RhoAfl/fl mice. Additionally, RA-prone mice had higher expression of receptors to extracellular matrix proteins collagen ($\alpha 2\beta 2$) and fibronectin ($\alpha 5\beta 1$) compared to control mice ($p=0.016$ and $p=0.011$ resp) and to RA-protected mice (GLC Rac1fl/fl and GLC RhoAfl/fl, $p=0.0004$ and $p=0.011$, resp). In total, both the number of FoxP3+ CD4 cells and the expression of $\alpha 5\beta 1$ receptors on CD4 cells correlated strongly with the synovitis score ($r=0.72$, $p=0.0017$ and $r=0.59$, $p=0.012$, respectively). GGTase1 gene lays under the control of HOX proteins essential for cell homing. Importantly, HOX regulate the expression of integrins. Studying the expression of HoxA genes in spleen, we found that RA prone GLC and GLC Cdc42 mice tended to have lower expression of HoxA2 and higher expression of HoxA9 compared to RA-protected GLC Rac1 and GLC RhoA and to control mice. The Hoxa9/Hoxa2 ratio was significantly higher in RA prone mice compared to RA-protected mice ($p=0.0085$) and control mice ($p=0.019$). This ratio correlated with $\alpha 5\beta 1$ receptors ($r=0.55$, $p=0.0084$), FOXP3+ CD4 cells ($r=0.50$, $p=0.017$), and the arthritis index ($r=0.50$, $p=0.033$).

Conclusion: Taken together this study shows that Rho proteins play divergent role in development of arthritis. Activation of Rac1 and RhoA by GGTase1 deletion changes the pattern of HOXA proteins and increases expression of integrin receptors, which facilitates leukocyte influx in the paw joints. Deletion of Rac1 and RhoA has RA-protective effect in GLC mice.

References:

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OP0335-PARE MODERNISATION OF PATIENT ORGANIZATION THROUGH ONLINE COURSES

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Background: A big and very crucial part of every membership organization is the continuing education of members and elected representatives in regional and local chapters.

Given the geographical long distances in Sweden, attending courses in person can be difficult, and expensive for the Swedish Rheumatism Association, the SRA. In 2019 the SRA developed a method to modernise our educational efforts by offering online courses to our elected representatives. Thus making it possible for anyone who has access to a computer to participate when it suits him or her. The courses can be done individually or in groups.

Objectives: The goal is to offer modern and cost effective ways to educate in matters selected by the SRA, and for elected representatives and members to be able to attend courses at any times.

Methods: The courses consists of films, texts and study exercises.

The staff of the SRA record films and make as much of the content themselves as possible. The content must be relaxed, easy to do and enhance the feeling of familiarity. Those who complete the courses should feel like they are in the same room as the lecturers.

Results: In 2019, the SRA completed two courses for elected representatives. The first course was on the matter of democratic structures at the annual general meeting of the SRA, and all decision making that applies for the SRA, for example, statutes, how to elect new board members and decide on an activity plan for the coming year. The course circled around these topics:

1. How do you write a motion and how do you send it to the annual meeting?
2. How do you prepare as a representative?
3. What happens at an annual general meeting?
4. How do you ask for the word and how do you argue for your cause?
5. How do you report from a general meeting to your local organization?

During the evaluation, the SRA received positive comments from those who attended. By the end of 2019, the SRA finished developing an online course on membership recruitment.

Conclusion: In 2020, the SRA plan to do three more online courses on the topic of systemic sclerosis, rheumatoid arthritis and fibromyalgia.

Online courses will not replace physical meetings, but offer a good alternative in the field of patient education.

Disclosure of Interests: None declared

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Improving work participation

OP0336-HPR RISK FACTORS FOR PERSISTENCE AND DEVELOPMENT OF FREQUENT MUSCULOSKELETAL PAIN IN ADOLESCENT ATHLETES

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Background: Physical activity has a positive impact on health, but adolescent athletes often report musculoskeletal pain (MP) which is negative in the aspect of sustaining physical activity over time. There is a lack of longitudinal assessments of MP and potential risk factors, such as timing of physical maturation, in adolescent athletes.

Objectives: To identify risk factors associated with the persistence or development of frequent MP at a 2-year follow-up in adolescent sport school students.

Methods: Fourteen-year-old sport school students ($n=233$) were invited to participate in this 2-year longitudinal study. Self-reports of MP was assessed as frequency, distribution, and intensity, and health status by EQ-5D. Physical maturation was calculated by the Mirwald equation (height, weight, and sitting height) (1), and categorized as early (>1 year), average (± 1 year), or late (<-1 year). Students were grouped at baseline and follow-up into infrequent (never to monthly) or frequent (weekly to daily) MP groups. Logistic regression analysis was used to study associations between frequent MP at follow-up and baseline variables.

