S1. IDENTIFICATION OF NOVEL OSTEOARTHRITIS GENES USING ZEBRAFISH

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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 chondroitin 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 changes in the joint in real time at a level impossible in other model organisms. In conclusion zebrafish can be 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?

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Background: Osteoarthritis is a frequent articular disease, especially in elderly people, and causes pain and disability. Major hallmarks of OA are cartilage degradation and chondrocyte death. Of note, there is no treatment for OA. Based on previous data on collagen-induced arthritis in mice, our purpose was to study the effect of adrenomedullin (AM) and an AM-derived peptide (22-52AM) on chondrocytes in vitro and in OA model in vivo. We investigated AM and AM receptor [Calcitonin Receptor Like Receptor (CLR)/Receptor Activating Modulated Protein (RAMP)] expression by articular chondrocytes under physiological (hypoxia) and OA (IL-1) environment. We also brought evidence highlighting the effects of AM and 22-52 AM on cartilage breakdown and chondrocyte apoptosis in vitro and in vivo.

Methods: In vitro studies: Chondrocytes were isolated from bovine articular cartilage. Cells were cultured (20%o2 or 3%o2) until they reached confluency then, detached and plated for subsequent experiments. MTT test, DAPI staining and Caspase 3 activity were performed on cells challenged with Fas-Ligand (Fas-L) and AM and/or 22-52AM to assess cell sensitivity to apoptosis. Influence of oxygen and IL-1 was tested on AM and AM receptor transcript expression variations (RT-qPCR), HIF-1α transcriptional modulation to the nucleus has been evidenced by immunofluorescence.

In vivo studies: C57/bi6 mice were used in the joint instability OA model after medial meniscectomy (Kadi et al 2008). Then, mice were injected 3 times a week with saline, AM or 22-52AM for 8 weeks. OA scoring on knee joints was performed to evaluate treatment effect on OA progression (Pritzker et al 2006).

Results: Our in vitro data clearly demonstrated that hypoxia induced a nuclear localization of HIF-1α in bovine chondrocytes, along with an increased AM/AM receptor expression and a decreased Fas receptor expression. When cells were exposed to IL-1, AM production (mRNA, secreted protein) and RAMP-3 mRNA expression were increased, suggesting an AM autocrine/paracrine survival effect. This suggests a potential anti-apoptotic gene expression pattern in chondrocytes which can improve cell survival. This is confirmed by AM antiapoptotic effect on Fas-induced apoptosis/cell death. 22-52AM which acts as a receptor antagonist had opposite effect. Interestingly, in vivo, 22-52AM significantly increased cartilage destruction in the joint instability OA model compared to saline (Pritzker’s score: 5.07 ± 2.37 versus 2.96 ± 1.27, respectively; p < 0.05).

Conclusions: This study highlighted the role of hypoxia on AM/AM receptor expression in articular chondrocytes. Moreover, we have demonstrated that AM protected chondrocytes from apoptosis and could act as a therapeutic agent in vivo whereas, despite its interesting properties highlighted in RA model, 22-52AM seemed to have a deleterious effect in OA model.

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

S3. INVESTIGATION OF THE ROLE OF UFM1 SPECIFIC PEPTIDASE 2 IN BEUKES HIP DYSPLASIA

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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 protease 2 (USP22). The mutation prevents USP22 from cleaving its target Ufm1. USP22 and Ufm1 are both components of a novel ubiquitin-like system of unknown function and where only one putative modification target 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 USP22 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 Uba5 and Ufc1, the E1 and E2 enzymes of Ufm1 pathway. RNA in situ hybridization experiments on mouse tissue showed expression of USP22 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 USP22 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
osteo genesis, 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

