S1. IDENTIFICATION OF NOVEL OSTEOARTHRITIS GENES USING ZEBRAFISH

Chrissy Hammond1
Biochemistry, University of Bristol, Bristol, United Kingdom

Background: Osteoarthritis (OA) affects tens of millions of people worldwide. Twin studies and human GWAS have shown that there is a strong genetic component to the disease, however, to date relatively few genes have been linked to OA onset and progression. Using zebrafish, a genetically tractable organism, we aim to identify novel genes relevant to OA.

Methods: We undertook a forward genetic screen of 600 zebrafish families, screening for cartilage and bone phenotypes by Alcian blue (cartilage) and Alizarin Red (bone) staining. We identified 5 families with phenotypes resembling OA, including progressive loss of joint mobility, cartilage matrix breakdown and osteophyte (bony spur) formation. We are mapping the causative genes using recombination distance based mapping techniques.

Results: We have identified one of the causative genes as chst11 (also known as C4ST1) a gene involved in the metabolism of cartodrin sulphate. Chst11 has also been implicated in human OA. Loss of chst11 leads to misassembly of the cartilage matrix and changes to chondrocyte cell behaviour, such as altered cell proliferation and premature chondrocyte hypertrophy. Additionally loss of chst11 leads to increased osteoblast differentiation in vivo.

Conclusions: Identification of a gene from a zebrafish forward genetic screen that has also been implicated in human OA pathogenesis acts as a proof of principle that zebrafish display sufficient similarities in their skeletal system to be a useful model in osteoarthritis. Zebrafish are not only genetically tractable, but are also transparent and use of fluorescent transgenic reporter lines allows us to track gene expression in the living fish in real time. We have generated numerous transgenic reporter lines marking chondrocytes at various stages of differentiation, as well as osteoblasts and important signalling molecules such as indian hedgehog, these allow us to track changes in the joint in real time at a level impossible in other model organisms.

In conclusion zebrafish can form a useful complement to existing models of OA.

Disclosure statement: The author has declared no conflicts of interest.

S2. IS ADRENOMEDULLIN A POTENTIAL THERAPEUTIC FOR OSTEOARTHRITIS, WHILE ITS TRUNCATED PEPTIDE 22-52 ACTS AS A PRO-DEGENERATIVE FACTOR?

Michal Dudek1 and Gillian A. Wallis1
University of Manchester, Manchester, United Kingdom

Background: Beukes Hip Dysplasia is an autosomal dominant disorder where the abnormal shape of the hip joint leads to secondary osteoarthritis. The locus of BHD has been previously mapped to 4q35 and screening of candidate genes within this region revealed a mutation in the gene encoding the Ubiquitin-fold modifier 1 specific 2 protease (UfSP2). The mutation prevents UfSP2 from cleaving its target Ufm1. UfSP2 and Ufm1 are both components of a novel ubiquitin-like system of unknown function and where only one putative target Ufm1 has been identified. This study aims to investigate the pathway affected by the BHD mutation and the way in which it leads to hip dysplasia.

Methods: In order to optimize an experimental system to identify targets for Ufm1, tagged and modified Ufm1 constructs have been generated and expressed in HEK293T cells using lentiviral expression vector. Proteins conjugated to the overexpressed Ufm1 were purified by Tandem Affinity Purification (TAP) and identified by Mass Spectrometry. Mouse tissue sections were probed for the expression of UfSP2 by radioactive RNA in situ hybridization to determine the tissues most likely involved in BHD pathology. Differentiation of mouse 2T3 osteoblast cell cultures was induced with rhBMP-2 and the pattern of expression of the Ufm1 pathway genes was determined by Real-Time PCR. Er stress was chemically induced in 2T3 osteoblasts and the pattern of expression of the Ufm1 pathway genes was determined by Real-Time PCR.

Results: HEK293T cells stably overexpressing tagged Ufm1 versions were generated. Ufm1 conjugated proteins were isolated using TAP and identified by Mass Spectrometry as Ubas5 and Ufco1, the E1 and E2 enzymes of UfSP2 pathway. RNA in situ hybridization experiments on mouse tissue showed expression of UfSP2 in the secondary ossification centre of the knee joint at two weeks of age with weak expression in cartilage but not hypertrophic cartilage and expression in the surrounding muscle. Induction of 2T3 osteoblasts with rhBMP-2 showed upregulation of the Ufm1 pathway in osteogenic differentiation. Chemically induced Er stress was also found to induce the Ufm1 pathway.

Conclusions: Higher expression of UfSP2 in bone and the secondary ossification centre as well as upregulation of the Ufm1 system during osteogenic differentiation suggests a role for the pathway during
osteoogenesis. Isolation and identification of Ufm1 conjugation targets by tandem affinity purification and mass spectrometry have confirmed that the modified forms of Ufm1 are processed down the Ufm1 targeting pathway. Ufm1 conjugation targets are currently being sought in osteogenic cell lines in order to establish the role of the Ufm1 pathway during osteogenesis.

Disclosure statement: All authors have declared no conflicts of interest.

S4. CANNABIDIOL BLOCKS THE INHIBITORY EFFECTS OF THE GPR55 AGONIST L-ALPHA-LYSOPHOSPHATIDYLinositol ON MECHANOSENSITIVE KNEE JOINT AFFERENTS

Kenneth Paton1, John Harris1, David A. Kendall2 and Sara Kelly2,3

1School of Biosciences, University of Nottingham, Sutton Bonington, United Kingdom; 2School of Biomedical Sciences, University of Nottingham, Nottingham, Nottingham, United Kingdom; 3Arthritis Research UK Pain Centre, University of Nottingham, Nottingham, United Kingdom

Background: The orphan receptor, GPR55, is expressed in dorsal root ganglia neurons and has been implicated in pain. A recent published study demonstrated that a synthetic GPR55 agonist reduced mechanical evoked activity in rat knee joint-associated afferents. Work in our lab has demonstrated inhibitory effects of the putative endogenous ligand for GPR55, L-alpha-lysophosphatidylinositol (LPI), on mechanically evoked response of knee afferents. The aim of the present study was to examine the role of GPR55 in the inhibitory effects of LPI on knee afferent mechanosensitivity using the putative GPR55 antagonist cannabidiol.

Methods: Male Sprague-Dawley rats (250-350 g) (n=24) were deeply anaesthetised with sodium pentobarbital (60 mg/kg, i.p.) and the external jugular vein and trachea cannulated. Extracellular recordings were made from knee joint-associated afferents (receptive fields (RFs) over the ipsilateral knee) in response to von Frey stimulation (0.6-15 g, 5 s each / 5 min). Once stable evoked responses were obtained, 100 g of LPI (150, 250 μM) (n=10) or vehicle (2 x 100 μl saline) (n=7) was peripherally injected (close i.a) and effects followed for 60mins. In separate rats (n=7), cannabidiol (50 μg/100 μl) was peripherally injected 30mins prior to LPI (250 μM/100 μl) and knee afferent mechanically evoked responses were followed for a further 60mins. Conduction velocities were estimated (RF electrical stimulation; A- and C-fibres).

Results: As previously reported LPI (150, 250 μM) did not control and dose-related inhibitory effects on mechanically evoked responses of knee joint afferents (P < 0.001, two-way ANOVA). Vehicle (saline) had no significant effects. Pre-administration of cannabidiol (50 μg/100 μl) significantly blocked the inhibitory effects of LPI (250 μM/100 μl). For example the median 15 g evoked response was 25% of control in the presence of LPI alone, whereas following pre-treatment with cannabidiol this value was 119% of control following LPI (p < 0.01, Mann Whitney).

Conclusions: A genetic knock out study suggests that GPR55 may have a role in pain. In our pharmacological study we were able to block the inhibitory effects of LPI with the putative GPR55 antagonist cannabidiol. Our findings and those of others indicate that GPR55 may have a functional role in modulating the mechanosensitivity of joint innervating afferents; findings that may have implications for the treatment of arthritic pain. Further studies are required to clarify the role of GPR55 in sensory neurones.

Disclosure statement: KP is supported by a BBSRC studentship and a BBSRC Strategic Skills Award. All other authors have declared no conflicts of interest.

Table 1.

<table>
<thead>
<tr>
<th>Follow-up (years)</th>
<th>nbDMARD N = 3543</th>
<th>Anti-TNF N = 11719</th>
<th>Etanercept N = 4072</th>
<th>Infliximab N = 1840</th>
<th>Adalimumab N = 1420</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age: Mean (SD)</td>
<td>60 (12)</td>
<td>56 (12)</td>
<td>56 (12)</td>
<td>56 (12)</td>
<td>56 (12)</td>
</tr>
<tr>
<td>Gender: N (%)</td>
<td>2552 (72)</td>
<td>8915 (76)</td>
<td>3138 (77)</td>
<td>2586 (75)</td>
<td>3194 (76)</td>
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<tr>
<td>Solid cancer: N</td>
<td>91</td>
<td>295</td>
<td>134</td>
<td>71</td>
<td>91</td>
</tr>
<tr>
<td>Solid cancer: Rate per 10000 yrs (95% CI)</td>
<td>63 (57, 71)</td>
<td>68 (57, 81)</td>
<td>61 (48, 77)</td>
<td>60 (48, 73)</td>
<td></td>
</tr>
<tr>
<td>Solid cancer: unadjusted HR (95% CI)</td>
<td>0.73 (0.58, 0.93)</td>
<td>0.73 (0.58, 0.93)</td>
<td>0.77 (0.58, 0.91)</td>
<td>0.69 (0.51, 0.92)</td>
<td></td>
</tr>
<tr>
<td>Solid cancer: Age and gender adjusted HR (95% CI)</td>
<td>0.94 (0.74, 1.20)</td>
<td>1.03 (0.78, 1.35)</td>
<td>0.91 (0.66, 1.24)</td>
<td>0.87 (0.65, 1.17)</td>
<td></td>
</tr>
<tr>
<td>Solid cancer: IPTW adjusted HR (95% CI)</td>
<td>0.88 (0.65, 1.17)</td>
<td>0.94 (0.68, 1.29)</td>
<td>0.87 (0.61, 1.25)</td>
<td>0.81 (0.57, 1.14)</td>
<td></td>
</tr>
</tbody>
</table>

ORAL ABSTRACTS 8: EPIDEMIOLOGY AND OUTCOMES

O37. THE RISK OF SOLID CANCER IN PATIENTS RECEIVING ANTI-TUMOUR NECROSIS FACTOR THERAPY FOR RHEUMATOID ARTHRITIS: RESULTS FROM THE BRITISH SOCIETY FOR RHEUMATOLOGY BIOLOGICS REGISTER

Louise Mercer1, James Galloway1, Audrey Low1, Kath Watson2, Mark Lunt1, William Dixon1, BSRBR Control Centre Consortium1, Deborah Symmons1 and Kimme Hyrich1, on behalf of the BSRBR1

1Arthritis Research UK Epidemiology Unit, The University of Manchester, Manchester, United Kingdom; 2British Society for Rheumatology, London, United Kingdom

Background: The use of anti-TNF in the management of rheumatoid arthritis (RA) has been coupled with concerns about tumourigenesis. Meta-analyses of randomized controlled trial (RCT) data have not found an increased risk of solid cancer. The short duration of RCT means latent events such as cancer may be missed. The aim of this study was to determine whether anti-TNF influences risk of cancer when used in routine UK clinical practice.

