300
PRESENCE OF KNEE OSTEOPHYTES IS HERITABLE AND LINKED TO REGIONS OF CHR. 19 AND 2 THAT CONTAIN TGFB1 AND SPP2

E.H. Sybouts †‡, E.E. Quillen †, T.E. Macrini §, M.C. Mahaney †§, L.A. Cox †§, D.P. Nicolella ¶, L.M. Havill †§. † *Texas BioMed. Res. Inst., San Antonio, TX, USA;* ‡ *Univ. of Texas Hlth. Sci. Ctr., San Antonio, TX, USA;* § *St. Mary's Univ., San Antonio, TX, USA;* ‖ *Southwest Natl. Primate Res. Ctr., San Antonio, TX, USA;* ¶ *Southwest Res. Inst., San Antonio, TX, USA*

Purpose: Osteophytes play a central role in clinical assessment of osteoarthritis presence and severity. These bony outgrowths capped with cartilage form primarily on the margins of articular surfaces of large synovial joints. Osteophyte presence and OA are common co-morbidities, but osteophytes can develop in the absence of cartilage degradation, expected in OA, and vice versa. Here we test the hypothesis that osteophyte formation, like other bone traits, is heritable, and perform a linkage scan to localize genetic effects.
Methods: Right distal femora from 567 baboons (394 females, 173 males; 3.2 to 33.3 years of age) were collected at necropsy and examined for presence/absence of osteophytes. All animals represent a single outbred pedigree and are genotyped at 359 microsatellite markers. Univariate polygenic models were fit using SOLAR with covariates selected using Bayesian model averaging, and multipoint linkage analysis was performed. Existing OA severity scores (1 = unaffected, through 4=severe) based on cartilaginous lesions, were used to relate osteophyte presence to OA status through a test for bivariate genetic correlation.
Results: Osteophyte presence showed a significant genetic effect ($h2 = 0.37$, $p = 0.008$). We found no age or sex effects. Significant linkage was detected on the baboon equivalent of human chromosome 19 (likelihood odds ratio (LOD score)) of 2.79; 28.6 to 48 megabases (Mb) from the p-terminus. Suggestive linkage (LOD 2.56) was detected on the baboon equivalent of human chromosome 2; 231 Mb from the p-terminus. Maximum OA severity score and percent of the joint surface affected each show significant covariate effects on osteophyte presence. Bivariate quantitative genetic analyses indicate, however, that the genes that affect variation between these two phenotypes and osteophyte formation are not completely overlapping (genetic correlations of ~0.5). Bivariate linkage analyses were performed to assess the shared genetic effect of these loci on the two traits. These analyses did not, however, improve the LOD scores for the osteophyte loci.
Conclusions: The regions identified in the multipoint linkage analysis contain previously identified candidate genes for bone growth and osteophyte development. The region of significant linkage on chromosome 19 contains TGFB1, and the suggestive linkage region on chromosome 2 includes SPP2, an inhibitor of BMP2. Our results indicate that osteophyte formation is under significant genetic influence, and that a substantial amount of this influence is independent of that on cartilaginous manifestations of OA. Osteophytes are undoubtedly commonly associated with OA, but these results support further investigation of the nature of the shared and non-shared genetic influences on these two components of knee OA.

301
EARLY RESPONSE TO IMPACT KNEE INJURY IN GENETIC STRAINS OF MICE REVEALS SIMILARITY IN CARTILAGE BUT DIFFERENCES IN SYNOVIUM

M.F. Rai, X. Duan, N. Holguin, J.D. Quirk, M.J. Silva, L.J. Sandell. *Washington Univ. Sch. of Med., St. Louis, MO, USA*

