437

SIDE DIFFERENCES OF THIGH MUSCLE CROSS SECTIONAL AREAS IN KNEES WITH THE SAME RADIOGRAPHIC OA (KL) GRADE, BUT UNILATERAL FREQUENT PAIN

T. Dannhauer[1,2], M. Sattler[1,2], M. Hudelmaier[1,2], A.M. Sänger[3], D.J. Hunter[4], K. Kwoh[5], F. Eckstein[1,2]. *[1]Paracelsus Med. Univ., Salzburg, Austria; [2]Chondrometrics GmbH, Ainring, Germany; [3]Salzburg Univ., Salzburg, Austria; [4]Univ. of Sydney, Sydney, Australia; [5]Univ. of Pittsburgh and Pittsburgh VAHS, Pittsburgh, PA, USA*

Purpose: It has been reported that thigh muscle status, specifically anatomical cross sectional areas (ACSAs) and maximal force, may play a role in the onset and progression of knee OA. It is, however, less clear whether changes in ACSAs and muscle force are associated with OA "per se", or with knee pain (that is associated with knee OA). As many, but not all knees with radiographic OA (rOA) are painful, we aim to take a first step in disentangling the above relationships by studying whether the pain status in knees with OA is associated with differences in thigh muscle status.

Our objectives therefore are (1) to determine whether ACSAs of the thigh extensors, flexor, and adductors display significant side-differences (same person) between the knee with frequent pain (i.e. painful knees) vs. those without pain (i.e. painless knees) with the same rOA grade (i.e. calculated KLG2 or 3, from OARSI atlas graded osteophyte and JSN status); (2) to analyze whether extensor and flexor muscle forces show significant side-differences in painful vs. painless rOA knees, and (3) to examine the correlation between force and ACSAs in painful vs. painless rOA knees.

Methods: A between-knee, within-person design was used to study knees with frequent pain (most days of the month within the past 12 months; P01SxKOA = 2) versus knees without pain P01SxKOA =0), in participants with the same rOA grade (P01OAGRD: KLG2 or 3) in both knees. All cases satisfying these criteria (that did not show a change in pain frequency >1 grade within the next 12 months) were drawn from the 4796 Osteoarthritis Initiative (OAI) participants (public-use data sets 0/1.2.2): 31 women, 17 men (n = 48; age 45–78 years). Public use MRI data (0.E.1.: T1-weighted SE sequence) and custom software were used to determine the ACSAs of the extensors, flexors and adductors in the axial image that was located at 35% of the femoral length (from distal to proximal; estimated based on body height) and in the two adjacent images (0.5 cm proximal and 0.5 cm distal). The vastus medialis (VMO) MSCA was determined in a slice located at 31% (distal to proximal). Maximal isometric extensor (V00_R/L_EmaxF) and flexor forces (V00_R/L_FmaxF) were taken from the OAI data base. These were measured using an isometric chair (Metitur Oy, Jyvaskyla, Finland). A paired t-test was used to compare between painful and contra-lateral painless knees within participants.

Results: Painful knees showed significantly lower extensor ACSAs (49.6 vs. 52.6 cm2; −6.2%; p = 0.00003), VMO ACSAs (16.5 vs. 17.6 cm2; −7.8%; p = 0.0007) and maximal extensor muscle forces (331 vs. 364 N; −15.4%; p = 0.003) compared with contra-lateral painless knees. In contrast, there were no significant differences in flexor and adductor ACSAs or flexor forces (all p > 0.39). Results were similar in men and women, and in KLG2 and KLG3 knees. The maximal force per unit ACSA tended to be lower in painful vs. painless knees (6.55 vs. 6.87 N/cm2, p = 0.058 for the extensors, and 4.39 vs. 4.48 N/cm2, p = 0.57 for the flexors) but the differences did not reach statistical significance. The correlations between extensor force vs. extensor ACSA were r = 0.64 in painful and r = 0.66 in painless knees, those between flexor force vs. flexor ACSA r = 0.44 in painful and r = 0.52 in painless knees, and those between extensor and flexor force 0.69 in painful and 0.79 in painless knees. Again, the correlations were similar in men and women and in KLG2 and KLG3 knees.