Methods: The analysis was conducted in the BSRBR, a national cohort study. Patients with RA starting anti-TNF (etanercept (ETA), infliximab (INF) or adalimumab (ADA)) and a biologic-naive comparison cohort taking non-biologic therapy (nbDMARD) were recruited from 2001. Subjects were followed for 5 years until 31/12/2009. Solid cancer or death, whichever came first. Subjects with prior solid cancer identified by record linkage with the UK cancer registry (NHS-IC) were excluded. Incident cancers were identified in 3 ways; lifelong flagging with NHS-IC; 6 monthly patient and physician questionnaires for 3 years and annual physician questionnaires thereafter. The first solid cancer per subject, confirmed by histology or NHS-IC, was analysed. Cancers occurring after stopping anti-TNF were attributed to the most recent anti-TNF. Rates of cancer in anti-TNF and nbDMARD cohorts were compared using Cox models adjusted using inverse probability of treatment weighting (IPTW) for age, gender, comorbidity, RA duration, NSAID, smoking and registration year. Each anti-TNF was then compared separately to nbDMARD. Site-specific analyses were performed for sites with >10 cancers in each cohort: colorectal, lung and female breast.

Results: 386 solid cancers were confirmed: 91 in 3543 nbDMARD patients and 295 in 11719 anti-TNF (84 v 63 per 10000 person-years (pyrs)) (Table 1). After adjusting for IPTW there was no difference in risk of solid cancer between the cohorts (adjusted hazard ratio (aHR) for anti-TNF 0.88 (95% CI 0.65, 1.17)). There was no difference in site-specific risk for anti-TNF vs nbDMARD; colorectal aHR 1.21 (0.54, 2.70), lung 0.89 (0.46, 1.74), breast 0.99 (0.51, 1.92). The risk did not vary with length of follow up.

Conclusions: In patients without prior solid cancer no increase in solid cancer risk was seen in this UK cohort of RA patients treated with anti-TNF followed for up to 5 years. Additional follow up is warranted to further assess site-specific risk and allow for longer latency.

Disclosure statement: O.T. received research grants from Abbott, Merck, Pfizer, Roche, Swedish Orphan Biovitrum and UCB. All other authors have declared no conflicts of interest.

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O38. SYSTEMIC RHEUMATOID VASCULITIS IN THE BIOLOGIC ERA

Elena Ntatsaki1, Richard A. Watts2,3, Janice Mooney4 and David G. I. Scott1,3
1Rheumatology, Norfolk and Norwich University Hospital, Norwich, United Kingdom; 2Norwich Medical School, University of East Anglia, Norwich, United Kingdom; 3Rheumatology, Ipswich Hospital NHS Trust, Ipswich, United Kingdom

Background: Systemic Rheumatoid Vasculitis (SRV) is a rare complication of rheumatoid arthritis (RA), characterized by the development of necrotizing vasculitis. The occurrence of SRV has been reported to have decreased since the 1990s, possibly due to the introduction of modern immunosuppressive therapy. The aim of this study was to review the incidence, outcome and clinical features of SRV in patients with early well-defined population in the biologic era (2001–2010) and to compare these with a pre-biologic cohort of patients (1986-2000).

Methods: Using NORVAS, a prospective register of patients with systemic vasculitis since 1988, all patients with a diagnosis of SRV from 1st January 2001 until 31st December 2010 were identified. SRV was defined according to the Scott and Bacon criteria (1984). The incidence was calculated using life tables method with cohort comparison. Clinical features were compared with the 1986-2000 cohort using a chi-squared test.

Results: The denominator population was 549,000 in 2007 (97% white Caucasian). 18 patients with SRV were identified (10 male), median age at SRV diagnosis was 72 years and average disease duration 15.6 years. The average annual incidence 2001-10 was 3.9 /million (male 4.5 /million, female 3.4/million). One-year mortality was 12% and 5-year mortality 60%. There was no difference in mortality between the two cohorts (p = 0.134). Prior to SRV diagnosis patients had used a median of 2 DMARDs (64% Methotrexate). Only 2 patients had previous therapy with biologic drugs (12.5%). Comparison of this cohort (2001-2010) with our previous cohort (1988-2000) showed that although the incidence decreased, the clinical manifestations of SRV remain similar.

Conclusions: The incidence of SRV in the new millennium is low compared with the 1990s. However mortality remains high. Modern immunosuppressive therapy for RA has been associated with a decrease in incidence of SRV but has had no influence on clinical features and outcome.

Table 1. Cohort comparison

<table>
<thead>
<tr>
<th></th>
<th>2001-2010</th>
<th>1988-2000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>18 (95% CI) 47 (85% CI)</td>
<td></td>
</tr>
<tr>
<td>Average annual incidence (/million)</td>
<td>3.9 (2.3-6.2) 9.1 (6.8-12.0)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>4.5 (2.2-8.3) 8.9 (5.7-13.3)</td>
<td></td>
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<td>Female</td>
<td>3.4 (1.4-6.6) 8.7 (5.6-12.9)</td>
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<td>1 year mortality, %</td>
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<td></td>
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<tr>
<td>5 year mortality, %</td>
<td>60 51</td>
<td></td>
</tr>
<tr>
<td>CLINICAL FEATURES</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>Rheumatoid Factor</td>
<td>18 100 42 89</td>
<td>0.358</td>
</tr>
<tr>
<td>Erosions Nodules</td>
<td>13 83 32 68</td>
<td>0.98</td>
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<tr>
<td>Cardiovascular</td>
<td>3/38 16/57 2</td>
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<tr>
<td>Gastrointestinal</td>
<td>0 0 2 4</td>
<td>0.990</td>
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</tbody>
</table>

Disclosure statement: All authors have declared no conflicts of interest.


Jennifer Humphreys1, Suzanne M. Verstappen2, Tanya Marshall3, Mark Lunt1, Kimmy Hyrich1 and Deborah P. Symmons1
1Arthritis Research UK Epidemiology Unit, School of Translational Medicine, University of Manchester, Manchester, United Kingdom; 2Department of Rheumatology, Norfolk and Norwich University Hospital, Norwich, United Kingdom

Background: The development of new classification criteria for rheumatoid arthritis (RA) has been a re-evaluation of RA incidence rates. The new criteria purport to have increased sensitivity to identify RA in patients with early inflammatory arthritis (EIA). We used the new criteria to estimate the age and sex-specific incidence of RA in Norfolk, England.

Methods: This study included all patients aged ≥16 notified to the Norfolk Arthritis Register (NOAR), a primary-care inception cohort of patients with EIA, from 1990-5 with symptom onset in 1990. The denominator population was the Norwich Health Authority population based on the 1991 census. Age and sex-specific incidence rates using both 1987 and 2010 classification criteria were calculated at baseline visit, annually for the first 3 years and at 5 years. At each follow up both criteria sets were applied cross-sectionally and cumulatively, i.e. once a patient satisfied a particular criterion that result was carried forward to future assessments.

Results: 283 patients were notified to NOAR with symptom onset in 1990. 23 patients were excluded as an alternative diagnosis was made by their rheumatologist. The overall incidence rate (IR) when applying the 2010 criteria at baseline assessment was 54/100 000 for women and 25/100 000 for men (Table 1). These rates were higher than when applying the 1987 criteria at baseline. Cumulative incidence rates using both criteria sets converged when applied cumulatively. However, 7.3% patients satisfied the 1987 criteria cumulatively after 5 years but never satisfied the new criteria, and 27.1% satisfied the new criteria but never satisfied the 1987 criteria.

Conclusions: The 2010 classification criteria aim to identify early, those patients with EIA who, in the absence of appropriate treatment, would go on to develop persistent, erosive and disabling RA. This study shows that the new criteria classify at baseline similar numbers of patients as having RA, that the previous criteria would have taken up 5 years to identify a small proportion of patients (13%) satisfied only one criteria set over 5 years. These results provide important information for health economics evaluation and healthcare planning.

Disclosure statement: All authors have declared no conflicts of interest.

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<td>0 0 2 4</td>
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Disclosure statement: All authors have declared no conflicts of interest.

O40. SMOKING AND RHEUMATOID FACTOR STATUS IN PREDICTING RESPONSES TO BIOLOGICS

Abdul Khan1 and David L. Scott1
1Rheumatology, Kings College Hospital, London, United Kingdom

Background: Only 70-80% of patients with active rheumatoid arthritis (RA) respond to biologics. Identifying potential non-responders will increase the cost-effectiveness and reduce the risks of treatment with tumour necrosis factor (TNF) inhibitors and rituximab. We tested the hypothesis that simple pre-treatment biomarkers - smoking status and rheumatoid factor (RF) positivity - predict biologic responsiveness in RA.

Methods: We studied 6-month changes in disease activity scores (DAS28) in 359 RA patients: 209 received TNF inhibitors and 150 rituximab (mean age 56/61 years, mean disease duration 8/13 years,
RF positivity 61/53%). Our primary outcome was NICE response criteria (DAS28 change >1.2); we also assessed mean changes in DAS28 both in NICE categorized smoking status as current, previous or never. We categories all patients as RF positive or negative; we also assessed anti-citrullinated peptide antibody (ACPA) status with rituximab.

Results: 68% of all patients given TNF inhibitors and 59% given rituximab were NICE DAS28 responders. Smoking status significantly predicted NICE DAS28 responders with both biologics (Table 1, P < 0.001 on Chi-Squared analyses). Few current smokers were responders (OR 0.15; 95% CI 0.04, 0.60); 27% rituximab - 20%, RF status predicted responses to rituximab but not TNF inhibitors; only 35% of RF negative patients responded to rituximab (P = 0.001). Smoking and RF status had an additive impact on rituximab responses; only 9% of RF negative smokers responded compared with > 80% of RF positive or negative “never” smokers. Combining ACPA with RF increased the prediction of rituximab responses; only 3% of RF/ACPA negative smokers responded. 6-month changes in DAS28 confirmed these findings. For example mean DAS28 only fell by 0.14 in 40 RF/ACPA negative smokers given rituximab compared with 2.77 in 46 RF/ACPA positive non-smokers.

Conclusions: Smoking exerts major effects on biologic responsiveness in active RA; the underlying reason for these effects are unknown and merit further research. As smoking and RF positivity identify 3-fold variations in biologic responses, the rationale for all patients following a single treatment pathway in active RA, which is promoted in NICE HTA appraisals, appears unsustainable. The balance of evidence suggests biologics are unlikely to be cost-effectiveness in RA patients who continue to smoke; this observation creates a complex ethical dilemma which needs to be addressed.

Disclosure statement: All authors have declared no conflicts of interest.