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Background: The orphan receptor, GPR55 is expressed in dorsal root ganglia neurones 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 endogenic 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, 100mg/kg 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) had significant and dose-related inhibitory effects on mechanically evoked responses of knee joint afferents (P < 0.0001, 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 knockout 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>
<thead>
<tr>
<th>Table 1.</th>
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<tr>
<td>Follow-up (pyrs)</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Age: Mean (SD)</td>
</tr>
<tr>
<td>Gender: % female</td>
</tr>
<tr>
<td>Solid cancer: N</td>
</tr>
<tr>
<td>Solid cancer: Rate per 10000 pyrs (95% CI)</td>
</tr>
<tr>
<td>Solid cancer: adjusted HR (95% CI)</td>
</tr>
<tr>
<td>Solid cancer: IPTW adjusted HR (95% CI)</td>
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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 Watson1, Mark Lunt1, William Dixon2, BSRR Control Centre Consortium3, Deborah Symmons4 and Kimme Hyrcyn,4 on behalf of the BSRRB5
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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 BSRRB, a national cohort study. Patients with RA starting anti-TNF (etanercept (ETA), infliximab (INF) or adalimumab (ADA)) and a biologic-naïve 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 weight (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 vs 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.
O38. SYSTEMIC RHEUMATOID VASCULITIS IN THE BIOLOGIC ERA

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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, potentially 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 a stable 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 diagnosis was established by retrospective review of medical notes and confirmed by an independent physician. Incidence figures were estimated using observed case numbers and 95% confidence intervals were calculated using the Poisson distribution. Survival was calculated using the Kaplan Meier method with log rank test for cohort comparison. Clinical features were compared with the 1988-2000 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 (Table 1).

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>1986-2000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>18 (95% CI) 47 (85% CI)</td>
<td>34</td>
</tr>
<tr>
<td>Average annual incidence (/million)</td>
<td>3.9 (2.3-6.2) 9.1 (6.8-12.0)</td>
<td>18.3</td>
</tr>
<tr>
<td>Male</td>
<td>4.5 (2.2-6.3) 8.9 (5.7-13.3)</td>
<td>20.0</td>
</tr>
<tr>
<td>Female</td>
<td>3.4 (1.4-6.9) 8.7 (5.6-12.9)</td>
<td>12.2</td>
</tr>
<tr>
<td>1 year mortality, %</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>5 year mortality, %</td>
<td>60</td>
<td>51</td>
</tr>
<tr>
<td>CLINICAL FEATURES</td>
<td>n (%)</td>
<td>(n (%)</td>
</tr>
<tr>
<td>Rheumatoid Factor</td>
<td>18</td>
<td>100</td>
</tr>
<tr>
<td>Erosions Nodules</td>
<td>13</td>
<td>83</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>3/9</td>
<td>38</td>
</tr>
</tbody>
</table>

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


Jennifer Humphreys1, Suzanne M. Verstappen2, Tarnya Marshall3, Mark Lunt4, Kimme Hyrich1, and Deborah P. Symmons1
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Background: The development of new classification criteria for rheumatoid arthritis (RA) allowed for a re-estimation of RA incidence rates. The new criteria purported 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 had 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 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 using the 1987 criteria, which, despite being lower for women, were similar for men (20/100 000 for women, 19/100 000 for men). Age and sex-specific incidence rates using both classification criteria were baseline similar to cumulative incidence rates using the 1987 criteria at 5 years. The incidence rates using both criteria sets converged when applied cumulatively. However, 73% patients satisfied the 1987 criteria cumulatively after 5 years but never satisfied the new criteria, and 27%(0%) 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 to 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.

