ultrastructure of the knockout strains AC revealed distinct differences between the mutants and CD1 mice.

**METHODS:** Mouse tibiae were dissected from adult (2-9 month old) and developing mice (1-18 days old) from each of the three knockout strains. The tibial plateaux were kept intact and fixed in formaldehyde in 0.1M Piperazine\_1, 4-bis-2-ethanesulphonic acid (PIPES) buffer (pH 8.5) for 24 hours, dehydrated in an ascending ethanol series, transferred to xylene, embedded in paraffin wax, sectioned at a thickness of 7.5µm and stained in picosirius red<sup>5</sup>. Sections were imaged using light and polarised light microscopy. Additional mouse tibiae were plunge frozen in propane cooled by liquid nitrogen, freeze substituted for 5 days in 58% acetone, 30% methanol, 10% acrolein and 2% tannic acid and a further 5 days in 100% acetone. Samples were then critical point dried,



Figure 1 shows perpendicularly aligned columns of chondrocytes(c) in the deep zone of 9 month old collagen IX null mouse AC after cryofixation and fracturing.

fractured and coated in platinum/palladium (80/20) for examination by scanning electron microscopy.

**RESULTS:** There was no discernible difference between normal and matrilin-1 mouse AC. Decorin and collagen IX null mutants showed, respectively, more rapid or retarded AC development than normal mice, although the final adult AC appeared normal. The rate of development varied from the normal by a few days only, but the results support suggestions describing the involvement of collagen IX and decorin in fibrillogenesis<sup>6, 7</sup>. In addition, altered physicochemical properties of the collagen matrix affected the chemical fixation of the collagen matrix ultrastructure in decorin and collagen IX adult tissue. The structural integrity of the collagen IX null AC also degraded with age and changes in the tissue ultrastructure were observed at 9 months, correlating with studies suggesting an early onset of osteoarthritis<sup>1</sup>.

**REFERENCES:** <sup>1</sup>Fässler *et al.*, (1994) *Proc. Natl. Acad. Sci. USA* **91**: 5070-5074; <sup>2</sup>Danielson *et al.*, (1997) *J. Cell Biol.* **136**: 729-743; <sup>3</sup>Aszódi *et al.*, (1999) *Mol. Cell Biol.* **19** (11): 7841-7845 <sup>4</sup>Hughes *et al.*, (2005) *Eur. Cell Mater.* **9**: 68-84; <sup>5</sup>Sweat *et al.*, (1964) *Arch. Pathol.* **78**:69-72; <sup>6</sup>Sholtzen *et al.*, (1994) *J. Biol. Chem.* **269** (45): 28270-28281; <sup>7</sup>Müller-Glauser *et al.*, (1986) *J. Cell Biol.* **102**: 1931-1939

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