O41. BREAST IS BEST. LIFECOURSE INFLUENCES ON THE DEVELOPMENT OF KNEE OSTEOARTHRITIS: THE NEWCASTLE THOUSAND FAMILIES STUDY

Ajay Abraham1, Mark S. Pearce1, Kay D. Mann1, Roger M. Francis2 and Fraser Birrell1

1Institute of Health and Society, Newcastle University, Newcastle upon Tyne, United Kingdom; 2Institute for Ageing and Health, Newcastle University, Newcastle upon Tyne, United Kingdom; 3Musculoskeletal Research Group, Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, United Kingdom

Background: There is a paucity of lifecourse research on osteoarthritis (OA). However, several studies shorter term have demonstrated the association between adult risk factors such as obesity and higher bone mineral density with subsequent knee OA. We performed a lifecourse analysis of risk factors for knee OA (defined by osteophytes on ultrasonic) at acting at different stages of life, including early life factors, among members of the Newcastle Thousand Families birth cohort.

Methods: Potential risk factors for knee osteoarthritis (including birth weight, breast feeding data and socioeconomic status) have been collected prospectively in this birth cohort of subjects aged 63 years (born in May-June 1947) and an aim a priori conceptual framework was developed. Subjects had both knees scanned by a trained musculoskeletal sonographer. Ultrasound protocols were derived from EULAR guidelines. The presence of knee osteophytes was assessed at the tibial and femoral sites, medially and laterally. These data were analysed in relation to a range of factors from across the lifecourse using logistic regression models.

Results: Among the 311 participants, the prevalence of knee osteoarthritis was 22%, 25% and 30% for right, left and “any” knee, respectively. While birth weight, exclusive breast feeding and social class at birth showed significant univariate associations with knee osteoarthritis, only exclusive breast feeding (among factors acting in early life) showed a significant association in the adjusted model (OR 1.85; 95% CI 1.02, 1.20; p = 0.01) and total hip bone mineral density at age 50 (OR 1.37 per 0.1 g/cm²; 95% CI 1.06, 1.78; p = 0.02) were the factors acting in adulthood that increased the risk of knee osteophytes at age 63. Increase in levels at age 50 (as surrogate marker of inflammation) showed a borderline significant association in the multivariate model (OR 1.68 per g/L; 95% CI 0.99, 2.85). The univariate effect of social class at birth on knee osteophytes was found to be mediated by its subsequent effect on breast feeding and total hip bone mineral density.

Conclusions: This is the first study to perform a lifecourse analysis of knee OA risk using prospectively collected data. While exclusive breast feeding is known to decrease risk of adult obesity and therefore knee OA, this study is the first to suggest that exclusive breast feeding is independently protective against knee OA. The mechanism might be reduced burden of infection and inflammation through the lifecourse: a testable hypothesis. If confirmed, this may lead to novel treatment strategies, such as early polyvalent vaccination, as well as reinforcing public health messages about breast feeding.

Disclosure statement: All authors have declared no conflicts of interest.

O42. ASSOCIATION OF SYSTEMIC SCLEROSIS WITH DIFFERENT AUTOANTIBODY SUBGROUPS AND MALIGNANCIES: A RETROSPECTIVE REGISTRY-BASED UK COHORT STUDY

Pia Moinazadeh1, Carmen Fonseca1, Martin Hellmich1, Arni Shah1, Cecilia Chighizola1, Christopher P. Denton1 and Yoon Ong1

1Centre for Rheumatology and Connective Tissue Diseases, UCL Medical School, Royal Free Hospital, London, United Kingdom; 2Dermatology, University of Cologne, Cologne, Germany; 3Institute of Medical Statistics, Informatics and Epidemiology, University of Cologne, Cologne, Germany; 4Division of Rheumatology, Johns Hopkins University School of Medicine, Baltimore, Maryland, United States of America; 5Rheumatology Division, Department of Internal Medicine, School of Medicine, Milan, Italy

Background: Systemic sclerosis (Scleroderma, SSc) is a heterogeneous multysystem connective tissue disease on the basis of vascular endothelial cell damage, altered immunological processes and abnormal fibrinotic response. Several studies and case series have reported an increased risk of cancer among patients with SSc reported. We assessed the risk of cancer in our single centre UK cohort for SSc patients, and evaluated the frequency of different cancer subgroups with risk ascertinement among patients with different autoantibody subgroups and the duration between cancer diagnosis and scleroderma onset.

Methods: We obtained information about SSc and cancer from our research database and medical records of patients attending the Centre for Rheumatology and Connective Tissue Diseases at the Royal Free Hospital. All of our patients meet the classification criteria of LeRoy as having limited (lcSSc) or diffuse (dcSSc) cutaneous systemic sclerosis. Statistical methods for contingency tables (Chisquare-test or Fisher’s exact t-test) and time-to-event data were used. Specifically, differences between SSc patients with and without history about malignancies were assessed using Kaplan-Meier curves and log-rank tests and multiple Cox proportional-hazards regression was used to assess the impact of autoantibody status on cancer risk.

Results: Among 2177 patients with SSc, 7.1% of patients had a history of cancer. 26% showed anti-centromere antibodies (ACA), 18.2% were positive for anti-ScI70 antibodies and 26.6% showed anti-RNA polymerase antibodies (RNAP). 42.2% of patients with cancer had breast cancer followed by haematological (12.3%), gynaecological (11.0%) and neurological (10.0%). The frequency of malignancies among patients with RNAP (14.2%) was significantly increased than those with anti-ScI70 (6.3%) and ACA (6.8%) (p < 0.001 and p < 0.001 respectively). Among the patients, who were diagnosed with cancer within 36 months of the clinical onset of SSc, there were more patients with RNAP (55.3%) than other autoantibody specificities (ACA 23.5%; p < 0.008 and anti-ScI70 antibodies 13.6%, p = 0.002 respectively) and SSc patients with anti-RNAP had two-fold increased risk of cancer compared to patients with ACA (p < 0.0001).

Conclusions: These findings provide independent confirmation of recent studies that SSc patients with anti-RNAP antibodies have an increased risk of cancer prior to or in the early stages of SSC.

Disclosure statement: All authors have declared no conflicts of interest.
ORAL ABSTRACTS 9: MOLECULAR MECHANISMS OF DISEASE—INFLAMMATORY ARTHRITIS I

S5. ANTI-VCA AND EBNA1 ANTIBODIES ARE PRODUCED IN THE RHEUMATOID SYNOVIAL IN THE PRESENCE OF ECTOPIC GERMINAL CENTRES AND CORRELATE WITH ACPA PRODUCTION
Cristina Croia1, Michele Bombardieri1, Alois Francescas2, Barbara Serafini2, Frances Humby1, Stephen Kelly1, Paola Migliorini3 and Costantino Pitalis1
1Experimental Medicine and Rheumatology, Queen Mary University of London, London, United Kingdom; 2Cell Biology and Neuroscience, Istituto Superiore di Sanita’, Rome, Italy; 3Internal Medicine, University of Pisa, Pisa, Italy

Background: We recently reported that ectopic lymphoid structures (ELS) are preferential sites for Epstein-Barr virus (EBV) latency and reactivation in the synovial tissue of rheumatoid arthritis (RA) patients. While synovial germinal centres have been shown to support the in situ production of anti-citrullinated protein/peptide antibodies (ACPA), it is unknown whether ELS are responsible for the generation of anti-EBV antibodies. In this work we aimed: 1) To investigate the presence of anti-VCA, EBNA1, EA and ACPA antibodies in the serum vs synovial fluid (SF) of RA patients; 2) To provide in vivo evidence of the synovial production of antibodies against unmodified and citrullinated EBV-proteins in the HuRA/SCID chimeric model; 3) To investigate whether EBV-infected synovial plasmacles produce ACPA.

Methods: Anti-EBNA1, anti-VCA, anti-EA and ACPA IgG were measured in paired serum and SF from RA and OA patients. The local production of unmodified and citrullinated (VCP1 and VCP2) anti-EBV antibodies was investigated by screening the serum of 30 SCID mice transplanted with ELS+ vs ELS- RA synovium. Finally, sequential double immunofluorescence for bilitinated citrullinated fibrinogen (BCI), BFRF1 (EBV lytic antigen) and CD138 was used to detect EBV reactivation in ACPA-producing synovial plasmacles.

Results: Serum and SF levels of anti-EBNA1, anti-VCA and ACPA IgG were significantly correlated. Sera of SCID mice transplanted with ELS+ (but not ELS-) RA synovium, displayed high levels of human ACPA, anti-EBNA1 and anti-VCA but not anti-EA antibodies. Interestingly, a proportion of SCID mice sera displaying high reactivity against anti-CCP and anti-EBNA1 antibodies were also found positive for anti-VCP1 and 2. Finally, in ELS+ RA synovium a subset of locally differentiated ACPA+ plasmacles surrounding ectopic germinal centres displayed EBV lytic infection as detected by reactivity for BFRF1.

Conclusions: Here we showed that antibodies against unmodified and citrullinated EBV epitopes are locally produced in the RA synovium in the presence of ELS and are closely associated with in situ ACPA production. Evidence of ACPA+ plasmacles showing lytic EBV infection strongly supports an important role for EBV in the activation and differentiation of autoreactive B cells within the RA synovium.

Disclosure statement: All authors have declared no conflicts of interest.

S6. TLR9 INDUCES TOLERANCE TO APOPTOTIC CELLS AND IS RESPONSIBLE FOR INDUCING REGULATORY B CELLS
Katherine Miles1, Jonathan Heaney1, Zaneta Sibinska1, Donald Salter1, John Savill1, David Gray2 and Costantino Pitalis1
1Queen’s Medical Research Institute, University of Edinburgh, Edinburgh, United Kingdom; 2Institute of Immunology & Infection Research, University of Edinburgh, Edinburgh, United Kingdom

Background: Apoptotic cells (AC) are immune-modulatory, dampening inflammation mediated by innate immune cells. They also protect mice from autoimmune mediated inflammation and we have previously shown that AC induce B cells to adopt an IL-10 secreting regulatory B cell phenotype (Breg). Marginal zone B (MZB) and peritoneal B1 cells are innate like B cells that have many self-reactive B cell receptors (BCR) and are selected by intracellular antigens expressed on AC; yet this is generally compatible with health. However AC express on their cell surface many of the antigens associated with autoimmune diseases and are subsequently thought to be a target of autoimmune responses. The B cell receptor (BCR) can deliver chromatin complexes from the AC to the endosome allowing Toll-like receptor-9 (TLR9) mediated signalling. Despite this, lupus-related renal disease is exacerbated not diminished in TLR9-deficient mice. We hypothesized that TLR9 plays a regulatory role in self-reactive B cells, maintaining tolerance to apoptotic cells. However if this pathway breaks down autoimmune mediated inflammation would be exacerbated.

Methods: B cells from mouse spleen, peritoneum and also from human peripheral blood was isolated and co-cultured with AC. IL-10 from these co-cultures was measured 72 hours later. Models of autoimmune mediated chronic inflammation including collagen induced arthritis (CIA) and experimental autoimmune encephalitis (EAE) were tested to ask if TLR9 expressed within B cells or chromatin complexes expressed on AC were crucial for the induction of regulation required to protect from autoimmunity.

