Objectives: We hypothesised that hyperuricemia affects neutrophil chemotaxis in CKD patients. Furthermore, we made use of a novel mouse model of chronic uric acid nephropathy.

Methods: Human study: Serum was collected and neutrophils isolated from CKD patients or healthy subjects. Serum BUN (blood urea nitrogen), creatinine and uric acid levels were measured. Neutrophil transwell assays were carried out and the number of migrated neutrophils towards fMLP, human IL-8 determined by flow cytometry. **Animal study:** Six week old Alb-creERT2;Glut9^{lox/lox} mice (ki/ki) and mice without active Cre (+/+) were injected with tamoxifen. The ki/ki mice received either a high fat diet with Inosine (HFD+Ino) to induce hyperuricemia-associated CKD or a chow diet with Inosine (Chow+Ino) to induce only hyperuricemia without CKD. Control +/-mice either received HFD+Ino or Chow+Ino diet. After two weeks, all groups were injected either with MSU crystals or vehicle into a preexisting air pouch, a mouse model for acute gouty arthritis. After 12 hours, neutrophil infiltration and the extent of inflammation were assessed via flow cytometry, ELISA, and colorimetric assays.

Results: Human study: Compared to healthy subjects, CKD stage 5 patients presented with significant higher levels of serum BUN (14.1 vs 52.1 mg/dl, $p=0.001$), creatinine (1.5 vs 9.3 mg/dl, $p=0.001$) and UA (2.3 vs 10.3 mg/dl, $p=0.001$). Neutrophils from CKD patients showed an impaired migratory ability due to the down-regulation of the adhesion molecules P-Selectin and $\alpha\beta$ Integrin.

Animal study: Two weeks post-HFD +Ino, ki/ki mice developed hyperuricemia-associated CKD (serum UA: 10–14 mg/dl; BUN: 80 mg/dl), whereas the ki/ki mice on Chow+Ino diet became hyperuricemic without CKD. Control +/-mice on both diets did neither develop hyperuricemia nor CKD. Interestingly, the number of infiltrating neutrophils into the air pouch was reduced in hyperuricemic ki/ki mice with CKD as well as in hyperuricemic ki/ki mice without CKD compared to +/-control mice. We observed less inflammation indicated by decreased IL-1 β , TNF α , CXCL1 and myeloperoxidase levels, and a down-regulation of adhesion molecules on infiltrated neutrophils in hyperuricemic ki/ki mice with CKD compared to +/-control mice.

Conclusions: Our data show that neutrophils from CKD patients are less able to migrate, which was consistent with data from our novel mouse model demonstrating that hyperuricemia impairs neutrophil chemotaxis in MSU crystal-induced inflammation. This indicated that the mechanism for defective neutrophil migration might be responsible for the lower incidence of acute gouty arthritis in hyperuricemic CKD patients.

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Big data in pre-clinical research

OP0359

EXPLORATION OF T-CELL SIGNATURES FOLLOWING TCR STIMULATION USING SINGLE CELL RNA-SEQ TO INFORM TREATMENT RESPONSE STUDIES IN RHEUMATOID ARTHRITIS

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Background: For rheumatoid arthritis (RA), as with many other rheumatic diseases, the importance of determining which therapy will work best, early in disease, to prevent further progression, is an important area of research. Progress in treatment response has been limited, possibly due to the complex interplay between various cell types. As such, specific T-cell signatures, determined by single cell RNA-Seq (scRNA-Seq), could be predictive of future response to treatments such as anti-TNF biologic therapies.

Objectives: Our aim was therefore to determine the optimal study design and to assess the potential of scRNA-Seq to identify T-cell signatures under resting and stimulated conditions to inform future studies.

Methods: Primary CD4 +T cells were either stimulated using anti-CD3/CD28 beads or subjected to the same conditions without stimulation for 4 hours. Single cells were isolated using the 10X Genomics Chromium Controller with a target recovery of 6000 cells. Each scRNA-Seq library was sequenced on 4 Illumina HiSeq 4000 lanes (~200K reads/cell) and processed using the cellranger pipeline. Further quality control and cluster analysis was performed using Seurat.

Results: For the unstimulated sample 5,586 cells were recovered and after quality control and filtering, 5,387 cells remained. Similarly, for the stimulated sample, 4,621 cells were recovered and 4,473 remained. This resulted in an average of 1094 and 1456 genes per cell. Similar clusters were seen after downsampling the stimulated dataset to 1 lane (~379M reads, ~82K reads/cell), suggesting that

CD4 +T cells are defined by large gene expression changes rather than subtle variations, consistent with protein expression data. Cluster exploration allowed the identification of several typical CD4 +T cell populations, including naïve, helper and regulatory. Furthermore, alignment of the two conditions in Seurat, identified classical and non-classical markers of activation, such as CD69, CCR7, MYC and PIM3. Finally, the relative cluster location and the expression of indicative markers suggested evidence of a progression from a naïve cell state to an 'active' effector state.