Purpose: Joint trauma can result in a spectrum of acute injuries potentially leading to post-traumatic osteoarthritis. We have shown that early molecular events, following tibial compression, include apoptosis and rearrangement of aggrecan distribution in C57BL/6J mice. We have also shown that two recombinant inbred mouse strains (LGXSM-6 and LGXSM-33), generated from a LG/J by SM/J intercross, differ in various phenotypes: LGXSM-6 has the capacity to heal full-thickness articular cartilage lesions and ear wounds and is relatively protected from developing post-traumatic osteoarthritis compared to LGXSM-33. In this study, we hypothesize that the two genotypes LGXSM-6 and LGXSM-33 will respond differently to impact knee injury.
Methods: We applied 6N, 9N, or 12N compressive forces on the right tibia of LGXSM-6 and LGXSM-33 mice. Knees were harvested at various time points (ranging from 5 to 56 days) following injury to evaluate the response of loading. Knee pathology and rate of anterior cruciate ligament (ACL) injury were determined by ex vivo magnetic resonance imaging. Quantification of ectopic calcification was done by micro-CT. Histology was conducted to evaluate changes in synovium and articular cartilage while immunohistochemistry was undertaken to observe matrix distribution. Cell apoptosis was measured by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay. QuantiGene Plex mRNA assay was conducted to gauge gene expression differences between the two mouse strains following loading.
Results: We observed a higher rate of ACL tear at higher loading forces in LGXSM-33 than LGXSM-6. Higher loading regimens resulted in a severe femoral cartilage lesion (characterized by loss of proteoglycan, apoptosis, and changes in the pattern of aggrecan and COMP expression). Aggrecan and COMP were present around the chondrocytes in the intact area while at the site of injury these molecules became internalized in the apoptosed chondrocytes. No such changes were observed in the contralateral non-loaded knees and no significant differences between the two strains were found. However, we noticed that LGXSM-33 showed significantly higher synovitis than LGXSM-6. Although, we observed an early (at 14-days) ectopic synovial chondrogenesis in LGXSM-33, LGXSM-6 exhibited severe synovial ectopic calcifications in the loaded knees at later (56-days) time point.
Conclusions: A difference was observed in ACL tear, which could indicate different material properties of the ligament between the strains. Once the ACL is torn, early cellular responses appear to be similar in the cartilage. In contrast, the synovial cell response differed between genotypes. These results suggest possible early interventions that prevent injurious molecular events.

302
POLYMORPHIC PREDISPOSITION FOR CLINICAL AND RADIOLOGICAL SEVERITY IN KNEE OSTEOARTHRITIS – A CASE CONTROL STUDY

R.N. Srivastava, A. Mishra, S. Raj. *KG Med. Univ., Lucknow, India*

Purpose: It has been established that age, gender, BMI, occupation and life style influences the onset and progression of knee osteoarthritis (KOA). Role of micro and macronutrient, environmental and genetic factors are being extensively studied to understand the patho-physiology of the disease and its application in prevention and therapeutic intervention. Genetic study resulted in identification of several susceptibility loci confirming the genetic association with KOA. Although ACR criteria for diagnosing the disease, WOMAC for clinical and KL grade for radiological severity are universally accepted, a discordance between the clinical and radiological features have been reported by many researchers. The cause of this discordance have been explored by many suggesting radiological features beyond those considered in KL grades to be responsible for pain in KOA. The objective of this study was to look for an association of genetic variants with clinical and radiological features of knee OA and also to determine whether clinico-radiological discordance is somehow explainable by genetic variants. In this study we evaluated 5 genetic polymorphisms (3 SNPs of CALM-1 gene and 1 SNP in GDF-5 and ESR-α gene), which have been found to be most commonly associated with KOA in most populations.
Methods: This case control study consisted of men and women $\geq$40 years that fulfilled American College of Rheumatology (ACR) clinical and radiographic criteria for KOA. 500 cases and 500 controls were recruited from the outpatient clinic of the Department of Orthopaedic Surgery from August 2008 to June 2013. For inclusion, cases were required to have knee pain for $\geq$6 months and at least one pain dimension of the WOMAC pain score $\geq$20%. Age and sex matched controls were otherwise healthy individuals attending Orthopaedic OPD for reasons other than knee problem. Blood were drawn for DNA isolation. PCR-RFLP for 4 SNPs and TaqMan assay for 1, were carried out to identify the SNPs. Data were summarized as Mean ± SD. Groups were compared by one way analysis of variance (ANOVA) and the significance of mean difference between the groups was done by Tukey's post hoc test. Discrete (categorical) groups were compared by chi-square ($\chi 2$) test. A two-tailed (α=2) p value less than 0.05 ($p<0.05$) was considered statistically significant. Analyses were performed on SPSS software (Windows version 17.0).
Results: For each SNP, on comparing the mean clinical features between genotypes, ANOVA revealed significant association of GDF-5 with WOMAC-pain ($F= 7.63$, $p<0.001$). Further, Tukey test revealed significantly ($p<0.01$ or p0.05) association with WOMAC pain. Other clinical features like WOMAC stiffness, physical function and VAS for knee pain failed to show any association with these 4 genotypes. ESR-α