telopeptides of collagen type II was quantified by CTX-II ELISA. Articular cartilage erosion was scored using histology after toludine blue staining of proteoglycans of the tibial and femoral plateau, where the eroded surface was quantified relative to the entire articular surface.

Results: Treatment with human OA cartilage explants with 100 nM salmon calcitonin significantly (P < 0.05) inhibited MMP-13 release from human articular cartilage explants by 38%. Extracts of pulverized human OA explants cultured with calcitonin had significantly higher levels of total proteoglycan pr. mg cartilage than control explants cultured without salmon calcitonin, 1 mM and 1 nM (P < 0.01); 10 nM and 100 nM (P < 0.05). In vivo, scoring of cartilage erosion by histology showed significantly more surface erosion in the OVX+vehicle group compared that of the sham operated animals (112%, P < 0.01). Treatment with oral calcitonin or estrogen completely reverted this to sham levels (P < 0.01). In joint extracts, ovariectomy resulted in 100% increased cartilage degradation products compared to sham operated animals. Treatment with oral salmon calcitonin restored this increase to sham levels.

Conclusions: In human OA articular cartilage, calcitonin decreased MMP-13 levels and furthermore increased proteoglycan content. The demonstrated in vivo effects indicate that calcitonin counteract the slow progression of cartilage degradation in a non-traumatic model of OA. The chondroprotective effect of salmon calcitonin may be a combination of direct effects on chondrocytes in addition to the well-established effect on bone resorption.

A55
LUMBAR DISC DISEASE SHOWS LINKAGE TO CHROMOSOME 19
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Purpose: Lumbar degenerative disc disease (LDD) forms part of the spine osteoarthritis (OA) phenotype and is known to be influenced by genetic factors. Using healthy twin volunteers we have shown both extent and severity scores of LDD on lumbar spine MRI scan to have heritability >70%. We studied dizygotic (DZ) twins having lumbar MRI scans from the previous heritability study and performed a genome-wide linkage study to determine whether specific chromosomal regions are linked with LDD. Twins offer particular advantages for the study of an age-related phenotype such as this: not only are twins within a pair age-matched, they are more closely matched for environmental factors than normal sib-pairs.

Methods: Ethics committee approval and patient consent were obtained. Dizygotic twins (N = 338) from the TwinsUK register who underwent MRI scanning of the lumbar spine 10 years ago were identified. DNA was isolated from venous blood by standard technique. Genotyping included 737 highly polymorphic microsatellite markers spaced every 10 cM. The estimated genotyping error was <1%.

Multipoint genome-wide linkage analysis was conducted using generalized linear modeling based on optimal Haseman and Elston methods in which the square of the sibling difference in OA phenotype (age-adjusted) is regressed on the estimated proportion of alleles identical-by-descent (IBD). An empirical p-value was estimated using a permutation approach in which 1,000 permutations of the dataset were performed for each LOD score, keeping IBD and family structure in tact.

Results: DZ twins were female and had mean age = 52 years. Linkage peaks (defined as maximum LOD > 3) were identified for LDD severity at three chromosomal regions. These included chromosome 1 (position 285 cM), chromosome 5 (position 175 cM) and chromosome 19 (position 80 cM). The peak on chromosome 19 had LOD = 4.06 and the empirical p = 6.7 x 10^-4 confirmed reliability of the linkage signal. A peak at a similar position on chromosome 19 has also been identified with hand OA in TwinsUK volunteers. This phenotype used a composite score derived from assessment of distal- and proximal interphalangeal joints and carpometacarpal joints.

Conclusions: LDD is known to be influenced by genetic factors. This is the first genome-wide linkage study of LDD and shows evidence of a linkage peak on chromosome 19. Of interest, we have found a similarly positioned peak for hand OA (in press), work which replicates the findings of others (Demissie et al 2002). The region of interest is large and is estimated to contain ~300 genes. These results suggest that there are predisposing genes common to hand OA and LDD in this region and fine mapping this area would be a reasonable next step.

A56
ANK/ANK MOUSE, AN OSTEOARTHRITIS MODEL
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Purpose: The ank/ank’ mouse is a spontaneous genetic mouse strain in which calcific deposits form in synovial joints with sub-sequent bone formation in peri-articular tissues leading to ankylosis. The purpose of the present study is to determine the sequence of morphologic and morphometric changes in chondrocyte histology within synovial joints of the ank/ank’ mouse.

Methods: Using 5μm Hematoxylin and Eosin sections from formalin fixed, paraffin embedded, decalcified bones (upper extremity, lower extremity), we analyzed the cartilage histological features of joints in 22 mice: 11 homozygotes (ank-/ank-), 5 heterozygotes (ank+/ank”) and 6 controls (ank+/ank+), ranging in age from 7 weeks to 18 weeks. Cartilage morphometry to determine the size distribution of chondrocytes was performed on knee joint articular cartilage using a Visiopharm image analysis system.

Results: ank/ank’ mice display similar histologic changes in all synovial joints with most advanced disease present in the most peripheral joints. We found that the earliest cartilage morphologic and morphometric change appears to be chondrocyte hypertrophy in the distal joints of ank/ank’ mice. This is followed by precipitation of calcific material in the synovial space and within synovium. Later, cartilage metaplasia of the

Fig. 1. Knee joint, articular cartilage, ank/ank’ mouse. The cartilage is thicker and the chondrocytes larger than the control (Fig. 2). Figure width = 100 μm.
capsule and periosteum occurs, followed by osseous metaplasia and ultimately ankylosis. With age, the sequence of histologic changes proceeds progressively to involve the proximal joints as well.