Conclusions: This data has provided important insights into future study design and confirmed the potential of scRNA-Seq to identify T-cell signatures. Importantly, despite obvious expression changes, cluster identity was maintained between stimulatory conditions. This implies it is possible to directly compare scRNA-Seq expression profiles between patient samples showing different disease activity without confounding the conclusions and enable the use of scRNA-Seq to investigate its predictive potential in RA treatment response. We are therefore in the process of expanding this work to study patient samples and different cell types. For example we have already generated similar data for monocytes on 3 RA samples and 3 healthy samples.

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OP0360

RNA-SEQUENCING OF 800 HUMAN BLOOD SAMPLES REVEALS SHARED AND UNIQUE EXPRESSION PROFILES ACROSS SEVEN SYSTEMIC AUTOIMMUNE DISEASES

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Background: Systemic autoimmune diseases (SADs) are chronic inflammatory conditions with limited treatment options. Although SADs encompass different clinical diagnoses, many of them share common pathophysiological mechanisms and have similar clinical manifestations. Therefore, defining a precise diagnosis and consequently an appropriate treatment is complex.

Objectives: We aimed to identify characteristic expression profiles for patients diagnosed with different SADs and find specific biomarkers for each disease based on whole blood RNA-seq data.

Methods: As part of the ongoing IMI PreciseSADS project we generated and analysed globin-depleted, polyA-selected RNA-seq data from an initial subset of 800 peripheral blood samples of healthy controls and patients with seven different SADs: systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), systemic sclerosis (SSc), Sjögren's syndrome (SSj), mixed connective tissue disease (MCTD), undifferentiated connective tissue disease (UCTD) and primary antiphospholipid syndrome (PAPS). Differential gene expression analysis was performed with DESeq2 and biomarker discovery was achieved using linear support vector classifier-based feature elimination and logistic regression-based estimator with cross-validation.

Results: We identified unique and common genes that were differentially expressed between controls and each of the seven SADs. The greatest extent of transcriptional dysregulation was found in SLE and MCTD patients, while UCTD, SSc and RA showed fewest differentially expressed genes. We found large and statistically significant overlaps between the lists of differentially expressed genes for each disease, with SLE, MCTD, SJS and UCTD showing most pronounced similarity at the gene expression level. The overlapping genes were enriched in interferon signalling pathway and the classical complement pathway. Low overlap was found between SJS and RA, and SJS and SSc.

We also looked for unique gene expression patterns in each disease with the aim of identifying potential biomarkers. We were able to define gene signatures differentiating between SADs and controls and also between SAD pairs.

Conclusions: Even though we were able to identify a limited number of disease specific signature genes, there are extensive and statistically significant overlaps in gene expression profiles of the seven investigated SADs. Similar to the clinical manifestations, the data presented here suggest that also on the molecular level, these diseases share a large portion of their pathophysiology. Work is ongoing to expand the dataset for confirmation of these preliminary data.

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How do you sleep?

OP0361-HPR SLEEPING PROBLEMS AND ANXIETY IS ASSOCIATED TO CHRONIC MULTISITE MUSCULOSKELETAL PAIN IN SWEDISH HIGH SCHOOL STUDENTS

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Background: The relationship between chronic multisite musculoskeletal pain (CMP) and sleep is complex, where pain can lead to sleeping problems and lack of sleep can intensify the pain perception. Most previous studies relates to adults, but adolescents may also suffer from CMP, and there is a need for more knowledge regarding the relationships between CMP and sleeping problems, stress, anxiety, depression, and health status.

Objectives: To study background factors associated to CMP in first year Swedish high school students.

Methods: First year Swedish high school students (n=296) were invited to complete questionnaires on chronic pain (mannequin with 18 body regions), sleeping problems (Uppsala Sleep Inventory, four items scored from 1–5), stress (ELO questions, scored from 1–5), anxiety and depression (Hospital Anxiety and Depression Scale, scored from 0–21), health status (EQ-5D, scored from 0 to 1, worst to best) and physical activity (International Physical Activity Questionnaire, categorised into low, moderate and high levels). Stress and sleeping items were dichotomized into 1–3 points (best) vs 4–5 points (worst). Individuals scoring at least severe problems (4 points) at one or more sleeping items were classified as having severe sleeping problems. HADS were categorised as non-cases (0–7), possible^{7–10} and probable cases (11–21 points). Students were grouped as having CMP (pain present in ≥3 regions) or not (no chronic pain or chronic pain in 1–2 regions). Multiple logistic regression analyses (adjusted for sex) with CMP as dependent variable were performed in SPSS, version 24.