Conclusions: Knees with rOA and frequent knee pain demonstrate reduced extensor muscle ACSAs and force compared to contra-lateral rOA knees without knee pain, but no differences in flexor ACSAs and force, or adductor ACSAs. Further longitudinal studies are needed to determine whether muscle status is "cause or consequence" of knee pain. Nevertheless, the current findings provide support to quadriceps strengthening programs for knee pain in rOA.

438

MACROSCOPIC AND HISTOPATHOLOGIC ANALYSIS OF HUMAN KNEE MENISCI IN AGING AND OSTEOARTHRITIS

C. Pauli[1], S.P. Grogan[2], S. Patil[2], S. Akihiko[1], J. Koziol[1], M.K. Lotz[1], D.D. D'Lima[2]. *[1]The Scripps Res. Inst., La Jolla, CA, USA; [2]Scripps Hlth., La Jolla, CA, USA*

Purpose: Meniscus lesions following trauma or associated with osteoarthritis (OA) have been described, yet meniscus aging has not been systematically analyzed. The objectives of this study were to (i) establish standardized protocols for representative macroscopic and microscopic analysis, (ii) improve existing scoring systems, and (iii) apply these techniques to a large number of human menisci.

Methods: Medial and lateral menisci from 107 human knees were obtained and cut in two different planes (triangle/cross-section and transverse/horizontal) in three separate locations (mid portion, anterior and posterior horns). All sections included vascular and avascular regions and were graded for i) surface integrity, ii) cellularity, iii) matrix/fiber organization and collagen alignment, and iv) Safranin-O staining intensity. The cartilage in all knee compartments was also scored.

Results: The new macroscopic and microscopic grading systems showed high inter-reader and intra-reader intra-class correlation coefficients. The major age-related changes in menisci in joints with no or minimal OA included increased Safranin-O staining intensity, decreased cell density, the appearance of acellular zones, and evidence of mucoid degeneration with some loss of collagen fiber organization. The earliest meniscus changes occurred predominantly along the inner rim. Menisci from OA joints showed severe fibrocartilaginous separation of the matrix, extensive fraying, tears and calcification. Abnormal cell arrangements included decreased cellularity, diffuse hypercellularity along with cellular hypertrophy and abnormal cell clusters. In general, the anterior horns of both medial and lateral menisci were less affected by age and OA.

Conclusions: New standardized protocols and new validated grading systems allowed us to conduct a more systematic evaluation of changes in aging and OA menisci at a macroscopic and microscopic level. Several meniscus abnormalities appear to be specific to aging in the absence of significant OA. With aging the meniscal surface can be intact but abnormal matrix organization and cellularity was observed within the meniscal substance. The increased Safranin-O staining appears to represent a shift from fibroblastic to chondrocytic phenotype during aging and early degeneration.

439

MOLECULAR ANALYSIS OF AGE- AND SEX-RELATED GENE EXPRESSION IN HUMAN MENISCAL TEARS WITH AND WITHOUT CONCOMITANT ANTERIOR CRUCIATE LIGAMENT TEAR

M.F. Rai, R.H. Brophy, Z. Zhang, A. Torgomyan, L.J. Sandell. *Dept. of Orthopaedic Surgery, Washington Univ. Sch. of Med., St. Louis, MO, USA*

Purpose: The meniscus plays a critical protective role for the knee joint by contributing to load transmission, shock absorption and joint stability. Little is known about gene expression in meniscal tears, particularly as it relates to injury pattern and patient age and sex. The purpose of this study was to test the hypothesis that gene expression in meniscal tears varies depending on patient age and sex and whether the anterior cruciate ligament (ACL) is also torn.

Methods: Meniscal tissue explants (n = 28) were removed at the time of clinically indicated partial meniscectomy in patients with meniscal tear (MT) alone or combined with ACL tear (MT+ACL tear). mRNA expression was examined by quantitative real-time PCR for several molecular markers of osteoarthritis (OA) including pro-inflammatory cytokines (IL-1α, IL-1β, IL-6, TNFα), chemokines (IL-8, CCL3, CCL3L1, CXCL1, CXCL3, CXCL6, CCL20), aggrecanases (ADAMTS-4, -5), metalloproteinases (MMP-1, -3, -9, -13), transcription factors (NFκB2, NFκBIA, IκBA) and matrix components (BMP-2, Col1a1, Col2a1, aggrecan).

