## 381

### NEGATIVE REGULATION OF GENE EXPRESSION OF CARTILAGE DEGRADING GLYCOSIDASES IN HUMAN OSTEOARTHRITIS AND RHEUMATOID ARTHRITIS SYNOVIAL FIBROBLASTS

M. Pásztói[1], **K. Wellinger**[2], G. Nagy[3], P. Géher[3], T. Lakatos[3], P. Pócza[1], B. György[1], M. Holub[1], Á. Kittel[4], K. Pálóczy[1], M. Mazán[1], A. Falus[1], E. Buzás[1], K. Tóth[2]
*[1]Dept. of Genetics, Cell and Immunobiology, Semmelweis Univ., Budapest, Hungary; [2]Univ. of Szeged, Szeged, Hungary; [3]Dept. of Rheumatology, Semmelweis Univ., Budapest, Hungary; [4]Lab. of Molecular Pharmacology, Inst. of Experimental Med., Hungarian Academy of Sci., Budapest, Hungary*

**Purpose:** We have shown earlier that exoglycosidases alone or in combination with matrix metalloproteinases, are capable of degrading the extracellular matrix of hyaline cartilage. While it is well established that the expression of matrix metalloproteinases is upregulated under certain conditions, there is no information concerning the regulation of cartilage degrading glycosidases.
Objective: The lack of information in this field has compelled us to carry out a systemic study to characterize gene expression regulation in synovial fibroblasts of patients with osteoarthritis and rheumatoid arthritis.
**Methods:** We tested the gene expression of β-D-hexosaminidase, β-D-glucuronidase, hyaluronidase, sperm adhesion molecule1 and klotho in synovial fibroblasts, synovial fluid and synovial membrane samples of patients with osteoarthritis and rheumatoid arthritis.
**Results:** We could detect the expression of β-D-hexosaminidase, β-D-glucuronidase, hyaluronidase, and klotho in synovial membrane samples, hexosaminidase being the major glycosidase expressed within the joints. We found that the gene expression of cartilage degrading glycosidases was moderately downregulated by proinflammatory cytokines including TNF-alpha, IL-1beta and IL-17. TGF-beta profoundly downregulated the expression of glycosidases in synovial fibroblasts derived from either osteoarthritis or rheumatoid arthritis patients.
**Conclusions:** These data suggest that gene expression of synovial fibroblast glycosidases does not differ significantly in patients with OA and RA, and it is under negative regulation by some locally expressed cytokines.

## 382

### FUNCTIONAL BETA-2-ADRENERGIC RECEPTOR STATUS COULD INFLUENCE THE INFLAMMATORY RESPONSE IN ARTHROSIC PATIENTS

**J.F. Horga**[1], A.M. Peiró[1], M. Simón-Talero[2], P. Giménez[3], H. Martínez[4], E. Montell[4], J. Vergés[4]
*[1]Clinical Pharmacology Div. Hosp. Gen. de Alicante, Alicante, Spain; [2]C.S La Florida, Alicante, Spain; [3]CIBERehd, Inst. de Salud Carlos III, Madrid, Spain; [4]Clinical Res. Unit. Scientific Med. Dept. BIOIBÉRICA, S.A., Barcelona, Spain*

**Purpose:** The beta-2-adrenergic receptor (ADRB2) represents a junction between sympathetic nervous system and immune system. In fact, inflammatory articular illnesses are associated to a lower number of ADRB2 in PBMC which can be correlated to a decrease of cell immune functionality and clinical evolution. We want to find the relation between ADRB2 haplotype in arthrosic patients and the response to adrenergic stimulation through cyclic AMP (cAMP) production.
**Methods:** In total, 30 arthrosic patients (mean age= 70 years; sex (M/F) 10/20) and 20 healthy controls were included (mean age= 71 years; sex (M/F) 12/8). We determinate: a) Lymphocyte cAMP production before and after isoproterenol (ISO, from $10^{-8}$ to $10^{-3}$) stimulation in vitro; b) Allelic forms of amino acid 16, 27 and 164 from ADBR2 gen in DNA-leukocyt. Also, patient's clinical data was registered.
**Results:** Significant decreases were found in AMPc (pmol/mL) in arthrosic patients vs. controls (mean values at ISO $10^{-8}$ were: 14 vs. 20 pmol/mL; at ISO $10^{-4}$ were: 41 vs. 78 pmol/mL, $p<0.05$) (Fig. 1). In arthrosic patients, although ADRB2 response was lower, we did not found a functional influence of these polymorphisms on cAMP level because presence of allelic variants was similar in both groups.