Results: 254 students (86% of total sample, 87 boys and 167 girls) with a mean age of 16.1 (SD 0.6) years participated in the study. CMP was present in 25 (9.8%) students with no differences between boys and girls (8.0% vs 10.8%; p=0.488). Having CMP was associated with reporting severe sleeping problems (OR 2.49, 95% CI: 1.06 to 5.81, p=0.035) with initiating sleep, maintaining sleep, early morning awakenings and/or not feeling restored after sleep in comparison to the other students. Students with CMP were more likely to be categorised as probable cases for anxiety (OR 3.06, 95% CI: 1.09 to 8.61, p=0.034), but there were no associations for possible cases for anxiety (OR 1.15, 95% CI: 0.38 to 3.51, p=0.800), possible cases (OR 2.03, 95% CI: 0.63 to 6.54), or probable cases for depression (OR 3.35, 95% CI: 0.33 to 33.83). There was a nearly significant association between stress and belonging to the CMP group (OR 2.31, 95% CI: 0.97 to 5.53, p=0.059). A higher self-reported health status was associated to a lower likelihood for CMP (OR 0.04, 95% CI: 0.01 to 0.27, p=0.001). Distribution of physical activity levels of low, moderate and high was not significantly associated to having CMP in comparison with not having it.

Conclusions: One in ten high school students fulfilled criteria for having chronic multisite musculoskeletal pain. CMP was associated to sleeping problems, anxiety, and a worse health status. The results from this study may be used by school health-care professionals in their preventive work to promote student's health.

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Stratifying connective tissue diseases

OP0362 NOVEL GENE VARIANTS ASSOCIATED WITH CARDIOVASCULAR DISEASE IN SYSTEMIC LUPUS ERYTHEMATOSUS AND RHEUMATOID ARTHRITIS

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Background: Patients with Systemic Lupus Erythematosus (SLE) and Rheumatoid Arthritis (RA) have increased risk of cardiovascular disease (CVD).

Objectives: We investigated whether single nucleotide polymorphisms (SNPs) at autoimmunity risk loci were associated with CVD in SLE and RA.

Methods: SLE patients (n=1045) were genotyped using the 200K Immunochip SNP array (Illumina). The allele frequency was compared between patients with and without different manifestations of CVD. Results were replicated in a second SLE cohort (n=1043) and in an RA cohort (n=824). We analysed publicly available genetic data from the general population, performed electrophoretic mobility shift assays and measured cytokine levels and occurrence of anti-phospholipid antibodies (aPLs).

Results: We identified two new putative risk loci associated with increased risk for CVD in two SLE populations, which remained after adjustment for traditional CVD risk factors. An *IL19* risk allele was associated with stroke/myocardial infarction in SLE (OR 2.3 (1.5–3.4), p=8.5×10⁻⁵) and RA (OR 2.8 (1.4–5.6), p=3.8×10⁻³), meta-analysis (OR 2.5 (2.0–2.9), p=3.5×10⁻⁷), but not in population controls. The *IL19* risk allele affected protein binding and SLE patients with the risk allele had increased levels of plasma-IL10 (p=0.004) and aPL (p=0.01). An *SRP54-AS1* risk allele was associated with stroke/transient ischaemic attack in SLE (OR 1.7 (1.3–2.2), p=2.5×10⁻⁵) but not in RA. The *SRP54-AS1* risk allele is an expression quantitative trait locus for four genes.

Conclusions: The *IL19* risk allele was associated with stroke/myocardial infarction in SLE and RA, but not in the general population, indicating that shared immune pathways may be involved in the CVD pathogenesis in inflammatory rheumatic diseases.

Disclosure of Interest: None declared

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OP0363 OPTIMISINGPRECISION MEDICINE BY USING GENETICS TO ASSIGN DIAGNOSTIC PRIOR PROBABILITIES TO PATIENTS WITH SYNOVITIS – PROOF OF PRINCIPLE

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Background: In patients with synovitis, the question is 'Which disease does this patient have?' However, traditional tests often only inform us about disease presence yes/no and disease discriminating symptoms often take a while to arise. Time independent information, such as genetics, might accelerate the diagnostic process. As increasing number of patients have genotyping data available in medical records prior to their visit, the question emerges: can genetic data facilitate disease differentiation in early disease?

Objectives: Proof of principle study to test the differentiating ability of genetic profiles in patients with synovitis.

Methods: We studied the most common rheumatologic diseases: rheumatoid and psoriatic arthritis, SLE, spondyloarthritis and Gout. The population level