Results: 131 students (79 boys and 52 girls) were included in the study. Development or persistence of frequent MP at follow-up ($n=61$) was associated with being a girl, late physical maturation (only boys were categorized as late), non-contact sports participation, frequent MP at baseline, and reporting ≥ 2 MP sites at baseline. Students with a better health status at baseline were less likely to belong to the frequent MP group at follow-up (Table).

Conclusion: Frequent MP is common in sport school students. MP in young athletes may become a future health problem and there is a need for recognition and interventions by coaches and health services to prevent MP from becoming persistent.

References:

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Table. Associations between background variables at baseline and frequent MP at follow-up based on crude logistic regression analysis controlling each variable for sex.

Baseline variables	Model	
	Infrequent MP vs. Frequent MP	
	OR	(95% CI; p-value)
Sex		
Boys	1.00	
Girls	2.76	(1.34–5.68; $p<0.01$)
Physical maturation		
Average (± 1 year)	1.00	
Early (>1 year)	0.41	(0.05–3.65; $p=0.42$)
Late (<-1 year)	3.83	(1.13–12.95; $p=0.03$)
Sport categories		
Contact	1.00	
Non-contact	5.16	(2.07–12.88; $p<0.001$)
MP groups		
Infrequent	1.00	
Frequent	2.74	(1.31–5.72; $p<0.01$)
MP intensity last week (NRS 0–10, best to worst)	1.15	(0.98–1.35; $p=0.10$)
Number of MP sites		
0	1.00	
1	2.32	(0.71–7.58; $p=0.16$)
≥ 2	2.87	(1.32–6.25; $p<0.01$)
EQ-5D (0.00–1.00, worst to best)	0.03	(0.001–0.58; $p=0.02$)

Disclosure of Interests: None declared

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New technologies in translational rheumatology

OP0337 DIFFERENTIAL METHYLATION OF PERIPHERAL BLOOD ADAPTIVE IMMUNE CELLS IN INDIVIDUALS AT HIGH RISK FOR RA AND WITH EARLY RA COMPARED WITH CONTROLS IDENTIFIES PATHWAYS IMPORTANT IN TRANSITION TO ARTHRITIS

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Background: The "Targeting Immune Responses for Prevention of RA" (TIP-RA) collaboration studies individuals at high risk for developing RA because of serum anti-citrullinated protein antibody positivity in absence of arthritis, and is focused on defining how they transition from at-risk to classifiable disease. One potential mechanism is through alterations in epigenetics patterns in adaptive immune cells.

Objectives: Previous studies showed that DNA methylation patterns of early RA (ERA) synovocytes differ from long-standing RA, suggesting that abnormal methylation occurs early in synovium and evolves over time. To extend these observations, we performed

a cross-sectional analysis in TIP-RA of DNA methylation signatures in peripheral blood cells in ERA, at-risk anti-CCP+ individuals and demographically matched CCP- controls. **Methods:** Genomic DNA was isolated from two independent cohorts of CCP- (cohorts 1 and 2, respectively: B cell: $n = 17/34$; memory T cell: $n = 21/34$; and naïve T cell: $n = 21/33$), CCP3+ (B cell: $n = 18/37$; memory T cell: $n = 20/36$; and naïve T cell: $n = 20/35$), and CCP3+ ERA (B cell: $n = 4/18$; memory T cell: $n = 5/18$; and naïve T cell: $n = 5/18$) after separating PBMCs using antibodies and magnetic beads. Methylation was measured by Illumina Infinium MethylationEPIC chip. Differentially methylated loci (DMLs) were identified using Welch's t-test and mapped to gene promoter regions to define DM genes (DMGs). Principal component analysis (PCA) was used to represent relationship among groups. Pathway analysis was applied by Reactome. **Results:** For the initial cohort, 1494, 1097 and 1330 DMLs were identified among CCP+, CCP- and ERA in B cells, memory T cells and naïve T cells, respectively. For the confirmatory cohort, 523, 793 and 548 DMLs were found in corresponding cell populations. The DML overlap between the 2 cohorts was highly significant ($p = 2.48E-77$). The DMLs were combined for both groups and corresponded to 411, 412, and 351 DMGs in B cells, memory T cells and naïve T cells. Of these, we found 246, 198 and 195 DMGs between CCP3+ and ERA in each peripheral blood cell population, respectively. PCA showed separation of CCP+, CCP- and ERA in each of the three blood cell types by DMLs (Fig. 1). DMGs were mapped to biological pathways to identify DM pathways. Although most were not significant, there were several highly significant differences comparing CCP+, ERA and CCP- in memory T cells involving pathways, including "Interferon gamma signaling" (FDR 7.48E-14), "PD-1 signaling" (FDR 8.71E-10), "Translocation of ZAP-70 to Immunological synapse" (FDR 4.75E-10), and "Phosphorylation of CD3 and TCR zeta chains" (FDR 8.71E-10).

Figure 1. PCA shows the separation of CCP+, CCP- and ERA patients in memory T cells in confirmatory cohort.