Table 1

<table>
<thead>
<tr>
<th>Age band</th>
<th>No. of patients with EIA</th>
<th>Female</th>
<th>1987 criteria cumulative 5 years IR (95%CI)</th>
<th>Male</th>
<th>1987 criteria cumulative 5 years IR (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15-24</td>
<td>17</td>
<td>18.6 (6.4, 40.6)</td>
<td>15.5 (9.0, 36.3)</td>
<td>0 (0, 11.1)</td>
<td>3.0 (0.1, 16.7)</td>
</tr>
<tr>
<td>25-34</td>
<td>23</td>
<td>20.3 (8.2, 41.8)</td>
<td>29.0 (13.9, 53.5)</td>
<td>5.6 (0.7, 20.3)</td>
<td>5.6 (0.7, 20.3)</td>
</tr>
<tr>
<td>35-44</td>
<td>34</td>
<td>56.6 (34.1, 88.4)</td>
<td>50.6 (25.9, 81.0)</td>
<td>12.1 (3.3, 30.9)</td>
<td>12.1 (3.3, 30.9)</td>
</tr>
<tr>
<td>45-54</td>
<td>53</td>
<td>86.6 (56.4, 124.4)</td>
<td>91.9 (61.6, 130.0)</td>
<td>34.5 (17.2, 61.7)</td>
<td>31.3 (15.0, 57.7)</td>
</tr>
<tr>
<td>55-64</td>
<td>58</td>
<td>91.8 (59.4, 135.5)</td>
<td>88.1 (56.4, 131.0)</td>
<td>42.1 (21.0, 75.3)</td>
<td>42.1 (21.0, 75.3)</td>
</tr>
<tr>
<td>65-74</td>
<td>74</td>
<td>90.1 (55.8, 129.6)</td>
<td>94.4 (61.7, 137.0)</td>
<td>58.3 (31.9, 91.0)</td>
<td>57.9 (31.9, 91.0)</td>
</tr>
<tr>
<td>75+</td>
<td>23</td>
<td>26.1 (10.5, 53.7)</td>
<td>29.8 (12.9, 58.7)</td>
<td>44.3 (17.9, 91.3)</td>
<td>57.9 (26.1, 108.1)</td>
</tr>
<tr>
<td>Total</td>
<td>261</td>
<td>53.9 (44.5, 64.7)</td>
<td>55.7 (46.2, 66.7)</td>
<td>24.5 (18.1, 32.4)</td>
<td>26.5 (19.3, 34.7)</td>
</tr>
</tbody>
</table>
RF positivity 61/53%). Our primary outcome was NICE response criteria (DAS28 change ≥1.2); we also assessed mean changes in DAS28. We categorized smoking status as current, previously smoking 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 compared with ≤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 > 86% 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 change in 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.

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

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Background: Systemic sclerosis (Scleroderma, SSc) is a heterogeneous multisystem 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 ascertainment 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 (Chi-square-test or Fisher’s exact t-test) and time-to-event data were used. Specifically, differences between SCC 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 auto-antibodies (ACA), 18.2% were positive for anti-Scl70 antibodies and 26.6% showed anti-RNA polymerase antibodies (RNAP). 42.2% of patients with cancer had breast cancer followed by haematological (12.3%), gastrointestinal (11.0%) and gynaecological cancers (11.0%). The frequency of malignancies among patients with RNAP (14.2%) was significantly increased compared to those with anti-Scl70 (6.3%) and ACA (6.8%) (p < 0.0001 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-Scl70 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.

Table 1. NICE DAS-28 responders by smoking and rheumatoid factor status

<table>
<thead>
<tr>
<th>Biologic</th>
<th>Rheumatoid factor</th>
<th>Current smoker</th>
<th>Previous smoker</th>
<th>Never smoked</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF inhibitors</td>
<td>All Negative Positive</td>
<td>27% 33% 24%</td>
<td>63% 57% 65%</td>
<td>90% 88% 92%</td>
<td>68% 71% 67%</td>
</tr>
<tr>
<td>Rituximab</td>
<td>All Negative Positive</td>
<td>20% 9% 45%</td>
<td>68% 56% 74%</td>
<td>98% 94% 100%</td>
<td>59% 35% 79%</td>
</tr>
</tbody>
</table>

FR positivity 61/53%). Our primary outcome was NICE response criteria (DAS28 change ≥1.2); we also assessed mean changes in DAS28. We categorized smoking status as current, previously smoking 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 compared with ≤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 > 86% 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 change in 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.

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

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Background: Systemic sclerosis (Scleroderma, SSc) is a heterogeneous multisystem 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 ascertainment 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 (Chi-square-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 auto-antibodies (ACA), 18.2% were positive for anti-Scl70 antibodies and 26.6% showed anti-RNA polymerase antibodies (RNAP). 42.2% of patients with cancer had breast cancer followed by haematological (12.3%), gastrointestinal (11.0%) and gynaecological cancers (11.0%). The frequency of malignancies among patients with RNAP (14.2%) was significantly increased compared to those with anti-Scl70 (6.3%) and ACA (6.8%) (p < 0.0001 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-Scl70 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.

Table 1. NICE DAS-28 responders by smoking and rheumatoid factor status

<table>
<thead>
<tr>
<th>Biologic</th>
<th>Rheumatoid factor</th>
<th>Current smoker</th>
<th>Previous smoker</th>
<th>Never smoked</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF inhibitors</td>
<td>All Negative Positive</td>
<td>27% 33% 24%</td>
<td>63% 57% 65%</td>
<td>90% 88% 92%</td>
<td>68% 71% 67%</td>
</tr>
<tr>
<td>Rituximab</td>
<td>All Negative Positive</td>
<td>20% 9% 45%</td>
<td>68% 56% 74%</td>
<td>98% 94% 100%</td>
<td>59% 35% 79%</td>
</tr>
</tbody>
</table>
S6. TLR9 INDUCES TOLERANCE TO APOPTOTIC CELLS AND IS RESPONSIBLE FOR INDUCING REGULATORY B CELLS

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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 CO27+ 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-AND B-CELL RESPONSES

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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-17; Th17) 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.

Methods: Wild type (WT) and IL-27RKO mice were treated with IL-6 or IL-27 to examine the role of IL-27 in the promotion of IL-17 responses and compared against WT controls. The role of gp130 signalling was examined using gp130-/- mice.

Results: The addition of IL-27 to IL-6RKO mice was sufficient to promote an increased Th17 cell response over control mice. Incubation of WT mice with IL-27 increased the number of IL-17 producing cells when compared against IL-6 treated WT controls. However, WT mice incubated with IL-6 did not show an increased IL-17 response over WT controls. Similarly, incubation of gp130-/- mice with either IL-6 or IL-27 did not promote an increased IL-17 response over WT mice. Incubation of IL-6RKO mice with IL-27 promoted higher IL-17 responses over control IL-6RKO mice. IL-27 was only effective in the promotion of the IL-6RKO Th17 cell response when present in the presence of IL-6.

Conclusions: The role of IL-27 in the arthritis response is dependent on the presence of IL-6 and the IL-27 receptor, gp130. IL-27 promotes the IL-6RKO Th17 cell response over IL-6RKO controls. This suggests that IL-27 is not sufficient to promote Th17 cell responses in IL-6RKO mice. It also suggests that the IL-27 type I receptor, gp130, is required for the activation of the IL-27 driven Th17 cell response.

Disclosure statement: All authors have declared no conflicts of interest.
Methods: Experimental inflammatory arthritis was induced in wild type (WT) mice and IL-27RKO mice. Disease severity was assessed through measurement of joint swelling and histological analysis of joint sections. Flow cytometry, immunohistochemistry and immunological assays were used to monitor the peripheral immune response and the cellular response within the synovium. To support in vivo studies, in vitro approaches investigated T helper cell responses to IL-27.

Results: IL-27RKO mice developed exacerbated inflammatory arthritis, displaying increased synovial infiltrates and bone erosions compared to WT mice. IL-27RKO mice also displayed increased peripheral Th17 cell responses and higher serum IL-17 levels. Surprisingly, these mice also had heightened B-cell responses associated with an increase in antigen-specific serum IgG levels. Immunohistochemical analysis of synovial infiltrates revealed increased activation of STAT3 was associated with disease exacerbation, further confirming a damaging role for local STAT3 activation in arthritis progression.