Results: We can now report that IL-10 production by MZB and B1a B cells, stimulated by contact with apoptotic cells results from the engagement of TLR9 within the B cell following recognition of DNA-containing complexes on the surface of apoptotic cells. Until now TLR9 ligation has been seen as an inflammatory signal, but we confirmed a hitherto unexpected immuno-regulatory role by demonstrating that the protective effect of apoptotic cells during EAE was absent in TLR9-deficient mice. In addition the protection seen when apoptotic cells are given to mice during the onset of CIA was abolished if DNA containing complexes expressed on the surface of the apoptotic cells was first removed with DNase. Human circulating CD27+ B cells also responded to DNA-bearing apoptotic cells, but not DNase treated cells, by secreting IL-10.

Conclusions: This data creates a new paradigm in which autoimmunity may arise if this tolerance mechanism mediated by innate like B cells is not re-imposed after episodes of inflammation or if the regulatory B cell response is subverted. Innate like B cells may then switch to secreting auto-antibodies at higher titre and affinity as well as secreting pro-inflammatory cytokines, thus driving an autoimmune phenotype.

Disclosure statement: M.G. has received honoraria from MSD. All other authors have declared no conflicts of interest.

S7. INTERLEUKIN-27 RECEPTOR-DEFICIENT MICE DEVELOP EXACERBATED INFLAMMATORY ARTHRITIS ASSOCIATED WITH HIGHER-T H- AND B-CELL RESPONSES
Gareth W. Jones1,2, Claire J. Greenhill1, Anwen S. Williams1, Mari A. Nowell1, Brendan J. Jenkins3 and Simon A. Jones1
1Institute of Infection and Immunity, Cardiff University, Cardiff, United Kingdom; 2Centre for Invasive Immunity and Infectious Diseases, Monash Institute of Medical Research, Clayton, Victoria, Australia

Background: Cytokine control of the adaptive immune response is a central process in the development of inflammatory diseases. T helper cells producing interleukin-17 (IL-17T) cells have emerged as a distinct T-cell subset implicated in a number of autoimmune diseases including rheumatoid arthritis (RA). As such, targeting of the inflammatory pathways promoting Th17 cell responses is of interest in developing new therapies to treat RA. The IL-6 family of cytokines share the ubiquitously expressed glycoprotein-130 (gp130) receptor for activation of intracellular signalling pathways and members of this family, most notably IL-6 and IL-27, have emerged as key regulators of the Th17 cell response. Through differential activation of signalling transducers and activators of transcription (STAT)1 and STAT3, IL-6 and IL-27 have opposing outcomes on the generation of Th17 cells. IL-6/STAT3 signalling promotes the differentiation of Th17 cells from naive T helper cells while IL-27 via STAT1 counteracts this IL-6-driven process. Accordingly, IL-6 receptor-deficient (IL-6RKO) mice are protected from inflammatory arthritis, display no T-cell infiltrates in the synovium and have an impaired Th17 cell response. Studies in IL-27RKO mice have highlighted an anti-inflammatory role for IL-27 in inflammatory diseases. However, the mechanisms linking IL-27 to arthritis progression remain unclear.
S6. ANTI-TNF ANTIBODY THERAPY, BUT NOT TNF RECEPTOR BLOCKADE, INDUCES IL-17 SUPPRESSING REGULATORY T CELLS
Jenny McGovern1,2,3, Dao X. Nguyen4,5,6,7, Claire A. Notley1,2,3, Claudia Mauri1,8,9, James Bateman1,2,3, James Bateman1,2,3, Maggie Allen1,2, Dipti Samani1,2,3, David Davies1,2,3
1Centre for Rheumatology, University College London, London, United Kingdom

Background: The highly inflammatory cytokine IL-17 has attracted considerable attention for being pivotal in the pathogenesis of several autoimmune diseases, including rheumatoid arthritis. The importance of IL-17 is underscored both by its relative resistance to control by regulatory T cells (Treg) and the propensity of Treg to produce this inflammatory cytokine.

Methods: We recruited 72 RA patients, fulfilling the revised classification criteria of the American College of Rheumatology for RA, and 15 healthy volunteers for this study.

Results: Here we demonstrate that Treg from rheumatoid arthritis (RA) patients responding to adalimumab (an anti-TNF antibody) inhibited IL-17 production in vitro, in contrast to Treg from healthy individuals or patients with active RA (p = 0.008). This capacity to suppress IL-17 was associated with a 2-fold reduction in RORγt+ Th17 cells in the peripheral blood (p = 0.002), and an increase in the percentage of Foxp3+ Treg (p = 0.001). Within the Treg compartment, it was observed that adalimumab treated patients had a greater proportion of cells expressing low levels of Helios and CD62L, consistent with an extra-thymic source. These Treg controlled Th17 responses via IL-10 and TGFβ, but Treg suppression of IL-17 was governed through a monocyte-derived IL-6 inhibition. The induction of peripheral Treg was not observed in RA patients responding to etanercept, a modified anti-TNF therapy. Indeed, Treg from etanercept treated patients did not acquire IL-17 or IFNγ suppressor function, and peripheral RORγt+ T cell numbers were similar to patients with active RA.

Conclusions: Collectively, this data suggests that adalimumab therapy induces a potent and stable population of Treg, which has the capacity to inhibit IL-17 associated inflammation in patients with RA. These results may provide mechanistic insight into the therapeutic benefit of switching between different anti-TNF agents and the differing incidence of tuberculosis between adalimumab and etanercept.

Disclosure statement: All authors have declared no conflicts of interest.

ORAL ABSTRACTS 10: EDUCATION

O43. NATIONAL RHEUMATOID ARTHRITIS SOCIETY WORKWISE WORKSHOPS’ ONLINE RESOURCES: TOOLS TO HELP PEOPLE WITH RHEUMATOID ARTHRITIS REMAIN IN THE WORKPLACE OR GET BACK TO WORK
Clare Jacklin1 and Alisa M. Bosworth1
1External Affairs, National Rheumatoid Arthritis Society, Maidenhead, United Kingdom

Background: In 2010 NRAS delivered 10 workshops in major cities across the UK focussing on providing targeted information and help for people worried about the impact of their disease on their job, or who, having previously had to give up work, wanted to return to work. The feedback from these workshops was extremely positive and so we determined to put the materials, both audio visual and written, into a format on our website to make them widely available to all. The workshops comprised sessions from experts in the areas of employment law, occupational health, occupational therapy and support from NRAS.

Methods: We subsequently filmed the experts delivering their workshop presentations and these videos are now available to view online at www.nras.org.uk/workwise. Also available are downloadable transcripts of the experts’ video presentations and slide presentations. We have also included an animated film about how the Equalities Act 2010 can help people with long term conditions in the workplace, as well as a video of an NRAS Member talking about her personal experience of working while living with RA.

Results: Results of the survey on the impact and usefulness of the online resources are collected via an online survey included in each section of the WorkWise web area and visitors are encouraged to fill this in. 50% of respondents categorized the online materials as excellent and that they would recommend them to others. The WorkWise online resources have been viewed 741 times since the facility was fully implemented in September 2011. The most viewed podcast was the one delivered by the expert in Employment Law which clarifies and summarizes the terms of the Equalities Act as it applies to people with RA. Anecdotal feedback from health professionals in rheumatology is that these tools are extremely useful to them and they sign-post their patients to this url.

Conclusions: NRAS believe that work should be an important health outcome of treatment for people with RA. The charity is committed to working towards this aim. The NRAS WORKWISE online resources contribute to our aims and provide a set of tools that can assist people with concerns about work issues. It is becoming acknowledged by government and the NHS that a person’s ability to remain in or return to work should be monitored alongside clinical outcomes as a clear indication as to how successful therapy/treatment of the person’s disease is.

Disclosure statement: A.B., C.J.: Workwise online resources have been viewed 741 times since the facility was fully implemented in September 2011. The most viewed podcast was the one delivered by the expert in Employment Law which clarifies and summarizes the terms of the Equalities Act as it applies to people with RA. Anecdotal feedback from health professionals in rheumatology is that these tools are extremely useful to them and they sign-post their patients to this url.

O44. DESIGNING VIRTUAL PATIENTS FOR MUSCULOSKELETAL EDUCATION: A GROUNDED THEORY QUALITATIVE STUDY
James Bateman1,2,3, Maggie Allen1,2, Dipti Samani1 and David Davies1
1Institute of Clinical Education, University of Warwick, Coventry, United Kingdom; 2Department of Rheumatology, University Hospitals Coventry and Warwickshire NHS Trust, Coventry, United Kingdom

Background: Virtual patients (VPs) are computer representations of clinical cases. They are increasingly used in undergraduate education, and recent technical inter-operability standards mean that they can now be shared between authors and institutions. VP design is potentially limitless, however two recent literature reviews highlight the lack of evidence to support any individual design features.

Methods: We created and piloted two web-based VP cases with a wide range of design properties, derived from the literature. Each case takes students approximately 30 minutes to work through, and covers two musculoskeletal (MSK) problems; polyarthritis and back pain. The cases were piloted by the authors, rheumatology specialists and non-specialists (general practitioners). The Warwick Biomedical Research
Ethics Committee approved the research. Final year undergraduates at Warwick Medical School were invited to participate in an education research session. Volunteers completed the two cases, written case evaluations and took part in a 1-hour digitally recorded focus group that was conducted to an established methodology with a facilitator and moderator. The principle investigator transcribed each focus group. Data was analysed using computer assisted qualitative data analysis (NVivo 9©) using a classic grounded theory approach, using a constant comparative method, generating theoretical hypotheses grounded in the data. Further focus groups were conducted using purposeful sampling. After six focus groups in year four and year two, no new themes emerged, and sampling was terminated. Focus group data was triangulated using a written case evaluation and online record of VP use for each student.

**Results:** We have derived a theoretical model of student interaction with VPs using established classic grounded theory methodology. The initial open coding was followed by axial coding around an emergent central phenomenon, ‘learning from the VP’. Three parent elements form the causal conditions for the phenomenon (‘clinical components’, ‘pedagogic properties’ and the ‘VP as an e-learning object’). This is also influenced by intervening conditions (student centred, environmental and organizational), and student-VP interaction strategies. Individual VP design features appear to have both a positive and negative affect on this model, resulting in different consequences when the VP is used, such as differing depths of student engagement with the case.

**Conclusions:** This study builds a theory for VP design grounded in the data. These elements explicitly designed to study relevant design variables in MSK cases. As well as informing future VP authoring, these results form the basis for a large multi-centre randomized trial of VP design variables in medical undergraduates from three UK medical schools. All learning materials from the study will be open access, reusable and modifiable education resources.

