Introduction: Females tear their anterior cruciate ligaments (ACLs) 3-10 times more frequently than males participating in similar athletic activities. The reason for this discrepancy is not known. Tissue remodeling occurs continuously in both normal and injured tissues. In this process, old or damaged structures are degraded and replaced with newly synthesized molecules. The balance between the degradative and biosynthetic arms of this process is controlled by the activities of matrix metalloproteinases (MMPs) and their inhibitors (tissue inhibitors of metalloproteinases, TIMPs). We hypothesize that gender differences in tissue remodeling contribute to the disparate susceptibilities of males and females to ACL injury. A key first step in testing this hypothesis is to determine which effectors of tissue remodeling are present in the human ACL. Accordingly, in this study we used polymerase chain reaction (PCR) to identify all known or putative MMPs and TIMPs in human ACL obtained from surgical specimens.

Methods: With Institutional Review Board approval, whole ACLs were obtained from total knee arthroplasties (TKAs) except for one tissue that was from an amputation. Each of the ACLs was undamaged and appeared to be free of aggressive inflammation. The ACLs were rinsed with saline, flash-frozen in N2 (l) within one minute of removal, and stored at -80ºC for up to one year. ACLs were discarded if they exhibited gross structural abnormality or a pannus indicative of an inflammatory response. Other human tissues used as controls (foreskin, placenta, and bone) were similarly processed. For one year. ACLs were discarded if they exhibited gross structural abnormality. Four individuals (Table 1) were pooled for use as PCR templates. Technological Inc.), and total RNA as template. First strand cDNAs from each reverse transcription for 60 min at 37ºC with an oligo-(dT) primer (Promega Corporation). Isolations from other tissues were done as for ACL. RNA quality was assessed for this tissue. Testis total RNA was purchased (Clontech Laboratories). RNA from an amputation. Each of the ACLs was undamaged and appeared to be frozen in N2 (l) within one minute of removal, and stored at -80ºC for up to one year. RNA from the positive control tissue. Using these criteria, we conclude that ten of sixteen known MMPs, one of two putative MMPs, and all four known TIMPs are present in human ACL. In this example primer pairs for MMP-16 yielded no RT-PCR product of the correct size (432 bp) with reverse-transcribed ACL RNA as template (+RT) and human genomic DNA (G) as templates.

Discussion: Target mRNAs were considered to be present in ACL if: 1) a PCR product of the correct size was obtained from a template of reverse-transcribed ACL RNA; 2) no product was obtained using the identical PCR conditions with mock reverse-transcribed condition; 3) no product was obtained using the identical PCR conditions with mock reverse-transcribed RNA and human genomic DNA as template; 4) no product was obtained using the identical PCR conditions with mock reverse-transcribed RNA from the positive control tissue. We did not detect obvious increases in cellular response (lymphocytes, plasma cells, or neutrophils) in either the synovia or the dense connective tissue of ACLs from osteoarthritic knees, although synovial hyperplasia was evident in arthritic specimens.

In such analyses we obtained RT-PCR products of the appropriate size for MMPs 1, 2, 3, 7, 9, 11, 14, 17, and 18; a partial MMP sequence designated A4; and TIMPs 1, 2, 3, and 4. RT-PCR products for MMPs 8, 10, 12, 13, and 16 were not obtained using reverse-transcribed RNA from ACL as template. However, products for these MMPs were successfully amplified from either testis or bone. No MMP-20 PCR product was obtained using templates from ACL, testis, bone, placenta, or foreskin. Available data for a second partial MMP-like sequence designated A8 was too limited to design an intron-flanking primer pair.

Discussion: Target mRNAs were considered to be present in ACL if: 1) a PCR product of the correct size was obtained from a template of reverse-transcribed ACL RNA; 2) no product was obtained using the identical PCR conditions with mock reverse-transcribed condition; 3) no product was obtained using the identical PCR conditions with mock reverse-transcribed RNA from the positive control tissue. Using these criteria, we conclude that ten of sixteen known MMPs, one of two putative MMPs, and all four known TIMPs are present in human ACL. Future studies will determine whether gender differences in expression of these genes contribute to female ACL injury.

References: