least 2 in at least 1 knee. 13.6% had an osteophyte score of at least 2 and 4.6% joint space narrowing of at least 2. Tibio-femoral osteoarthritis was present in 35.9% of patients. The mean intake of vitamin D was 3.17 micrograms a day, which is well below the recommended daily intake. Vitamin D intake was negatively associated with tibia femoral K&L score (p=0.028) and osteophyte score (p=0.013) but not tibio-femoral joint space score (p=0.28). The correlations were stronger in females than males, although the difference was not statistically significant. There were no associations between vitamin D intake and patello-femoral joint space score. There was no association between supplementary vitamin D intake and any of the radiographic variables.
Conclusions: Low vitamin D intake is associated with an increase prevelance of radiographic osteoarthritis and this is particularly driven by osteophytosis. There is no association with patello-femoral disease. Further work exploring vitamin D intake and osteoarthritis is recommended.

A33

A SNP LOCATED DOWNSTREAM OF BMP5 IS ASSOCIATED WITH ALLELIC EXPRESSION IMBALANCE AT THE GENE AND WITH PRIMARY OSTEOARTHRITIS

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Purpose: We have previously observed that the bone morphogenetic protein 5 gene BMP5 demonstrates a high degree of differential allelic expression (DAE) in articular cartilage chondrocytes, implying the presence of polymorphism within cis-regulatory elements of the gene. Our hypothesis is that this polymorphism confers OA susceptibility. The aim of this study was to map the variants that correlate with DAE at BMP5 and to then test their association with OA in a case-control cohort.
Methods: We genotyped 41 haplotype-tagging SNPs within a 416 kb interval encompassing and flanking BMP5 in 23 OA patients for whom we had determined the allelic expression profile. DAE status and SNP genotype were then correlated using the nonparametric Mann-Whitney test. Overall levels of BMP5 expression in chondrocytes were determined using real-time (RT)-PCR. Association analysis was performed on a cohort of 605 females with primary hip OA and 730 female controls, all of UK Caucasian origin. Ethical approval was obtained from local ethics committees.
Results: A SNP located 5kb downstream of BMP5 demonstrated significant correlation with DAE status ($P < 0.005$). The minor allele of the SNP was also correlated with an overall reduction in BMP5 expression as assessed by RT-PCR ($P = 0.03$). Homozygotes for the minor allele were significantly more prevalent in our cases (5.5%) than our controls (2.1%) with a P-value of 0.002 and odds ratio of 2.7 (95% CI 1.5-5.1). A subsequent detailed analysis of the HapMap revealed that the associated SNP is part of an extended haplotype block up to 80kb in length.
Conclusions: We have generated both genetic and functional data that support a role for downstream cis-acting regulatory elements of BMP5 as OA susceptibility loci.
Research into Ageing and the Arthritis Research Campaign supported this study.

A34

RISK PREDICTION OF KNEE OSTEOARTHRITIS USING MULTIPLE GENES

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Purpose: Primary osteoarthritis (OA) is the most common cause of joint disability in the developed world. A number of phenotypic risk factors have been identified and genetic factors are also major determinants of disease. The objective of this study was to assess how much of the risk of knee OA could be predicted by combinations of genetic polymorphisms associated in other populations with hip and/or knee OA.
Methods: Genetic polymorphisms in OA candidate genes were genotyped in 298 men and 305 women aged 50-86 diagnosed with knee OA assessed clinically and radiographically, and in 300 male and 299 female age and ethnicity matched controls. 18 OA candidate genes which had been previously reported to influence knee or hip OA risk or both were studied: *FRZB, COMP, COL2A1, VDR, AACT, ADAM12, CILP, BMP2, LRCH1, ESR1, OPG, TNA, COX2, CD36, NCOR2, TNFAIP6,ASPN, CALM1*. Genotype frequencies at a total of 37 polymorphisms in the above genes were compared between cases and controls separately by gender. We fitted a multivariate model with estimated knee OA risk as a function of all the polymorphisms in genes which were involved in this population as well as in independent studies. A new "OA genetic risk" variable was derived using the best fit in women with a minimum value of 0 and a maximum of 6. This same model was then tested in men.
Results: We found that SNPs in 12 genes were significantly associated with disease susceptibility in women in our cohort and SNPs in 8 of the genes were associated in men. The proportion of individuals affected with knee OA in the case-control study indicated that a much higher prediction of OA risk can be achieved by combining several genes which have consistently shown to be involved in OA genetic susceptibility. In particular we found that comparing women with a high "OA genetic risk" (>4) to those with low genetic risk (<2.5) resulted in an odds ratio of 11.15 (95%CI 5.1, 24.6$p < 1 \times 10^{-16}$). In men the same comparison resulted in OR= 4.27 (95%CI 2.4, 7.7 $p < 1 \times 10^{-7}$).
Conclusions: These data indicate that using a multiple gene approach, a higher genetic risk prediction is achievable in women with OA.