**Disclosure statement:** All authors have declared no conflicts of interest.

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**O45. HAVE YOU BEEN TO CAPRI? WWW.CAPRI.SCOT.NHS.UK: EARLY RESULTS OF A CLINIC FOR ARTHRITIS PATIENTS IN REMISSION ON THE INTERNET**

Helen E. Harris1, Suzanne Brannan2, Gavin Venters3, Alan McQuillan3, Fiona Lovegrove1, Jane Gibson1, David Chinn4 and John S. McLaren1

1Fife Rheumatoid Diseases Unit, NHS Fife, Kirkcaldy, United Kingdom; 2Information Technology Department, NHS Scotland, Edinburgh, United Kingdom; 3Ophthalmology Department, Queen Margaret Hospital, Dunfermline, United Kingdom; 4Research and Development, NHS Fife, Dunfermline, United Kingdom

**Background:** The current management of stable Rheumatoid Arthritis (RA) patients in Fife and nationally is out-patient attendance (OPD) 6-12 monthly where review includes completion of the Disease Activity Score (DAS28) and often ultrasound (US) examination. We aimed to see if patient use of a web-based DAS28 score was a safe and acceptable alternative to routine out-patient based care over a 12 month period.

**Methods:** Patients with a DAS28 score of 2.6 or less and negative US 3 joints were invited to participate in CAPRI. Patients registered online at www.capri.scot.nhs.uk when they completed the study questionnaires and reviewed pictorial instructions before scoring a DAS28. Subsequently, they were requested every 3 months to score the DAS28 on-line. If the score was < 3.3 the patient was asked to log on again in 3 months, with reminders sent by email. If the score was >=3.3 the patient was automatically sent an appointment for reassessment in the OPD. Patients completed the Short Form 36 (SF36) and Health Assessment Questionnaire Disability Index (HAQDI) at the beginning and end of the study. After 12 months of low DAS 28 scores patients were sent a routine OPD appointment for review. The site was launched in December 2009 and in September 2011 the platform for CAPRI was extended using the “Looking Local” system to include broadband TV, Wi and Bluetooth enabled phones. CAPRI patients were emailed instructions on how to score the DAS28 using the TV remote red button if they were Sky or Virgin customers, through Wi or using the Looking Local mobile phone App.

**Results:** To date, 75 patients have enrolled in CAPRI and 5 patients have completed 4 DAS28 scores (9 months follow-up). In total, 62 DAS 28 scores were completed of which 52 were less than 3.3, thus avoiding 26 six-monthly OPD appointments. Ten scores were higher than 3.2: these patients were reviewed in clinic and have now exited the study. Nine patients have completed the end of study patient satisfaction survey. There has been an average of 56 hits per day on the site after launch. In the first 2 months of availability 28 users have accessed the site using Looking Local and 9 patients have scored a DAS28 using either the red button, Wi or mobile phone App.

**Conclusions:** We believe this is the first attempt worldwide to set up an online self assessment clinic for RA patients. By avoiding the potential saving of 26 OPD appointments so far demonstrates that CAPRI may be a potentially efficient mechanism for monitoring RA patients in remission. Further analysis of CAPRI data will be undertaken to determine correlation of scores done online by patients with clinician scores in OPD. Both high and low scores SF36 and HAQDI data will also be analysed. CAPRI may offer the opportunity to increase the frequency of monitoring of RA patients while conserving hospital resources for patients that require direct clinical assessment.

**Disclosure statement:** H.H. has received honoraria from Pfizer and MSD, and an educational grant from Pfizer. All other authors have declared no conflicts of interest.

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**O46. THE EVALUATION OF AN ARTHRITIS EDUCATION INTERVENTION PROGRAMME FOR BLACK AND ETHNIC MINORITY COMMUNITIES**

Chandrika Gordhan1, Rebecca J. Stack1, Kanta Kumar1, Ishraga Awad4, Karim Raza1,2 and Paul Bacon3,1

1Rheumatology Research Group, School of Immunity and Infection, University of Birmingham, Birmingham, United Kingdom; 2Rheumatology Department, Sandwell & West Birmingham Hospitals NHS Trust, Birmingham, United Kingdom; 3Birmingham Arthritis Resource Centre, Birmingham Central Library, Birmingham, United Kingdom; 4Public Health Medicine, Sandwell Primary Care Trust, West Bromwich, United Kingdom

**Background:** Chronic musculoskeletal (MSK) conditions such as rheumatoid arthritis (RA), systemic lupus erythematosus and osteoarthritis are challenging for people from Black and Minority Ethnic (BME) communities who suffer from greater pain and disability, require longer periods of absence from work, and have lower understanding of MSK conditions. Many BME groups face language barriers making access to health education difficult. A Royal College of Physicians report urged professionals to engage with BME communities to empower them to take more control of their health. Providing MSK health education interventions to people from BME groups is a priority.

**Methods:** An outreach arthritis education intervention was developed, based on educational programmes run by Birmingham Arthritis Resource Centre. The intervention aimed to provide people from BME groups with arthritis and self-management knowledge in a culturally sensitive environment. During the intervention, English for Speakers of Other Languages (ESOL) tutors taught attendees the English words and phrases for arthritis related topics and provided additional language skills where necessary. Interventions for Afro-Caribbean, South Asian, Yemeni and Somali communities were run over 12 weeks and evaluated through a post-intervention questionnaire.

**Results:** Of the 248 attendees 27% were Somali, 5% Afro-Caribbean, 17% Yemeni and 51% South Asian. 67% were female. 51% of attendees self-reported a diagnosis of ‘arthritis’, 23% cared for someone with arthritis and 28% reported an interest in arthritis primarily because they believed that they or a relative had an undiagnosed form of arthritis. Among groups ANOVA found a significant difference between BME groups rating (using a 10 point rating scale, 10 = most positive) of the intervention’s helpfulness and ease of understanding. The post hoc analysis revealed that people from an Afro-Caribbean background rated the interventions helpfulness (mean = 8.77) and understanding (mean = 8.63) lower than people from a South Asian, Yemeni and Somali background/helpfulness mean was 9.75, 9.6 and 9.72 respectively, while mean understanding was 9.67, 9.8, 9.65 respectively). No significant difference was found between South Asian, Yemeni and Somali ratings of the intervention. The intervention promoted positive behaviour change in 48% of attendees, who went on to individually participate in further activities including exercise and ESOL classes.

**Conclusions:** All participants rated the intervention highly. People from South Asian, Yemeni and Somali backgrounds rated the intervention higher than individuals from an Afro-Caribbean background. This maybe because the ESOL tutor took a more prominent role in interventions for people from South Asian, Yemeni and Somali communities. Our data suggests that this model may be acceptable to these communities. Further work is needed to fully assess its impact.

**Disclosure statement:** All authors have declared no conflicts of interest.
O47. PATIENT EXPERIENCES, ATTITUDES AND EXPECTATIONS TOWARDS RECEIVING INFORMATION ABOUT ANTI-TNF: A QUALITATIVE STUDY

Paul Arkell1,2, Sarah Ryan2, Ann Brownfield2 and Jonathan Packham1,2
1Primary Care and Health Sciences Department, Keele University, Keele, United Kingdom; 2The Haywood Hospital Rheumatology Department, University Hospital of North Staffordshire, Stoke-on-Trent, United Kingdom

Background: Anti-TNF is an important advance in the treatment of many autoimmune diseases including Rheumatoid Arthritis (RA). Despite having the potential for significant adverse effects, there has been limited research into patient preferences for the information they receive before starting these medications. The aim of this study was therefore to explore patient experiences, expectations and attitudes towards pre-therapy counselling for anti-TNF.

Methods: Patients with RA taking anti-TNF were purposively sampled to give a variety of ages, disease durations, pre-medication disease activities and agents. Data were collected from two gender-specific focus groups of five individuals and 1½ hours duration. Topic-led discussions were recorded and written notes were taken to collect non-verbal cues. Transcripts were independently and repeatedly read and annotated by two researchers. Themes were identified and developed through discussion. Participants were provided with written summaries of findings and asked to provide comments.

Results: Three key themes were identified. Firstly, participants discussed weighing the risks and benefits of starting the new medication. There was a general perception that taking anti-TNF increased the risk of both cancer and infection. However, participants attached limited importance to this, reporting that at the time of anti-TNF initiation, the strong desire for RA symptom control was overriding. Some participants revealed that they worried more about having to stop anti-TNF than getting cancer, and one admitted deliberately concealing an illness in order to continue her medication.

Secondly, participants discussed how much information about anti-TNF they should receive. While they wanted differing amounts of information, most agreed that counselling should occur at an earlier stage in the management of their RA, as they found it challenging to absorb information during an RA flare-up severe enough to precipitate anti-TNF initiation.

Thirdly, participants discussed the process of starting anti-TNF. Many identified that their major concern was whether they would be eligible for the new medication. They remembered little about the investigations they underwent, and none said they would have objected to being tested for blood borne viruses.

Conclusions: This qualitative study has enriched the understanding of what patients feel is important with regards the benefits and disadvantages of taking anti-TNF, particularly when they are making decisions about treatment. Findings may be useful to professionals throughout the multidisciplinary team in guiding patient counselling about these new medications. Further research is needed to validate findings and expand on themes including patient fears about stopping anti-TNF, deliberate concealment of illness, preferences for timing of information, and attitudes towards testing for blood borne viruses and cancer screening.

Disclosure statement: All authors have declared no conflicts of interest.

O48. NATIONAL RHEUMATOID ARTHRITIS SOCIETY SURVEY: IMPACT OF RHEUMATOID ARTHRITIS ON THE FAMILY

Clare Jacklin1, Ailsa M. Bosworth1 and Kate Wilkinson1
1External Affairs, National Rheumatoid Arthritis Society, Maidenhead, United Kingdom

Background: Much research has been done on the effects of RA on the individual with RA, but by comparison there is relatively little data on the impact on the wider family and principal carer. It is this area that The National Rheumatoid Arthritis Society wishes to explore in greater depth. We hear of the burden carried by carers via our helpline, members' forum and anecdotal feedback from attendees at NRAS group meetings and events. We also conducted an impromptu workshop for ‘carers’ at the inaugural meeting of one of our groups during 2011. It was attended by approximately 21 people, mainly men and mainly spouses of people attending the launch, although some were daughters/sons or siblings. We were struck by the anger in the room: many felt that no-one had adequately explained to them what the impact of their partner having RA would be on them. We asked them to fill in a questionnaire to gauge areas of impact for further research. Results are detailed below and this has led to our next major survey for publication in 2012 ‘The Impact of RA on the Family’

Methods: An independent communications specialist, experienced in the fields of health and care, was commissioned to work with NRAS to carry out a national. An email was sent to NRAS members asking for partners and family members interested in taking part in some qualitative interviews to further explore themes identified by the group. Open questions were agreed between the interviewer (Kate Wilkinson) and NRAS to use during the interview process. 7 interviews took place, primarily with partners/spouses during Oct/Nov 2011.

Results: The pilot survey completed by 21 people attending the group session comprised 71% men and 29% women. Key findings were 71% of respondents felt frustrated that they cannot do more to help their spouse with the management of their disease; 57% were worried about what the future held for them both and 67% felt that their spouse’s RA had had a considerable impact on family life. However, 48% felt that there had been some positive impacts too. The interviews revealed similar findings but enabled the interviewer to drill down and explore specific concerns in more depth. These findings will inform development of the final survey.