Conclusions: We have demonstrated a protective role for IL-27 in inflammatory arthritis through regulation of T- and B- cell responses and modulation of local STAT1/3 activation. Excessive activation of STAT3 within the joint contributes to joint pathology and modulating the STAT1/3 axis may provide a potential therapeutic approach. In this regard, targeting of the IL-27 signalling pathway may be of future benefit for clinical intervention strategies to treat RA.

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

S6. ANTI-TNF ANTIBODY THERAPY, BUT NOT TNF RECEPTOR BLOCKADE, INDUCES IL-17 SUPPRESSING REGULATORY T CELLS
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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 highly 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 Th1 responses via IL-10 and modulation of local STAT1/3 activation. Excessive activation of STAT3 within the joint contributes to joint pathology and modulating the STAT1/3 axis may provide a potential therapeutic approach. In this regard, targeting of the IL-27 signalling pathway may be of future benefit for clinical intervention strategies to treat RA.

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

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
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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, 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 - also available in transcript format. Downloads of a general Workwise fact sheet and FAQs are also available. There are downloadable PDFs of two booklets entitled ‘I want to work’ A self help guide for people with RA and ‘When an employee has RA’ An Employer’s Guide, providing information for employers about how to help their employee with RA. We also implemented a brief survey to gauge how useful these tools are.

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 Work Wise 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 return or stay in 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 are supported by a grant from Abbott.

O44. DESIGNING VIRTUAL PATIENTS FOR MUSCULOSKELETAL EDUCATION: A GROUNDED THEORY QUALITATIVE STUDY
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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
O45. HAVE YOU BEEN TO CAPRI? www.capri.scot.nhs.uk: EARLY RESULTS OF A CLINIC FOR ARTHRITIS PATIENTS IN REMISSION ON THE INTERNET

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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 28 (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 scores patients 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 detailed of which 52 were less than 3.3 for 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 centre for RA patients, demonstrating 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 hard copy and MSF36 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.
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.
S9. INHIBITION OF NAMPT (PBEF/VISFATIN) DECREASES THE ABILITY OF HUMAN NEUTROPHILS TO GENERATE REACTIVE OXIDANTS, BUT DOES NOT IMPAIR BACTERIAL KILLING

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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. Namp (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. Namp also functions intracellularly in the regulation of NAD metabolism, and Nampt inhibitors have been shown to have anti-inflammatory potential in 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 (APO866) (1-100 nM) in vitro. A variety of neutrophil functional assays were then performed including, receptor expression, chemotaxis, phagocytosis, degranulation, ROS production, bacterial killing and apoptosis.

Results: Exogenously added Nampt (100 ng/mL) delayed both constitutive neutrophil apoptosis, and the turnover of the anti-apoptotic protein McI-1. However, it did not have any significant effect on other functions tested, such as chemotaxis, receptor expression or release of intracellular granule enzymes. However, inhibition of the enzymatic activity of Nampt (using the inhibitor FK866) decreased the activity of the respiratory burst in a time and dose-dependent manner, but did not impair neutrophil phagocytosis or bacterial killing.

Conclusions: These data confirm the importance of Nampt in regulating neutrophil apoptosis, but reveal a new function for Nampt in controlling functions that would contribute to the inflammatory process. Decreased ROS was not accompanied by impairment of bacterial killing, presumably because the residual intra-phagosomes activity of the NAPDH oxidase in Nampt-inhibited cells was still sufficient to create an anti-microbial environment. Therapeutic inhibition of Nampt in inflammatory diseases would be predicted to decrease the release of tissue-damaging ROS, but without a concomitant impairment of bacterial killing.