Results: Expression of ADAMTS-4 (p = 0.003), -5 (p = 0.001), MMP-1 (p = 0.007), -9 (p = 0.002), -13 (p = 0.01) and NFκB2 (p = 0.01) was significantly higher in MT patients under 40 (Fig. 1A). Similarly, the expression of ADAMTS-4 (p = 0.002) and -5 (p = 0.02), and MMP-1 (p = 0.02) and -13 (p = 0.002) was higher in MT+ACL patients <40 (Fig. 1B). In MT+ACL tear patients, the expression of IL-1β (p = 0.01), TNFα (p = 0.02), MMP-13 (p = 0.004), CCL3 (p = 0.03), and CCL3L1 (p = 0.03) was significantly higher while that of aggrecan (p = 0.03) was lower than in patients with only meniscal tears (Fig. 1C). The only sex-based difference

in expression of these genes was a higher level of IL-8 in males compared to females for MT ($p < 0.0001$) and MT+ACL tear ($p < 0.05$).

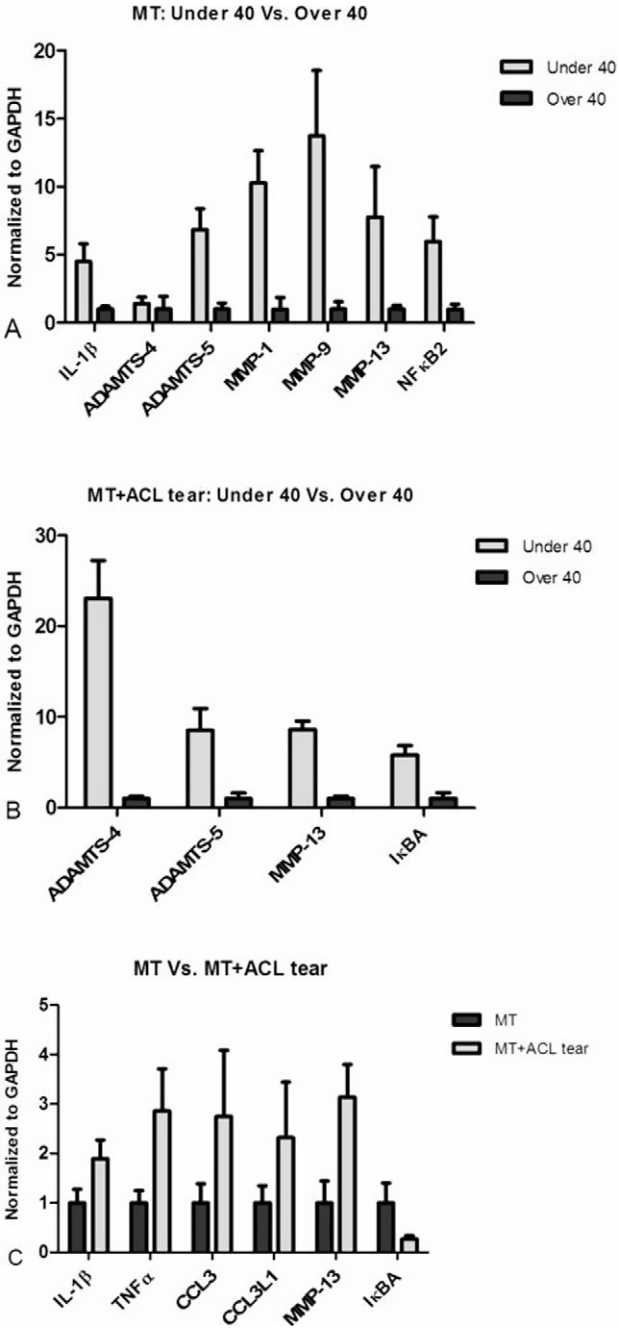

Fig. 1. Gene expression pattern in age-related meniscal tears with and without concomitant ACL tear. All results presented here are statistically significant ($p < 0.05$) within each graph between the two categories.