A35

RECOMIBINANT HUMAN CARTILAGE-DERIVED RETINOIC ACID SENSITIVE PROTEIN (CD-RAP) - A NOVEL TREATMENT OPTION FOR OSTEOARTHRITIS

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Purpose: Osteoarthritis is a significant worldwide health problem owing to the progressive and debilitating nature of the condition, which results in high morbidity and a marked decrease in the quality of life. It is the most common articular disorder in humans. New treatment options for this disease are required because currently no consistently working treatments are available.
Cartilage-derived retinoic acid sensitive protein (CD-RAP) is a highly specific marker for chondroid differentiation and has been shown in *in vitro* and *in vivo* assays to be a crucial determinant in cartilage differentiation and maintenance. It was evaluated whether CD-RAP alleviates or prevents cartilage degradation in an animal model of osteoarthritis.

Methods: 24 skeletally mature New Zealand White rabbits underwent anterior cruciate ligament transection. 6 additional sham operated animals were used as controls. 10 days after the surgery the transected animals received an intraarticular injection of CD-RAP encapsulated in liposomes or liposomes alone as vehicle control. Three different dose levels of CD-RAP were tested. Thereafter the animals were injected every 10 days until the end of the 9-week treatment period. The animals were analysed macroscopically, radiologically, and histologically. To score the radiological and histological data, the Kellgren and a modified Mankin score, respectively, were used.
Results: CD-RAP treatment resulted in reduced osteophyte formation and reduced joint space narrowing. This effect was significant as shown by the radiological scoring There was a trend towards middle and high dose CD-RAP being more efficacious than the low dose group but this was not statistically significant. The histological evaluation by the modified Mankin score, which evaluated cartilage structure, cells, matrix (safranin-O), and tidemark integrity, confirmed the radiological results. The average scores for sham, vehicle, and treatments groups (low, mid and high dose CD-RAP) were 1.12±1.19, 7.28±2.19, 7.22±2.69, 4.08±0.8, 4.47±1.93. Both middle and high dose CD-RAP groups showed a significant improvement over the vehicle group. Looking at the individual parameters, CD-RAP appeared to impact structural integrity as well as matrix content.
Conclusions: These data suggest that treatment with the cartilage specific protein CD-RAP may be a new therapeutic option for osteoarthritis patients. Next steps will include the long-term evaluation of liposome-encapsulated CD-RAP in a large animal model.