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

S10. NEUTROPHIL MICROPARTICLES AS POTENTIAL NOVEL EFFECTORS OF JOINT DISEASE

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Background: Microparticles (MPs) are small membrane-bound vesicles released by cells upon activation. Numerous reports have detected MPs in the synovial fluid of RA patients. However, the pathophysiological role of MPs in RA has not been established. The aim of the PhD studentship is to unveil the role of MPs in RA, particularly in physiological role of MPs in RA has not been established. The aim of these MPs were cultured with or without TNFα (10 ng/mL) for 30 min to induce MP release in supernatant. MPs were stained with Bio-Maleimide (BODIPY) dye, PE-conjugated CD66b (a marker for neutrophil-derived MPs) and analysed by BD LSFortessa applying the Fluorescence versus Side Scatter method. Further analyses were performed by triple staining with BODIPY, CD66b and either CD14-Macroglobulin (A2M), Ceruloplasmin (CP) or Heat Shock Protein-70 (HSP70) specific antibodies. These MPs were previously identified during proteomic analysis of PMN-derived MPs. Micromasses (MMs) were generated by culturing the human chondroblastoma C28/I2 line in high-density. MMs were stimulated with IL-1β (3 ng/ml) control- or TNFα-derived MPs for 24 h and extracellular matrix (ECM) production was measured using 1% Alcian Blue 8GS dye (AB) as previously described. ELISA for IL-6 production was performed on MMs culture supernatant.

Results: Compared to resting cells, the absolute number of MPs derived from TNFα-stimulated PMN was significantly higher (1444 ± 120 vs. 2650 ± 325 p < 0.01), with >80% events positive for CD66b. The number of MPs expressing A2M, CP and HSP70 increased upon stimulation (A2M: 64.3% ± 1.4 vs. 69.1% ± 0.8 p < 0.01; CP: 65.5% ± 1.2 vs. 70.5% ± 0.8 p < 0.01; HSP70: 74.3% ± 2.5 vs. 77.6% ± 3.7, ns). MMs stimulation with IL-1β (3 ng/ml) led to a significant decrease in ECM production (2.26 ± 0.05 vs. 2.02 ± 0.08 p < 0.05) as measured by AB staining. Treatment of MMs with TNFα-derived MPs protected from IL-1β-induced ECM loss (2.42 ± 0.2 p < 0.05). IL-6 production was markedly increased upon MM stimulation with IL-1β (95 ± 23 vs. 1598 ± 35 pg/ml) which was marginally yet significantly altered by the addition of TNFα-derived, but not control MMs (1604 ± 20 vs. 1491 ± 11 pg/ml, p < 0.01 n = 10).

Conclusions: Enhanced MP numbers were associated with activated PMN following stimulation with TNFα (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 TNFα-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 determinants involved in these effects.

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

S11. RESOLVIN D1 LIMITS PMN RECRUITMENT TO INFLAMMATORY LOCI: RECEPTOR DEPENDENT BIOACTIONS

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Background: Omega-3 polyunsaturated fatty acids (PUFA) are known to bestow protective clinical effects in the cardiovascular system and inflammatory disorders including rheumatoid arthritis. Recently, novel omega-3-derived lipid mediators were identified in the resolving phase of an acute, self-limited inflammatory response. These mediators, termed resolvins and protectins, display potent anti-inflammatory and pro-resolving effects, and exert their protective actions via specific G-protein coupled receptors (GPCRs). Recent studies identified two separate GPCRs that Resolvin D1 (RvD1) specifically binds on human leucocytes, the lipoxin-A4/Axinexin-A1 receptor (FPR2/ALX) and the orphan receptor GPR32 (which has no known murine orthologue). However, the contribution of its specific receptors in limiting polymorphonuclear cell (PMN) recruitment remains elusive.