Conclusions: We believe that health professionals will be in a better position to help the individual with RA and the family if they understand, in more detail, the issues that carers and the wider family face. The initial phase of our study has demonstrated the need to assess the impact of RA on the family. It suggests that with appropriate help and support for carers, the individual with RA may be able to enjoy a better quality of life. The full report of the survey will be available in April 2012.

Disclosure statement: All authors have declared no conflicts of interest.
S9. INHIBITION OF NAMPT (PBEF/VISFAFIN) DECREASES THE ABILITY OF HUMAN NEUTROPHILS TO GENERATE ROS AND IMPAIR BACTERIAL KILLING

Kate J. Roberts¹, Robert J. Moote² and Steven W. Edwards¹
¹Institute of Integrative Biology, University of Liverpool, Liverpool, United Kingdom; ²Institute of Ageing and Chronic Disease, University Hospital Aintree, University of Liverpool, Liverpool, United Kingdom

Background: Neutrophil apoptosis is required for the effective resolution of inflammation, and defects in the regulation of this process are implicated in inflammatory diseases such as rheumatoid arthritis. Nampt (nicotinamide phosphoribosyltransferase, pre-B-cell colony enhancing factor (PBEF) or visfatin) regulates a number of neutrophil functions, such as priming and apoptosis, and it has been shown that neutrophils can express this molecule. Nampt also functions intracellularly in the regulation of NAD metabolism, and Nampt inhibitors have been shown to have anti-inflammatory potential in vivo models of inflammation. In view of these effects of Nampt on neutrophil apoptosis, and the potential of Nampt inhibitors as anti-inflammatory agents, the aims of this work were to determine the role of Nampt in the regulation of neutrophil function. As neutrophils play a key role in protection against microbial infections, the role of Nampt in the regulation of bacterial killing was also investigated.

Methods: Neutrophils were isolated from venous blood of healthy volunteers and either stimulated with exogenous Nampt (100 ng/mL) or else Nampt enzymatic activity was inhibited by a 30-60 min incubation with FK866. Activity of Nampt (using the inhibitor FK866) decreased the activity of the NAPDH oxidase in Nampt-inhibited cells was still significantly altered by the addition of TNF-α, but not control MPs (1604 ± 74 vs. 1491 ± 11 ng/ml, p < 0.01).

Results: Enhanced MP numbers were associated with activated PMN following stimulation with TNF-α (a pro-inflammatory cytokine highly abundant in the RA joint), C28-I2 cell MMs cultures could produce ECM and was used as surrogate for native cartilage chondrocytes. MPs elicited different effects on IL-1β-stimulated proteoglycan and IL-6 production; Control MP did not affect the catabolic and pro-inflammatory phenotype induced by IL-1β, whilst TNF-α-derived MP prevented the reduced ECM deposition, accompanied by a modest effect on IL-6 release. We will continue testing the biology of these MPs and attempt to identify the molecular determinant(s) involved in these effects.

Disclosure statement: All authors have declared no conflicts of interest.
S1. THE MESENCHYMAL STROMAL CELL MARKER CD248 REGULATES INFLAMMATORY ARTHRITIS AND BONE FORMATION

Amy Naylor1, Eman Azzam2, Stuart Smith1, Adam Croft1, Jeremy Duffield3, David Huso6, Steffen Gay3, Caroline Ospelt3, Amy Naylor1, Eman Azzam2, Stuart Smith1, Adam Croft1, Jeremy Duffield3, David Huso6, Steffen Gay3, Caroline Ospelt3, Claire J. Greenhill1, Anwen S. Williams 1, Gareth W. Jones 1, Mari A. Nowell1, Abdul N. Moideen1, Marcela Rosas1, Phil R. Taylor1, Ian R. Humphreys1 and Simon A. Jones 1

Background: Interleukin 10 (IL-10) is an immuno-regulatory cytokine that terminates the inflammatory response. In inflammatory arthritis IL-10 is elevated in the serum and synovial fluid of patients with rheumatoid arthritis (RA) and has been implicated in various pro- or anti-inflammatory processes. IL-10 may also be involved in preventing bone degradation by inhibiting cytokines involved in bone resorption, such as IL-1β, which is generated by the cytosol complex called the inflammasome. We hypothesize a potential cross talk between IL-10 and the inflammasome, which may impact how IL-10 exerts its anti-inflammatory activities during inflammatory disease and regulate bone erosion.

Methods: To examine the role of IL-10 during RA, we used the antigen induced arthritis (AIA) model in IL-10KO mice and compared disease severity with wild type (WT) controls by histological staining and x-rays to assess bone erosion. To analyse pro-inflammatory cytokine induction, cartilage and bone destructive markers, and expression of inflammasomes components, quantitative real time PCR (Q-PCR) was undertaken on synovial mRNA and ELISAs on serum. Mice were injected with a florescence matrix metalloproteinases (MMPs) probe to assess the extent of potential cartilage damage and scanned prior to, and following arthritis induction. To characterize IL-1β expression following AIA, the production and localization of this cytokine in the joint as well as infiltrating macrophages, neutrophils and lymphocytes was assessed by immunohistochemistry.

Results: In IL-10KO mice, the inflammatory histopathology associated with arthritis induction in AIA model was significantly enhanced and prolonged as compared to wild type (WT) controls. Interestingly, histological and radiographic analysis of joint sections from these studies suggested that IL-10 is required to prevent excessive bone degradation (e.g. via modulation of bone pathology associated with inflammasome activation) (IL-10KO). A separate evaluation of pro-inflammatory regulators during experimental arthritis in IL-10KO mice showed specific augmentation of inflammasome components (e.g. NALP3, caspase-1, IL-33) and attenuation of its negative regulators (i.e. Caspase12), which was accompanied by synovial increases in IL-1β expression. In contrast, TNFα regulation (e.g., TNFα, ADAM-17) following arthritis induction in IL-10KO mice was comparable to that seen in WT. In this regard, arthritis IL-10 KO mice showed similar levels of matrix metalloproteinase (MMP) activity, as assessed by in vivo whole body imaging and synovial MMP expression (MMP-1, MMP-3, MMP-9, MMP-13), to that observed in WT controls. Immunohistochemistry data indicated no regulation by IL-10 of macrophages or neutrophils in the synovium, however IL-10KO mice had augmented T cell marker expression following AIA induction.

Conclusions: These data point toward a hitherto unidentified crosstalk between IL-10 and the inflammasome, which may impact arthritic processes such as leukocyte infiltration and bone pathology.

Disclosure statement: All authors have declared no conflicts of interest.

S2. THE REGULATION OF ARTHRITIC BONE EROSIONS BY IL-10

Claire J. Greenhill1, Anwen S. Williams 1, Gareth W. Jones 1, Ian R. Humphreys1 and Simon A. Jones 1

Background: Interleukin 10 (IL-10) is an immuno-regulatory cytokine that terminates the inflammatory response. In inflammatory arthritis IL-10 is elevated in the serum and synovial fluid of patients with rheumatoid arthritis (RA) and has been implicated in various pro- or anti-inflammatory processes. IL-10 may also be involved in preventing bone degradation by inhibiting cytokines involved in bone resorption, such as IL-1β, which is generated by the cytosol complex called the inflammasome. We hypothesize a potential cross talk between IL-10 and the inflammasome, which may impact how IL-10 exerts its anti-inflammatory activities during inflammatory disease and regulate bone erosion.

Methods: To examine the role of IL-10 during RA, we used the antigen induced arthritis (AIA) model in IL-10KO mice and compared disease severity with wild type (WT) controls by histological staining and x-rays to assess bone erosion. To analyse pro-inflammatory cytokine induction, cartilage and bone destructive markers, and expression of inflammasomes components, quantitative real time PCR (Q-PCR) was undertaken on synovial mRNA and ELISAs on serum. Mice were injected with a florescence matrix metalloproteinases (MMPs) probe to assess the extent of potential cartilage damage and scanned prior to, and following arthritis induction. To characterize IL-1β expression following AIA, the production and localization of this cytokine in the joint as well as infiltrating macrophages, neutrophils and lymphocytes was assessed by immunohistochemistry.

Results: In IL-10KO mice, the inflammatory histopathology associated with arthritis induction in AIA model was significantly enhanced and prolonged as compared to wild type (WT) controls. Interestingly, histological and radiographic analysis of joint sections from these studies suggested that IL-10 is required to prevent excessive bone degradation (e.g. via modulation of bone pathology associated with inflammasome activation) (IL-10KO). A separate evaluation of pro-inflammatory regulators during experimental arthritis in IL-10KO mice showed specific augmentation of inflammasome components (e.g. NALP3, caspase-1, IL-33) and attenuation of its negative regulators (i.e. Caspase12), which was accompanied by synovial increases in IL-1β expression. In contrast, TNFα regulation (e.g., TNFα, ADAM-17) following arthritis induction in IL-10KO mice was comparable to that seen in WT. In this regard, arthritis IL-10 KO mice showed similar levels of matrix metalloproteinase (MMP) activity, as assessed by in vivo whole body imaging and synovial MMP expression (MMP-1, MMP-3, MMP-9, MMP-13), to that observed in WT controls. Immunohistochemistry data indicated no regulation by IL-10 of macrophages or neutrophils in the synovium, however IL-10KO mice had augmented T cell marker expression following AIA induction.

Conclusions: These data point toward a hitherto unidentified crosstalk between IL-10 and the inflammasome, which may impact arthritic processes such as leukocyte infiltration and bone pathology.

Disclosure statement: All authors have declared no conflicts of interest.
S14. DUAL SPECIFICITY PHOSPHATASE 1 IS A CRUCIAL NEGATIVE REGULATOR OF INFLAMMATORY OSTEOLOGY AND MEDiates THERAPEUTIC EFFECTS OF DEXAMETHASONE IN COLLAGEN-INDUCED ARTHRITIS

Youridies Vattakuithi1, Nicole J. Horwood1 and Andy R. Clark2
1Kennedy Institute of Rheumatology, London, United Kingdom

Background: Erosion and aberrant remodelling of bone are common and debilitating features of rheumatoid arthritis (RA), resulting from enhanced activity of osteoclasts (OCL) in the inflamed joint. OCL hyper-activation is believed to be driven, both directly and indirectly, by pro-inflammatory factors such as tumour necrosis factor α (TNF-α). The p38 mitogen-activated protein kinase (MAPK) pathway is a critical driver of inflammatory osteolysis, regulating both, the expression of pro-inflammatory factors such as TNF-α and the differentiation / activation of OCL. Dual specificity phosphatase 1 (DUSP1) dephosphorylates and inactivates p38 MAPK. Expression of DUSP1 is increased by many pro-inflammatory factors as a negative feedback mechanism to limit the strength and duration of the inflammatory response. It is also upregulated by glucocorticoids (GCs), a mechanism that we have previously shown to contribute to anti-inflammatory actions of GCs in isolated macrophages. In the present study we used collagen induced arthrits, an experimental model of RA, to determine whether DUSP1 limits inflammatory osteolysis and mediates anti-inflammatory effects of GCs in vivo.