A36

MODULATION OF COLLAGEN NETWORK FORMATION TO IMPROVE FUNCTIONAL PROPERTIES IN CARTILAGE REGENERATION

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Purpose: Osteoarthritis is characterized by the progressive destruction of articular cartilage. Damage to the collagen network is often considered irreversible, whereas proteoglycan loss is reversible. Understanding mechanisms of repair of the collagen network is therefore key for functional repair of cartilage. The present study was designed to test whether collagen synthesis and subsequent crosslinking could be influenced such that a functional collagen network is formed.
Methods: Bovine articular chondrocytes were cultured in alginate beads for 5 weeks in the presence or absence of the lysyl oxidase inhibitor BAPN (0.25 mM) followed by 5 weeks without BAPN (+/- or -/-). Collagen deposition and amout of crosslinks (HPLC) and gene expression of collagen type 1 and 2 and SOX9 (RT-QPCR) were measured. Susceptibility of the extracellular matrix to *in vitro* degradation by MMP-1 was examined after 70 days of culture by overnight incubating 4 alginate beads with 100nM MMP-1. Alginate constructs of 3 mm thick and 6 mm in diameter were used to determine the equilibrium modulus and de secant modulus by unconfined compression. Here, an extra control was added to examine the effect of no crosslinks after 10 weeks of culture with BAPN (+/+).
Results: Complete inhibition of collagen crosslinking for 5 weeks by BAPN increased the collagen deposition 1.8 times, with a concomitant increase in tissue stiffness (the equilibrium modulus increased from 451 Pa to 879 Pa), despite the absence of crosslinks in the BAPN condition. When after 10 weeks the amount of collagen is further increased, crosslinks also seem to be important in improving the stiffness of the matrix. From week 8 onwards, i.e. 3 weeks after removal of the crosslink inhibitor, the crosslinks returned approaching the level of the control condition at week 10. With equal amount of crosslinks (-/- and +/-) the equilibrium modulus is higher when more collagen was present. However, the total absence of collagen crosslinks together with more collagen resulted in a lower secant modulus in the +/+ condition, indicating a higher permeability of the matrix. Incubation of 10-week-old beads in buffer with collagenase MMP-1, lead to a 2.5 fold increase of collagen release in the supernatant in the condition without crosslinks (+/+) than in the two conditions with crosslinks (-/- and +/-). SOX9 expression was not influenced by addition of BAPN whereas the COL2/COL1 ratio was higher, indicating that inhibition of crosslinks results in more collagen without affecting chondrocyte phenotype significantly.
Conclusions: Modulation of the amount of crosslinking and subsequently collagen deposition, during cartilage tissue regeneration has an effect on the functional properties of the construct. After 5 weeks, more collagen alone led to an increase of stiffness of the matrix. However, when more collagen was deposited after 10 weeks, crosslinks were also needed to improve matrix stiffness. The quality of the matrix indicated by MMP-1 degradation susceptibility is not influenced by the transient crosslink inhibition. These data suggest that modulation of the collagen network may be a feasible approach to induce cartilage regeneration.

A37

CARTILAGE ENGINEERING AND REPAIR: CONTRIBUTION OF HUMAN GLYCOSYLTRANSFERASES IN THE BIOSYNTHESIS OF MATRIX GLYCOSAMINOGLYCANS

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Purpose: Osteoarthritis is the most prevalent chronic human disease characterised by a deterioration of cartilage. Despite its important socio-economical impact, there is, until now, no satisfactory way to treat the disease. Symptomatic treatment with antalgics or anti-inflammatory drugs does not stop the progression of cartilage degeneration, and often presents side-effects, which limit their chronic use. Our goal is to design new therapeutic approaches, keeping in mind that cartilage is one of the rare human tissues which is unable to regenerate. The loss of matrix components, especially glycosaminoglycans (GAG), is an early event of cartilage damage.
Methods: An innovative strategy of cartilage bioengineering consists in overexpression by gene transfer, of glycosyltransferases able to stimulate the anabolic activity of chondrocytes, in order to promote cartilage repair. In that context, the research of our group is mainly devoted to the identification, cloning and structural characterisation of key-glycosyltransferases involved in the biosynthesis of GAG of the cartilage matrix. The work has been especially focused on the human galactose β 1,3-glucuronosyltransferase-I (GlcAT-I) which is involved in the final step of the biosynthesis of the common GAG-protein tetrasaccharidic linkage sequence, GlcA β1-3Gal β1-3Gal β1-4Xyl-O-.
Results: We have shown that any variation of the GlcAT-I activity in chondrocytes or cartilage explants, either upon overexpression, or, in contrast, by repression with antisense RNA, could increase, or decrease the GAG content in cartilage, respectively. Interestingly, overexpression of this enzyme was able to completely counteract the proinflammatory cytokine, interleukine 1β-induced depletion of GAG. The neosynthesised GAG were qualitatively similar to those present in the original cartilage ma-