Methods: To assess leucocyte-endothelial interactions, HUVEC were plated in Ibidi µ-Slides V0.4 and stimulated with TNFα (10 ng/mL, 4 h; R&D Systems), Human PMN were perfused over the endothelium at 3 dyne/cm², and capture, rolling and adhesion were quantified after 8 min. Zymosan stimulated peritonitis (1 mg/mouse) was used an acute model of inflammation. Bio-gel elicted macrophages harvested from WT or Fpr2 null mice were used to test the phagocytic actions of RvD1. Macrophages were pre-incubated with vehicle or RvD1 (0.01-100 nM,
30 min) prior to addition of fluorescently labelled zymosan (1:20 ratio), and phagocytosis determined using a NOVOstar plate reader.

**Results:** RvD1 drastically reduced PMN-endothelial interactions; significantly reducing initial PMN capture and firm adhesion to activated endothelium in a concentration-dependent manner. Receptor-specific antibodies blocked these anti-inflammatory actions of RvD1, with low (1 nM) concentrations sensitive to GPR32 blockade, whilst the higher (100 nM) concentration appeared FPR2/ALX-specific. Interestingly, PMN FACs expressing FPR2/ALX but not GPR32 increased following activation with pro-inflammatory stimuli, corresponding with secretory vesicle and specific granule mobilization. In the murine system, CD248 was identified as counteracting Fpr2 knockout, namely Fpr2 was essential for the actions of RvD1. Indeed, RvD1 reduced zymosan-induced PMN infiltration at 4 and 24 h, with doses as low as 1 ng/mouse. Importantly, this action of RvD1 was abolished in Fpr2 null mice, indicating the crucial role of this GPCR in the mouse. Moreover, the pro-resolving properties of RvD1 were also FPR2/ALX specific, as RvD1 enhanced zymosan phagocytosis by 30% in WT but not Fpr2 null murine macrophages.

**Conclusion:** Unveiling the molecular targets responsible for pro-resolving lipid mediators, such as RvD1 and the two GPCRs studied here, can inform innovative drug discovery programmes for inflammatory pathologies. Supported by the Arthritis Research UK (Foundation Fellowship 18445 to LVJ); this work was part supported by the National Institutes of Health USA GM38765 and DE019938 to CNS. 

**Disclosure statement:** C.S. is an inventor on composition of matter licensed for clinical development, and retains founder stock in Resolvyy. All other authors have declared no conflicts of interest.

### S12. THE MESENCHYMAL STEM CELL MARKER CD248 REGULATES INFLAMMATORY ARTHRITIS AND BONE FORMATION

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**Background:** CD248 (TEM1/Endosialin) is a membrane receptor involved in leukocyte trafficking and resident tissue macrophage plasticity. Genetic deletion of CD248 in the mouse results in high bone mass due to increased osteoblast-mediated bone formation. The cause of this is impaired PGDF signalling, which normally acts as a break on osteoblast differentiation. There is an unmet clinical need to address the bone loss which commonly occurs in rheumatoid arthritis patients and these findings suggest that targeting CD248 in inflammatory arthritis may have the benefit of increasing bone mass as well as reducing inflammation.

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

### S13. THE REGULATION OF ARTHRITIC BONE EROSIONS BY IL-10

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**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 cytoplasmic complex called the inflammasome. We hypothesise a potential cross talk between IL-10 and the inflammasome, which could help explain 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 inflammasome components, quantitative real time PCR (Q-PCR) was undertaken on synovial mRNA and ELISAs on serum. Mice were injected with a fluorescent matrix metalloproteinases (MMPs) probe to assess the extent of potential cartilage damage and scanned prior to, and following arthritsis 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 the induction of AIA 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 (eg. via regulation of bone pathology associated cytokines in RA). A cross talk 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 (e.g. 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, arthritic IL 10KO 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 towards 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 OSTEOLYSIS AND A MEDIATOR OF THERAPEUTIC EFFECTS OF DEXAMETHASONE IN COLLAGEN-INDUCED ARTHRITIS