Conclusions: Gene expression in meniscal tears varies by patient age, sex and injury pattern. Our findings suggest that elevated expression levels of OA-specific markers indicate an increased catabolic (inflammatory) response in young patients with MT as well as MT+ACL tear. Furthermore, higher expression of inflammatory markers in MT+ACL tear compared to MT alone provide molecular evidence suggesting that the combined injury pattern is more likely to lead to the development of OA. These findings suggest clinically relevant differences in the response of the knee to meniscus and ACL tears based on patient age and sex. Catabolic activity may be predictive of patients at risk for progression of OA following partial meniscectomy and ACL reconstruction.

440

CALCIUM DEPOSITION AND GLYCOSAMINOGLYCAN PRESENCE IN OSTEOARTHRITIC HUMAN MENISCAL ATTACHMENTS

A.C. Abraham, T. Haut Donahue. *Michigan Technological Univ., Houghton, MI, USA*

Purpose: Meniscal attachments are unique graded tissue interfaces that diffuse hoop stress from the meniscal body to the tibial plateau. Identified enthesopathic alterations at other tissue interfaces have shown, amongst other changes, an increase in water-affine proteoglycan content, which may result in extracellular matrix swelling and disruption of the fiber network. If the attachments become structurally compromised, excessive transverse meniscal extrusion results, and such extrusion is a known precursor to knee osteoarthritis. Coupling this information with noted catabolic activity within the arthritic joint gives rise to the supposition that the meniscus-to-bone interface is a potential disease-forming pathway, possibly predating other harbingers of degradation. To date there have been no studies examining bio-chemical changes at this interface.

Methods: Tibial plateaus from healthy ($n = 4$) cadavers and patients with end-stage knee osteoarthritis ($n = 3$) (undergoing total knee arthroplasty) were obtained with IRB approval. All four (medial anterior, lateral anterior, medial posterior, and lateral posterior) meniscal attachments from ligamentous zone to subchondral bone were excised, fixed in 10% neutral buffered formalin, dehydrated in graded ethanol, and embedded in glycol methacrylate. Specimens were sectioned using a diamond wafering blade, ground using progressive SiC sheets on an autopolishing wheel and cleared in xylene. Sections were stained using the Von Kossa (VK) technique for calcium deposits and counterstained using toluidine blue (TB) to identify glycosaminoglycans (GAG).

Changes in GAG presence in the insertion zones were quantified using Bioquant OSTEO software to identify the thickness of the TB stained and the TB+VK stained regions (Figure 1). Each region was outlined and measurements were performed at 20 μm intervals. Calcium deposition was scored using a modified grading scale as described by Sun et al. 2010 (Table 1). Two blind reviewers examined each section independently and results were averaged between them.

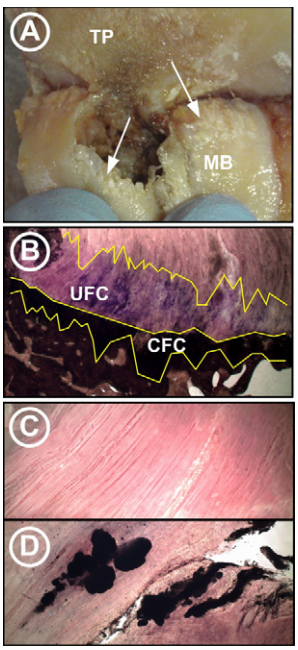

Fig. 1. (a) Gross inspection of insertion site reveals apparent calcium deposition on the surface. TP – Tibeal Plateau, MB – Meniscal Body. (b) Meniscal insertion stained for GAG and calcium. Yellow lines show the regional identification used for thickness measurements. AC – Articular Cartilage, UFC – Uncalcified FibroCartilage, CFC – Calcified FibroCartilage. Color thresholding (not pictured) used to aid in regional identification. (c) Fibrous attachment with no calcium deposition. (d) Calcium deposits within the fibrous attachment (left) and articular cartilage (right).