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### Introduction
For reasons which remain unclear, the clinical prognosis of combined ligament injuries is worse than isolated ligament tears. Joint laxity and instability are more likely to occur in combined rather than isolated injuries. One potential explanation for this outcome is that the mechanical stresses on the healing structures may simply exceed their repair capacities. Another possibility is that the altered stress environment may affect the quality of the repair, predisposing the tissues to lengthen or elongate, resulting in the excessive joint laxity and poor mechanical properties.

In contrast to studies of the mechanical properties of the medial collateral ligament (MCL) in a model of a combined ligament injury, no studies have been performed comparing the gene expression of extracellular matrices and cytokines between isolated MCL injury and combined anterior cruciate ligament (ACL) and MCL injury. The molecular mechanisms whereby ACL deficiency could impact MCL healing are of particular interest since, if understood, they could potentially be controlled to improve scar healing.

The purpose of this study was to test the hypothesis that concomitant ACL transection would increase inflammation-related cytokines, and decrease collagen type I mRNA levels in the healing MCL.

### Materials and Methods
Seven skeletally mature female NZW rabbits (weighing 5.3 +/- 0.2 kg) had bilateral injuries to their MCLs, with a sharply cut MCL at the joint line. At the time of MCL transection, both knees were opened, and the ACL and MCL in one leg were transected while the other leg simply had sham ACL surgery after the MCL was transected. The side receiving the combined injury was randomly assigned. Six weeks after surgery, all of the rabbits were sacrificed and hind limbs were dissected aseptically. Each MCL was then isolated, weighed and snap frozen in liquid nitrogen prior to RNA extraction using the TRIspin method [1]. Total RNA yield was quantified fluorometrically using the SYBR® Green reagent (Molecular Probes) and 1 ug of RNA reverse transcribed into cDNA using the StratScript RNase H- kit (Stratagene, LaJolla, CA). Semiquantitative PCR was performed as previously reported [2] using rabbit specific primer sets for collagen type I, collagen type III, collagen type V, lumican, decorin, biglycan, fibromodulin, TGF-β, IL-1, TNF-α, and a housekeeping gene, GAPDH. PCR products, visualized by agarose gel electrophoresis and ethidium bromide staining were photographed, and then band densities analyzed using commercially available image analysis software (Scanaalytics). Levels of mRNA were normalized using the corresponding GAPDH value. The data were assessed using standard paired t-test. A significance level was set at p<0.05.

### Results
The mean quantity of total RNA in the MCL scar of ACL deficient knees and ACL intact knees were 0.84 +/- 0.16 ug/mg wet weight and 0.71 +/- 0.11 ug/mg wet weight, respectively (p<0.05). The quantity of RNA in the MCL was significantly greater in ACL deficient knees. With regard to mRNA levels for matrix molecules, collagen type I mRNA level was significantly lower in ACL deficient MCL scars than that in ACL intact MCL scars (Fig 1). On the other hand, ACL transection did not affect collagen type III, collagen type V, lumican, decorin, biglycan, and fibromodulin mRNA levels of MCL scars. The ratio of collagen type I/III of ACL intact and ACL deficient MCL scars were 1.44 and 0.78, respectively (p<0.05). In contrast with collagen type I mRNA levels, TNF-α mRNA levels were significantly greater in the MCL scars with ACL deficient knee than in the ACL intact knees (Fig 1). ACL transection did not significantly change the mRNA levels for either TGF-β or IL-1.

### Discussion
In a previous study, we demonstrated biomechanically that an ACL/MCL combined ligament injury leads to inferior MCL scars with increased MCL laxity, increased cyclic creep, increased unrecovered creep, and decreased tensile strength [3]. In the present study, collagen type I mRNA levels were lower in MCL scars healing in an ACL deficient environment when compared to isolated MCL injury alone, suggesting a possible molecular mechanism leading to the inferior biomechanical properties. Indeed, the present study also demonstrated that TNF-α mRNA levels were increased in MCL scar of ACL deficient joints. It has previously been reported that TNF-α decreases collagen type I levels and increases collagenase synthesis. This cytokine may be critical in the pathway towards inferior scar properties. In this study, we also showed that the type I/III collagen mRNA ratio of ACL deficient MCL scars was lower than that of ACL intact MCL scars. It has been previously reported that type I/III collagen mass ratio is lower during healing of injured MCLs in ACL deficient knees. Longer healing intervals will have to be examined and tested both biomechanically and for gene expression to further understand this process.

The present study is the first report regarding the effects of ACL injury on the healing of injured MCLs with respect to the mRNA level. The present study may explain why scars in unstable joints are mechanically inferior to those found in stable joints at equivalent healing times. While it remains to be confirmed that the observed mRNA differences between MCL scar tissue from unstable and stable knees is reflected by corresponding changes in protein levels, the findings do support the concept that MCL scar cell metabolism is uniquely influenced by the combined injury environment.

### Figure 1. mRNA levels for matrix molecules and cytokines in MCL scars of ACL intact and ACL deficient knees.

**References:**

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