Methods: Dusp1+/+ and Dusp1−/− mice were immunized with type II chicken collagen to induce chronic arthritis. After onset of disease mice were treated with dexamethasone, injected intra peritoneally for 10 days. Development of disease and bone resorption of the affected paws were assessed by histologic examination, TRAP staining and micro-CT analysis. Furthermore, anti-collagen IgG antibodies were measured in the serum and T cell responses of immunized mice were analysed ex vivo.

Results: Compared to Dusp1+/+ mice, Dusp1−/− mice showed higher incidence, earlier development and more severe disease, characterized by increased numbers of OCLs and bone loss in affected joints. Dexamethasone treatment reduced clinical and histological scores in Dusp1+/+ mice but was less effective in Dusp1−/− mice. Surprisingly, serum anti-collagen IgG2a levels were significantly lower in Dusp1−/− compared to Dusp1+/+ mice and levels of anti-collagen IgG1 showed no difference. However, the production of inflammatory cytokines from T cells of draining lymph nodes was higher in Dusp1−/− mice compared to Dusp1+/+ mice.

Conclusions: These observations show that DUSP1 is an important negative regulator of inflammatory osteolysis and mediates therapeutic effects of GCs in an experimental model of RA. In this model, DUSP1 does not appear to exert its protective effect by limiting the humoral immune response to collagen immunization.

Disclosure statement: All authors have declared no conflicts of interest.

ORAL ABSTRACTS 12: GENETICS

O09. GLOBAL GENE EXPRESSION ANALYSIS OF DEDIFFERENTIATED CHONDROCYTES

Alan J. Mueller1, Elizabeth G. Laird1, Simon R. Tew1 and Peter D. Clegg2
1Musculoskeletal Biology, Institute of Ageing and Chronic Disease, University of Liverpool, Neston, United Kingdom

Background: Chondrocytes in two-dimensional culture are well known to lose their functional phenotype rapidly; progressive subculture alters the synthetic profile with loss of collagen type II and aggrecan hallmarks of this process. This is also termed ‘dedifferentiation’. The study hypothesized that the perturbations in functional phenotype following multiple passage is a consequence of differential expression of genes associated with an earlier stage of cartilage development.

Methods: Tissue was obtained from 12 week old, male, F344 rats (n = 10). This comprised of cartilage from the coxo-femoral and femoro-tibial joints (8 samples). Tissue was digested and cultured in standard growth medium. Monolayer cultures were expanded to 90% confluence and then sub-cultured at a 1:2 ratio for five occasions. In addition, native cartilage was harvested against which to compare dedifferentiated tissue (n = 2). RNA was extracted using TriReagent (Ambion/Invitrogen Biosciences) and this was amplified and bioin- labelled CRNA suitable for hybridization to the Illumina RatRef-12 v1.0 BeadChip® array (Illumina, Inc., USA) was produced. Raw bead-level intensity data was manipulated using the R programming platform (R 2.12.0, R Core Development Team, 2010) and the ‘Beadarray’ open-source package. Results are presented as: log fold change, false discovery rate, and log odds ratio of differential expression.

Results: Cluster analysis: Native cartilage (nC) was shown to cluster distinct to dedifferentiated chondrocytes (dC). Heatmap representation suggested homogeneity of gene expression intensity in comparisons with other mesenchyme-derived cell lines. Homeobox gene expression: In pairwise comparisons between dC and nC there was evidence of differential expression of the paired-type homeobox gene PITX1 (2.9, 9.2E-06, 5.8). Moreover, in pairwise comparison between dC and nC, up-regulation of the homeobox genes PPRX2 (2.6, 1.1E-06, 8.3), FOXP1 (2, 6.2E-07, 9), and HOXC10 (2.1, 9.5E-05, 3) were evident. In contrast, the articular homebox gene HOPX was down-regulated in dC relative to nC (2.6, 1.3E-11, 21).

Conclusions: Homeobox genes encode transcription factors that have roles in embryonic positional identity, ensure correct cell differentiation and faithful expression to ensure homeostasis of adult tissues. PITX1 has been shown to be upregulated and suggested to be uniquely up-regulated in dC compared to nC, and is expressed in a hind-limb restricted manner in development. Targeted inactivation results in a failure of normal hind-limb development with an absence of cartilage. Global gene expression analysis of dedifferentiated chondrocytes relative to native cartilage and other mesenchyme-derived tissue would suggest that loss of functional phenotype in monolayer culture encourages the up-regulation of genes associated with dedifferentiated chondrocytes during development. Further investigation to verify these findings is on-going.

Disclosure statement: All authors have declared no conflicts of interest.

O50. LARGEST UK RHEUMATOID ARTHRITIS GENOME WIDE ASSOCIATION STUDY TO DATE OF 8,300 SAMPLES STRENGTHENS CONFIRMED LOCI AND HIGHLIGHTS MORE POTENTIAL RA GENETIC RISK FACTORS

Gisela Orozco1, Steve Eyre1, John Bowes1, Edward Flynn1, Anne Barton1 and Jane Worthington1
1Arthritis Research UK Epidemiology Unit, University of Manchester, Manchester, United Kingdom

Background: The number of unequivocally confirmed rheumatoid arthritis (RA) loci currently stands at 31, but many lines of evidence indicate this is unlikely to be the final number, and that additional, well powered genome wide association studies (GWAS) will still be required to develop a full picture of the genes involved in RA. The objective of this study was to extend our previous Wellcome Trust Case Consortium RA GWAS adding more independent cases and control samples, with the aim to increase power to confirm previously identified loci and to detect novel association signals for the susceptibility to RA.

Methods: We had available 3223 UK RA cases and 5272 UK controls, which adds 1361 cases and 2334 controls to the original GWAS. All samples were genotyped using Affymetrix or Illumina arrays. The genotype data for all samples was imputed using IMPUTE2, with the 1000 Genomes Project and HapMap3 data as reference panels, to increase and unify the genomic coverage between samples. After a stringent QC was applied, we had 3034 cases and 5271 controls and 1831729 SNPs. Association analysis was performed using PLINK, which adds 1361 cases and 2334 controls to the original GWAS.

Results: We confirmed association to 22 of the 31 previously known RA susceptibility loci. For 15 of them, increased evidence of association was found when compared to the WTCCC GWAS. We also found association evidence for 2 loci that were at a suggestive significance level from the latest meta-analysis, CD247 and UBE2L3. We did not find association with 5 of the known RA loci. However, these were not associated with RA in the original WTCCC GWAS either, so it is possible that they have a modest effect in RA predisposition in UK population. We could not find proxies for 4 of the known RA loci. 12 novel loci were associated with disease at genome-wide significance level. In addition, we found 10 novel RA loci in the next tier of significance (P < 10−7) that have been associated with other autoimmune diseases or have shown suggestive evidence of association with RA in previous meta-analysis of GWAS data. These 22 novel loci were selected for follow up. After Bonferroni correction for multiple testing, we replicated the association of the 22 loci with RA.

Conclusions: We present results on the largest UK RA GWAS performed to date. We have identified a new RA risk loci mapping to 22q12, which has shown association to multiple other autoimmune diseases.
OS1. FINE MAPPING IN OVER 14,000 RHEUMATOID ARTHRITIS CASES AND 18,500 CONTROLS REVISES ASSOCIATIONS TO KNOWLOCII, INDICATES MULTIPLE INDEPENDENT EFFECTS AND REVEALS NOVEL ASSOCIATIONS

Steve Eye1, John Bowes1, Anne Barton1, Chris Amos2, Dorothee Digo2, Annette Lee2, Leonid Padyukov5, Eli A. Stahl1, Javier Martin1, Solbrit Rantapaa-Dahiqvist1, Soumya Raychaudhuri3, Robert Plenge4, Lars Klareskog2, Peter Gregersen1 and Jane Worthington5
1Musculoskeletal Research Group, The University of Manchester, Manchester, United Kingdom; 2Epidemiology, University of Texas, Houston, Texas, United States of America; 3Division of Genetics, Harvard Medical School, Boston, Massachusetts, United States of America; 4Centre for Genomics and Human Genetics, Feinstein Institute for Medical Research, New York, New York, United States of America; 5Instituto de Paratolisi, CSIC Granada, Granada, Spain; 6Rheumatology, Umea University, Umea, Sweden; 7Rheumatology, Karolinska University Hospital Solna, Stockholm, Sweden

Background: Genome-wide association studies (GWAS) have been tremendously successful in identifying loci associated with a range of traits and disorders. Indeed, there are over 240 confirmed susceptibility loci reported for nine autoimmune diseases alone, many of which are shared between the diseases. However, GWAS are unlikely to identify all causal SNPs, as they contain only a relatively small proportion of the total genetic variation. It will therefore be necessary to fine map these regions in order to help pinpoint the likely causal SNPs.

Aim: To identify novel RA susceptibility loci and fine map known and novel loci in a large cohort of rheumatoid arthritis (RA) cases and controls using the custom Illumina Immunochip.

Methods: DNA sample from Caucasian RA cases (n = 14,056) and controls (n = 18,583) were assembled from a number of previously described studies from the UK, US, Sweden and Spain. The Immunochip was designed by a consortium of researchers investigating 14 autoimmune diseases and represents all known genetic variation of dbSNP, 1Kx and sequencing projects for approximately 200 validated loci. The genotyping for the RA cases was performed in multiple centres and therefore all raw genotyping data was collated centrally for combined clustering and analysis. The data was first re-clustered and after applying strict QC metrics (99% SNP and 99% sample) the samples were subjected to further pruning for relatedness and ancestral outliers.

Results: Comparison of all case against all controls for 125,688 SNPs (M = 0.999) revealed association to markers at 13 loci at genome wide significance (p < 5 x 10^-8) five of these are novel RA susceptibility loci (rs345386443 YKTPY, rs17374797 IRAK1, rs10026988 TLE3, rs2240339 PADI4 and rs8192284 IL8R). Strong evidence of association was detected to all previously confirmed loci (p < 10^-50). When comparing QQ plots for novel loci (n = 7,222) with controls for 4 loci (2 novel) were identified at genome wide significance: rs8043085 RASGRP1, rs13303176 IRAK1, rs8464647 CCR6 and rs23825527 MMEL. Conditioning on the top negative patients with controls markers for ANKRDS5 and the MHC region reached genome wide significance. The signal over MHC was different in CCP negative compared to positive and did not peak over HLA DRB1. For the majority of loci the association signal now lies within a gene sequence.

Conclusions: Preliminary analysis of the Immunochip data for RA has revealed 7 novel RA susceptibility loci. Five additional potential loci (p < 1 x 10^-5, -p < 5 x 10^-8) will require validation in independent cohorts. Some of the identified loci are specific to either CCP positive or CCP negative disease, whilst other are associated with both subgroups. Fine mapping analyses are underway.

Disclosure statement: All authors have declared no conflicts of interest.