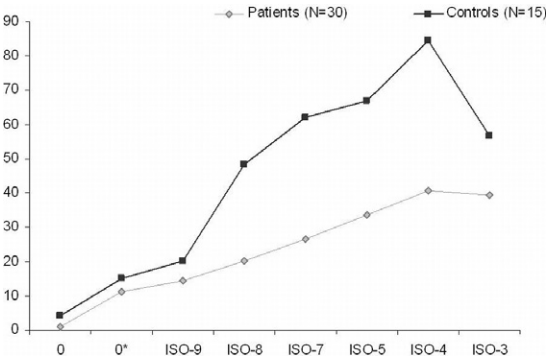


Figure 1. Cyclic AMP level under different isoproterenol concentration stimulus from $10^{-3}$ to $10^{-9}$.

**Conclusions:** It seems that functional ADRB2 status is a factor that could influence the inflammatory response in arthrosic patients.

# Imaging

## 383

### KNEE JOINT SHAPE ASSESSED BY ACTIVE SHAPE MODELLING OF PLAIN RADIOGRAPHS IS RELATED TO OSTEOARTHRITIS SEVERITY

**R.J. Barr**[1], J.S. Gregory[1], K. Yoshida[1], S. Alesci[2], D.M. Reid[1], R.M. Aspden[1]
*[1]Univ. of Aberdeen, Aberdeen, United Kingdom; [2]Discovery Translational Med., Wyeth Res., Collegeville, PA*

**Purpose:** The use of plain radiographs for the assessment of Osteoarthritis (OA) is well established. In a recent study, we demonstrated that Active Shape Modelling (ASM) of the hip joint, measured on radiographs, can quantify the severity of hip OA and may be able to predict the risk of rapid OA progression. ASM describes the shape of the joint in an image in terms of "modes of variation", which are assigned scores describing how many standard deviations each mode lies from the mean mode score of all the images used in the modelling.
The aim of this study was to determine whether a knee ASM could be developed for knee radiographs to assess the relationship between shape and OA severity, assessed by Kellgren-Lawrence grade (KLG).
**Methods:** 107 subjects were recruited using the local Radiology Information System. All subjects had undergone bilateral knee radiographs in the previous 12 months. Each knee was assigned a KLG. The cohort encompassed the full spectrum of KLG (0-4).
A new 85 point model template was developed using the ASM toolkit (Manchester University, Manchester, UK) and applied to digitized knee radiographs. The model included the femur, tibia, femoral and tibial osteophytes, tibial plateau, femoral condyles and intercondylar notch (Figure 1). Pearson correlation analysis was used to test the relationship between mode scores and KLG.

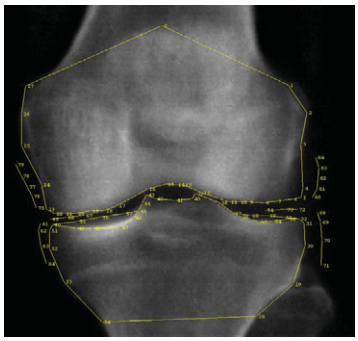

Figure 1

**Results:** The first 6 out of 15 ASM modes were significantly correlated with KLG; 5 of these remained significant after Bonferroni correction. Two of the first 6 modes were also significantly correlated with age (modes 4 and 5) although, after application of the Bonferroni correction for the number of modes compared ($P<0.0033$), only Mode 5 remained. An increase in KLG was significantly associated with a decreased mode score in modes 1 to 5, and an increase in Mode 6 score (Table 1).

Table 1. Correlation between shape modes and KL

| | Mode 1 | Mode 2 | Mode 3 | Mode 4 | Mode 5 | Mode 6 |
|---|---|---|---|---|---|---|
| KL Correlation coefficient | -0.38 | -0.48 | -0.17 | -0.31 | -0.28 | 0.23 |
| *P* values | <0.001 | <0.001 | 0.013 | <0.001 | <0.001 | 0.001 |
| Age Correlation coefficient | -0.16 | -0.09 | 0.04 | -0.24 | 0.33 | 0.07 |
| *P* values | 0.12 | 0.37 | 0.68 | 0.018 | 0.001 | 0.51 |

A one-way ANOVA (followed by Sidak post-hoc test) was used to explore further the relationship between KLG and each of the first 6 modes of variations. All 6 modes showed an overall significance across the 5 KL grades (Mode 1, 2, 4 and 5 $P<0.001$, Mode 3 $P=0.021$, Mode 6 $P=0.019$). However, the post hoc test identified significant differences in mode scores between different KLG in modes 1, 2, 4, and 5 but not modes 3 and 6.

Osteophyte formation was observed in all six modes although it was more pronounced in Modes 1 and 2. Uniform joint space narrowing was observed in Mode 1 whereas mode 2, 3 and 4 showed unilateral JSN. Variation in the shape of the medial tibial plateau was observed in modes 5 and 6. Similarly variations in condylar shape were observed in modes 2 and 5.