**Conclusions:** The ank/ank mouse demonstrates chondrocyte hypertrophy, joint space calcification and excessive peri-articular ossification, all features that occur to more or less extent in osteoarthritis. As these features develop and progress over 18 weeks of life, we conclude that the ank/ank mouse is a useful model for investigating the pathogenesis and regulation of these articular morphologic features of osteoarthritis.

**A57**

**CHONDROGENESIS OF INFRAPATELLAR FAT PAD AND SYNOVIAL MEMBRANE CELLS IN COMPARISON TO ARTICULAR CARTILAGE CHONDROCYTES**

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**Purpose:** Cartilage is frequently damaged and only has a limited capacity for repair. There is growing interest in the use of cell based therapies for enhancing cartilage repair. Autologous chondrocytes have been used in the repair of focal articular cartilage defects, although there are issues with donor site morbidities. There has been recent interest in the use of undifferentiated adult progenitor cells from other sources, which under appropriate culture conditions can be differentiated into chondrocytes. Such cells can be sourced from a variety of tissues, including the synovial membrane (SM) and the infrapatellar fat pad (IPFP). In this study we identified the chondrogenic potential of canine cells sourced from the SM and the IPFP, and compared their potential with articular cartilage chondrocytes (ACC). We hypothesised that IPFP and SM derived cells would have comparable chondrogenic potential in comparison to ACC in an *in-vitro* chondrogenesis model.

**Methods:** IPFP, SM and articular cartilage were harvested from the femoropatellar joint of 4 skeletally mature dogs (aged 3-5 years) which had no signs of joint disease. Cells were isolated by collagenase digestion and proliferated for two passages in monolayer culture. 3D cell aggregates were formed from 500,000 cells by centrifugation. The cell aggregates were placed in chondrogenic media and cultured for 2 weeks. Chondrogenesis was assessed by: Wet weight, Histologically (H&E, safranin O staining and collagen-II immunohistochemistry), Gene expression (real-time PCR quantification of collagen-I, II, aggrecan), and Biochemically (Total glycosaminoglycan (GAG) and DNA content).

**Results:** ACC derived cell aggrecates were significantly bigger than aggrecates derived from either IPFP or SM cells (p < 0.05), with the mean wet weight of the aggrecates being 5.2mg for the ACC cells, 1.2mg for the IPFP cells and 1.1mg for the SM cells. Histologically the IPFP and SM cell derived aggrecates showed significantly lower matrix accumulation in comparison to the aggrecates from ACC cells, and stained much more poorly for both GAG and Collagen-II. Similarly, both total GAG and DNA content of the aggrecates were significantly lower in both the SM and IPFP cell derived aggrecates in comparison to the ACC cell derived aggrecates. Average GAG content per cell aggrecate was over five fold lower in aggrecates derived from SM cells, and three fold lower in aggrecates derived from IPFP, in comparison to those derived from ACC. ACC derived cell aggrecates expressed high levels of both collagen-II and aggrecan genes, although collagen-I gene expression was of a similar magnitude to that of collagen-II expression. Average ratio of collagen-II to collagen-I gene expression per cell aggrecate was over 500 fold lower in aggrecates derived from SM cells, and nearly a 100 fold lower in aggrecates derived from IPFP, in comparison to those derived from ACC.

**Conclusions:** Under the culture conditions used in this study, cell aggrecates derived from ACC were able to produce a cartilage like matrix with the cells showing gene expression indicative of a chondrogenic phenotype. However, the ACC cells showed similar levels of collagen-I gene expression in comparison to collagen-II gene expression indicating a failure to fully recapitulate the phenotype of cells found within hyaline articular cartilage. Using the cell extraction protocol and culture conditions used in this study, neither SM nor IPFP derived cells demonstrated ability to produce a cartilage like matrix, nor did these cells demonstrate a gene expression profile consistent with chondrogenic differentiation.

**A58**

**PREVALENCE OF RADIOGRAPHIC OSTEOARTHRITIS OF KNEE AND LUMBAR SPINE, AND ITS ASSOCIATION WITH PAIN: THE RESEARCH ON OSTEOARTHRITIS AGAINST DISABILITY (ROAD) STUDY**

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**Purpose:** Although osteoarthritis (OA) of knee and lumbar spine is a major cause of disability in the elderly, few epidemiologic studies have been performed. We established a large-scale nationwide clinical study called ROAD (research on osteoarthritis against disability) in 2005 to clarify the environmental and genetic backgrounds. We began the study by creating a comprehensive and systemic database including clinical and genomic information in two cohorts of urban and rural areas. From their baseline data, we investigated the prevalence of radiographic OA of knee and lumbar spine, as well as its association with the respective local pain.

**Methods:** We recruited 1,885 inhabitants older than 50 years (mean age=73.5 yrs.); 1,030 (355 men & 675 women; mean age=76.9 yrs.) from the urban and 855 (317 men & 538 women; 69.3 yrs.) from the rural areas. The radiographic severity of OA was determined according to the Kellgren/Lawrence (KL) grade (0-4) at femoral-tibial joints of bilateral knees and at intervertebral spaces from L1/2 to L5/S1 of the lumbar spine by a blinded orthopaedic surgeon. Logistic regression analysis was performed after adjustment for age to determine the association.

**Results:** Prevalence of radiographic OA (KL> or = 2) in either