OS2. INVESTIGATION OF IDIOPATHIC INFLAMMATORY MYOPATHY FOR SHARED GENETIC RISK FACTORS WITH OTHER AUTOIMMUNE DISORDERS

Meghna Jan1, Hector Chinoy1,2, Janine Lamb2, Platt Hazelf, Lucy Wedderburn3, Jiri Vencovsky5, Katalin Danko3, Ingrid Lundberg2, Albert Selva O’Callaghan2, Timothy Radstake3, William C. Oliver4 and Robert G. Cooper1
1Rheumatic Diseases Centre, Salford Royal NHS Foundation Trust, Salford, United Kingdom; 2Centre for Integrated Genomic Medical Research, University of Manchester, Manchester, United Kingdom; 3Rheumatology Unit, Institute of Child Health, University College London, London, United Kingdom; 4Rheumatology, Institute of Rheumatology, Prague, Czech Republic; 5Division of Clinical Immunology, Medical and Health Science Center, Uniformed Services University of Debrecen, Debrecen, Sweden; 6Rheumatology Unit, Karolinska Institutet, Stockholm, Sweden; 7Internal Medicine Department, Vall d’Hebron General Hospital, Barcelona, Spain; 8Department of Rheumatology, Redbord University, Nijmegen Medical Center, Nijmegen, Netherlands

Background: The idiopathic inflammatory myopathies (IIM) are autoimmune disorders characterized by acquired proximal muscle weakness, inflammatory cell infiltrates in muscle biopsies and myositis-specific or associated autoantibodies. Myositis may present as a primary disorder, or may overlap with other connective tissue diseases such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) or systemic sclerosis. The aetiology of IIM is largely unknown, but is thought to include a combination of both genetic and environmental factors. Numerous recent genome-wide association studies (GWAS) have identified many genetic variants associated with autoimmune disorders, several of which are common to multiple diseases. We tested the hypothesis that genetic risk factors associated with other autoimmune disorders also predispose to IIM.

Methods: Single Nucleotide Polymorphisms (SNPs) significantly associated with SLE, RA, juvenile idiopathic arthritis, coeliac disease, Crohn’s disease, ulcerative colitis, psoriasis, type 1 diabetes, multiple sclerosis or systemic sclerosis were identified from published Caucasian GWAS and from the national human genome research institute (NHGRI) catalogue of published GWAS. 234 unique SNPs were identified (p < 5 x 10^-8), of which 99 had not been directly genotyped or captured through our MYOGEN GWAS. The SNPs were genotyped on the Sequenom platform in a sample of 1043 European Caucasian individuals with definite or probable adult or juvenile dermatomyositis or polymyositis. Genotype data was obtained from controls of European ancestry and imputation carried out for non-genotyped SNPs using HapMap Phase 3 and One Thousand Genomes data. Preliminary analysis was carried out using logistic regression in PLINK incorporating multi-dimensional scaling factors derived from the GWAS data as covariates to correct for population substructure.

Results: 1041 individuals and 83 SNPs were successfully genotyped and passed quality control filtering criteria. Two SNPs within the HLA region were significantly associated with IIM (rs2040406 near HLA-DOA, p = 6.1 x 10^-10, rs15672 near HLA-DRB1, p = 1.5 x 10^-5). Although no SNPs outside the HLA region achieved Bonferroni corrected significance levels for the number of SNPs tested, four SNPs previously associated with RA and SLE reached nominal significance (RA: rs13385731 in TNFAIP3, p = 0.001; SLE: rs13385731 in RASGRF3, p = 0.001, rs5029939 in TAF1P3, p = 0.004; rs230926 in TAF1P3, p = 0.005).

Conclusions: Association of HLA SNPs confirms the autoimmune nature of IIM. The nominal association of four SNPs previously associated with SLE and RA suggests that IIM may share genetic risk factors with other autoimmune disorders. These results require confirmation in an independent replication sample.

Disclosure statement: All authors have declared no conflicts of interest.

OS3. ANALYSIS OF THE IMMUNOCHIP IN A LARGE COHORT OF OLIGO- AND POLYARTHRITIS JUVENILE IDIOPATHIC ARTHRITIS CASES CONFIRMS PREVIOUS AND IDENTIFIES NOVEL ASSOCIATIONS

Joanna Cobb1, Anne Hinks1, John Bowes1, Kathryn Steel1, Marc Sudman1, Miranda C. Marion1, Mehdi Keddache1, Lucy R. Wedderburn1, Johannes P. Haas2, David N. Glass3, Carl D. Langefeld4, Wendy Thomson4 and Susan D. Thompson5
1Arthritis Research UK Epidemiology Unit, University of Manchester, Manchester, United Kingdom; 2Cincinnati Children’s Hospital Medical Center, Cincinnati Children’s Hospital, Cincinnati, Ohio, United States of America; 3Department of Biostatistical Sciences, Wake Forest University School of Medicine, Winston-Salem, North Carolina, United States of America; 4Division of Human Genetics, Cincinnati Children’s Hospital Medical Center, Cincinnati, Ohio, United States of America; 5Institute of Child Health, University College London, London, United Kingdom; 6German Centre for Rheumatology in Children and Young People, Garmisch-Partenkirchen, Germany

Background: Genome wide association studies (GWAS) have been highly successful in the identification of susceptibility loci for autoimmune diseases. One interesting outcome of these studies is...
the observation that many of the loci are shared across these diseases. Regions identified now require more detailed fine-mapping to localize the association signal, identify potential pleiotropic effects and identify putative functional variants. To this end the Immunochip consortium was established to pool confirmed loci from 12 diseases to include on a custom genotyping chip. The Immunochip, based on the Illumina Infinium platform, investigates ~200 established autoimmune susceptibility loci. For each locus, all known genetic variation from dbSNP, 1000 Genome and other sequencing projects was included. Juvenile idiopathic arthritis (JIA) is the most common arthritic disease of childhood. Candidate gene and GWAS have identified a number of common autoimmune genes that confer susceptibility to JIA. However, JIA has been less well studied using GWAS approaches and thus genotyping using the Immunochip has the potential for not only fine-mapping of previously associated regions but also to identify novel loci for JIA.

Methods: Genotyping was performed using the Immunochip, in a large cohort from the UK, US and Germany comprising 1749 JIA oligoarthritis and RF negative polyarthritis cases and 8854 controls. SNPs failed QC based on a call rate < 0.4, Samples failed QC based on a call rate < 0.5. Outliers of mean heterozygosity, related individuals and ancestral outliers were removed. Final sample size after QC was 1609 cases and 7153 controls. Analysis was performed using logistic regression adjusting for the top 5 principal components in PLINK vers1.07.

Results: Initial analysis has not only confirmed previously associated JIA loci (HLA, PTPN22, IL2, STAT4, PTPN2 and SH2B3/ATXN2) but has strengthened their association, such that all now reach genome-wide significance. A number of novel loci have been identified, some of which showed some evidence previously, such as IL2RA, IL7R and IRF1, and others which have never been associated with JIA to date, such as RUNX1, FAS and ANKRD55. These will require validation in independent cohorts.

Conclusions: The Immunochip project enables cost-effective fine-mapping of autoimmune loci in diseases such as JIA. This preliminary analysis has confirmed and strengthened the association of a number of previously associated genes, as well as identified novel susceptibility loci for JIA. Further analysis of this data will help characterize all associated variants and identify the likely causal variants for future functional studies.

Acknowledgements: Childhood Arthritis Prospective Study (CAPS), Childhood Arthritis Response to Medication Study (CHARMS), and BSR/ARCO study group.

Disclosure statement: All authors have declared no conflicts of interest.

OS4. GENOME-WIDE ASSOCIATION STUDY OF METHOTREXATE RESPONSE IDENTIFIES NOVEL GENES IN A LARGE COHORT OF EUROPEAN JUVENILE IDIOPATHIC ARTHRITIS CASES

Joanna Cobb1, Anne Hinks1, Edward Flynn1, Shashi Hirani2, Fiona Patrick1, Laura Kassem1, Simona Ursu1, Halima Moncrieffe1, Maja Bulatovic1, Marek Bohm5, Bertrand van Zeit1, Pavla Dolezalova1, Robert de Jonge6, Nico Wulffraat4, Stan Newman1, Wendy Thomson1 and Lucy Wedderburn3

1Arthritis Research UK Epidemiology Unit, University of Manchester, Manchester, United Kingdom; 2School of Community & Health Sciences, City University London, London, United Kingdom; 3Institute of Child Health, University College London, London, United Kingdom; 4Department of Paediatric Immunology, Wilhelmina Children’s Hospital, University Medical Centre Utrecht, Utrecht, Netherlands; 5First Faculty of Medicine, General Faculty Hospital, Prague, Czech Republic; 6Department of Rheumatology, Erasmus University Medical Centre, Rotterdam, Netherlands

Background: The drug methotrexate (MTX) is the first line treatment for children with Juvenile Idiopathic Arthritis (JIA). Approximately 45% of children treated with MTX for arthritis achieve 70% improvement as defined using internationally agreed JIA core set criteria, however, a proportion of children will not respond to MTX treatment. Currently there are no reliable predictors to identify children likely to fail to respond. In order to identify these children early, and thus target their treatment during the apparent short window of opportunity in which disease can more readily be brought into remission, the Childhood Arthritis Response to Medication Study (CHARMS) was established. Growing evidence suggests that multiple genes contribute to the genetic component of treatment response in arthritis. With this in mind, and using the CHARMS cohort, this study aimed to perform a genome wide association study (GWAS) to identify genomic loci associated with response to MTX in JIA.

Methods: Genotyping of the Illumina OmniExpress Beadchip was performed in a large cohort of 795 JIA cases from the UK, Netherlands and Czech Republic. Single nucleotide polymorphisms (SNPs) failed QC based on a call rate < 0.5 and/or cluster separation score < 0.4, leaving 705,684 SNPs available for analysis. Samples failed QC based on a call rate < 0.5, in addition outliers of mean heterozygosity, related individuals and ancestral outliers (identified using principal components analysis) were removed. The final sample size after QC was 730 cases. MTX response was defined using the internationally developed ACR Pedi categories (non-responder, ACR Pedi 30, ACR Pedi 50 and ACR Pedi 70). Analysis was performed using logistic regression adjusting for the top 10 principal components in PLINK vers1.07.

Results: In a preliminary analysis, 117 non-responders were compared to 232 responders meeting the ACR Pedi 70 criteria at 587,822 SNPs (all MAF > 5%) across the entire genome. Approximately 42 regions showed association with response at a significance of P < 0.0001, with the most highly associated SNPs found near the genes SLC2A13 (P = 3.79 x 10^-6), ZX121 (P = 1.12 x 10^-5) and CAMK2B (P = 1.36 x 10^-5). These findings will require validation in independent cohorts. Further analysis is underway using the entire cohort, giving us greater power and the ability to perform conditional logistic regression and haplotype analysis in order to determine the number of independent effects in each associated region.

Conclusions: These preliminary results suggest a role for novel pathways in MTX response. Further investigations utilizing all available samples will afford greater power to dissect the genetic basis of MTX response, thus moving us towards our ultimate goal of prediction of response to MTX for children with JIA.

Acknowledgements: Childhood Arthritis Prospective Study (CAPS) and Childhood Arthritis Response to Medication Study (CHARMS).

Disclosure statement: All authors have declared no conflicts of interest.