**Conclusions:** This is the first report on the application of ASM to knee radiographs of patients with Osteoarthritis. The results show clear relationships between the shape of the knee-joint and disease severity. The modes identify variations in osteophytes, joint space narrowing and the size and shape of the condyles and tibial plateau and can distinguish between their unilateral, medial or tibial occurrence.

## 384

### DGEMRIC IN THE ANKLE: FEASIBILITY AND PRELIMINARY RESULTS AFTER MATRIX ASSOCIATED AUTOLOGOUS CHONDROCYTE IMPLANTATION (MACI)

**S.E. Domayer**[1], G. Welsch[1], D. Stelzeneder[1], C. Hirschfeld[1], S. Quirbach[2], R. Dorotka[1], S. Nehrer[1], T. Mamisch[1], S. Trattnig[1]
*[1]Med. Univ. of Vienna, Vienna, Austria; [2]Med. Univ. Innsbruck, Innsbruck, Austria*

**Purpose:** Autologous chondrocyte implantation (ACI) techniques aim to prevent early osteoarthritis (OA). Repair tissue (RT) ultra structure influences the long-term clinical outcome. Delayed Gadolinium Enhanced MRI of Cartilage (dGEMRIC) can directly visualize the RT glycosaminoglycan (GAG) content in the knee, however the technique has not been applied to the ankle yet. Clinical studies on cartilage repair in the ankle are limited by numbers and biopsy availability. dGEMRIC is a non-invasive, quantitative measure of repair tissue composition and could enhance clinical research on ACI in the ankle.

This preliminary study aimed to (I) explore the feasibility of dGEMRIC with a high resolution sequence at 3 T in the ankle and (II) to gain first data on the composition of cartilage repair tissue after matrix associated ACI (MACI).

**Methods:** An isotropic 3D sequence (True FISP) was used to plan the field of view (FOV) of subsequent T1 imaging (voxel size of 0.3×0.3×0.3 mm, FOV 160×150 mm, Averages = 2, TR 9.65 ms, TE of 4.16 ms, acquisition time 9 min 49s). For quantitative T1 mapping a 3D GRE sequence, Volume Interpolated Breathhold Examination (VIBE) was used (TR 15 ms, TE 1.95 ms, FOV 160×160 mm, matrix size 384×384, in plane resolution 0.4×0.4 mm, slice thickness 3 mm, total 16 slices, bandwidth 480 Hz/pixel, flip angles 5° and 26°). The sequence was used both before and after intravenous administration of contrast agent (0.2m M/kg, Magnevist, Schering, Germany).

The interval until complete contrast agent diffusion was determined in volunteers and 45 minutes were found sufficient. Ten patients after ACI and MACI in the ankle were subsequently assessed (Figure 1).

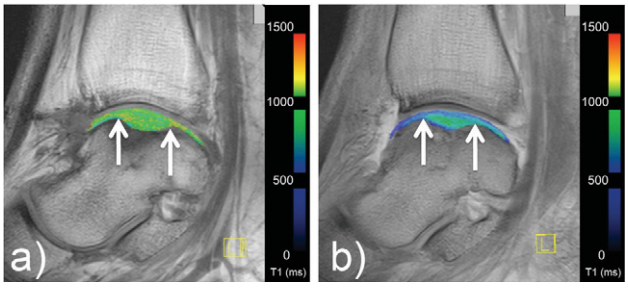


Figure 1. a) Pre contrast, b) post contrast.

T1 map region of interest (ROI) analysis included each one ROI in repair tissue and in normal, hyaline reference cartilage (RC) on contiguous slices to cover the whole repair site. Delta relaxation rates (DR1) of corresponding pre and post contrast ROIs and subsequent individual relative DR1 (rDR1) were calculated for both RT and RC: R1pre = 1/T1pre, R1 post = 1/T1post, DR1 = R1 post - R1pre, rDR1 = DR1 RT/DR1 NC

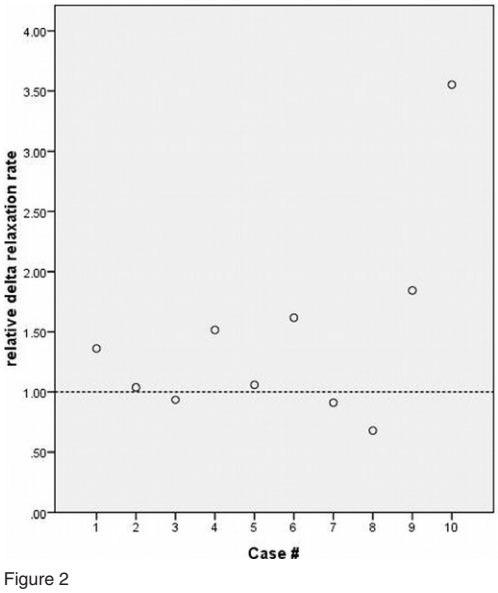


Figure 2