Conclusion: We identified reproducible methylation signatures of CCP-, CCP+, and ERA in peripheral blood B cells, memory T cells and naïve T cells in initial and confirmatory cohorts. The methylome of ERA also demonstrated a distinctive pattern from CCP+, indicating that progression to RA is accompanied by epigenetic remodeling, especially in T cell signaling and interferon responses. These signatures identify critical pathways in CCP positivity and classifiable RA and could provide the basis of novel interventions to prevent disease.

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OP0338

MASS SPECTROMETRY IDENTIFIES NOVEL BIOMARKERS IN GIANT CELL ARTERITIS, USEFUL IN PATIENTS ON INTERLEUKIN-6 RECEPTOR BLOCKADE

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Background: Acute phase reactants (erythrocyte sedimentation rate [ESR], C-reactive protein [CRP]) have limited utility in GCA, even in patients treated with prednisone alone. Furthermore, the lack of reliable biomarkers in patients receiving interleukin (IL)-6 blockade therapy is a major unmet need.

Objectives: To identify biomarkers of disease activity in GCA patients treated with prednisone monotherapy and with prednisone in combination with tocilizumab (TCZ).

Methods: We mapped the serum proteome of GCA patients with active and inactive disease in an unbiased manner using high-throughput multiplexed mass spectrometry. Proteomic analyses were performed in 5 µl serum samples with 11-plexed tandem mass tag (TMT) technology using an Orbitrap Lumos mass spectrometer. A SEQUEST-based database search engine was employed for peptide identification. Quantification was based on TMT reporter ion intensities. All patients were sampled during their participation in the GACTA trial,¹ in which they received TCZ plus 26 weeks of prednisone (TCZ group) or placebo plus 26 or 52 weeks of prednisone (PRED group). Active disease was defined as the presence of cranial or PMR symptoms requiring treatment intensification regardless of ESR and CRP levels. Samples were selected if patients were in clear states of active or inactive disease at GACTA systematic sample collection timepoints (baseline and weeks 4, 12, 24, 48). An exhaustive leave-2-out strategy was used to identify classification markers. All possible pairs of samples were isolated as test samples and the remaining training samples were used to identify the protein markers. Proteins with an absolute log2 fold concentration difference ≥ 0.5 between active and inactive samples and a P-value < 0.1 were retained and sorted based on the metric $-\log_{10}(P\text{-value}) \times \text{absolute}(\log_2 \text{ fold change})$. Top markers within each training set were selected to generate normalized ranks {0,1} across all samples. A mean rank was calculated for every sample. The set of normalized ranks for the test samples across all sets of top markers were bootstrapped for each test sample 100 times with replacement. The bootstrapped rankings were evaluated by determining areas under the curves (AUC) of receiver operator characteristic (ROC) curves.

Results: The PRED group included 21 patients (active, $n = 16$; inactive, $n = 5$) and the TCZ group included 21 patients (active, $n = 14$; inactive, $n = 7$). Using high-throughput sample preparation methods without applying any depletion of known highly abundant serum proteins, we quantified 760 proteins across all samples and 344 proteins in at least half the samples. Compared to inactive PRED-treated patients, active PRED-treated patients showed significant overexpression of several acute phase reactants including serum amyloid A1 and 2 (SAA1, SAA2) and complement factor H (CFH) (Fig. 1a). The magnitude of concentration change and the level of statistical significance observed for SAA1, SAA2 and CFH in PRED-treated patients were higher than those of CRP (Fig. 1a). Compared to inactive TCZ-treated patients, active TCZ-treated patients demonstrated significant overexpression of multiple biomarkers including haptoglobin, haptoglobin precursor, SAA2 and complement factor 4A, and underexpression of peptidase inhibitor 16 (Fig. 1b), a protein involved in vascular and regulatory T cell biology. Sets of 10 biomarkers resulted in a classification of active versus inactive disease with ROC AUCs of 0.89 (95% CI 0.79-0.96) in the PRED group (Fig. 2a) and 0.97 (95% CI 0.95-0.97) in the TCZ group (Fig. 2b).

Conclusion: We identified several differentially expressed serum proteins in GCA patients with active and inactive disease receiving prednisone monotherapy or TCZ-based treatment regimens. In both treatment groups, a signature of biomarkers classified disease activity status with high accuracy. Haptoglobin, a readily available laboratory test, may be useful in monitoring disease activity in GCA patients receiving IL-6 blockade therapy.

References:

[1] Stone *et al.* *NEJM* 2017

Figure 1. Differentially expressed serum proteins in giant cell arteritis patients with active and inactive disease

(a) Top 10 markers in patients treated with prednisone monotherapy (PRED group); (b) top 10 markers in patients treated with tocilizumab and prednisone (TCZ group)

Figure 2. Accuracy of top biomarkers for discrimination between active and inactive giant cell arteritis

(a) Top 10 markers in patients treated with prednisone monotherapy (PRED group); (b) top 10 markers in patients treated with tocilizumab and prednisone (TCZ group)