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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 the anti-inflammatory actions of GCs in isolated macrophages. In the present study we used collagen induced arthritis, 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 every second day for ten 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

O49. GLOBAL GENE EXPRESSION ANALYSIS OF DEDIFFERENTIATED CHONDROCYTES

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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 aggregan 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 cocco-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/Ambion Biosciences) and this was amplified and biotin 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 (9.9, 9.2E-06, 5.8), furthermore, in pairwise comparisons 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 atypical homeobox gene HOPX was down-regulated in dC relative to nC (-2.6, 1.3E-11, 21.4).

Conclusions: Homeobox genes encode transcription factors that have roles in embryonic positional identity, ensure correct cell differentiation and faithful expression in order to ensure homeostasis of adult tissues. PITX1, PPRX2, FOXP1 and HOXC10 are 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 chondrogenesis. Global gene expression analysis indicates that these genes are up-regulated in 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 normal hyaline cartilage. 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

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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, 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 chondrogenesis. Global gene expression analysis indicates that these genes are up-regulated in 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 normal hyaline cartilage. Further investigation to verify these findings is on-going.

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

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

OS1. FINE MAPPING IN OVER 14,000 RHUMATOID ARTHRITIS CASES AND 15,500 CONTROLS REFINES ASSOCIATIONS TO KNOWN LOCI, INDICATES MULTIPLE INDEPENDENT AFFECTS AND REVEALS NOVEL ASSOCIATIONS

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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 12 autoimmune diseases and represents all known genetic variation from dbsNP, 1xG and sequencing projects for approximately 200 validated loci. The genotyping for the RA samples 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 125,658 SNPs (MAF > 0.01) revealed association to markers at 13 loci at genome wide significance (p < 5x10^-8) five of these are novel RA susceptibility loci (rs34536443 TYK2, rs17374791 IRAK1, rs8026888 TLR3, rs2240339 PADI4 and rs8192284 IL6R). Strong evidence of association was detected in 12 previously confirmed loci (p < 10^-3). When comparing CCP positive cases (n = 7,222) with controls 4 loci (2 novel) were identified at genome wide significance (p < 5x10^-8) five of these are novel RA susceptibility loci (rs2040406 near HLA-DQA1, p = 6.21x10^-23; rs15672 near HLA-DRB1, p = 1.65x10^-8). Although no SNPs outside the HLA region achieved genome wide significance the number of the SNPs tested, four SNPs previously associated with RA and SLE reached nominal significance (RA: rs13315591 in FAM107A, p = 0.001; SLE: rs13385731 in RASGRF3, p = 0.001, rs5029939 in TAF15P3, p = 0.004; rs2230926 in TAF15P3, p = 0.005).

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

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

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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 of 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 disorders. 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, coellic 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. 233 unique SNPs were identified (p < 5x10^-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-DQA1, p = 6.21x10^-23; rs15672 near HLA-DRB1, p = 1.65x10^-8). Although no SNPs outside the HLA region achieved Bonferroni corrected significance levels for the number of the SNPs tested, four SNPs previously associated with RA and SLE reached nominal significance (RA: rs13315591 in FAM107A, p = 0.001; SLE: rs13385731 in RASGRF3, p = 0.001, rs5029939 in TAF15P3, p = 0.004; rs2230926 in TAF15P3, p = 0.005).

Conclusions: The 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 diseases. These results require further confirmation in an independent replication sample.

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

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

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Background: Genome wide association studies (GWAS) have been hugely 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 cases and 8854 controls. SNPs failed QC based on a call rate <0.4. Samples failed QC based on a call rate <98%. 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 IRE1, 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 BISP4 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

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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.

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 <98% and/cluster separation score <0.4. Samples failed QC based on a call rate <98%, 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%). 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), ZNF1 (P = 1.12 x 10^-5) and CA9 (P = 1.36 x 10^-6). 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.