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Seeking the pathophysiology of rheumatoid arthritis and spondylarthritis

OP0266 SYNOVIAL TISSUE PROFILING IN AUTOANTIBODY POSITIVE AT RISK INDIVIDUALS REVEALS GENE SIGNATURES ASSOCIATED WITH LATER DEVELOPMENT OF RHEUMATOID ARTHRITIS

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Background: Previous work has suggested subtle infiltration of synovial T cells[1] in the absence of overt synovial inflammation[2] in individuals at risk of developing rheumatoid arthritis (RA).
Objectives: To study the molecular changes in synovium preceding arthritis development in preclinical RA.
Methods: We included sixty-seven individuals who were IgM rheumatoid factor (RF) and/or anti-citrullinated protein antibody (ACPA) positive and without any evidence of arthritis. All individuals underwent mini-arthroscopic synovial biopsy sampling of a knee joint at inclusion and were prospectively followed. First, an explorative genome-wide transcriptional profiling study was performed on synovial biopsies obtained from 13 individuals using Agilent arrays (test cohort). Survival analysis was used to identify transcripts with a significant association with arthritis development. The expression level of differentially expressed genes was validated using quantitative real-time PCR in the total cohort. Immunohistochemistry was used to study gene candidates at protein level *in situ*.
Results: Six of the 13 individuals in the explorative study developed RA after a median follow up time of 20 months (IQR 2–44). The 7 individuals who did not develop RA had a median follow up time of 85 months (IQR 69–86). Using a False Discovery Rate of <5% we found that increased expression of 3151 transcripts correlated with a higher risk of arthritis development, and increased expression of 2437 transcripts correlated with a lower risk. Gene Set Enrichment Analysis revealed that synovial biopsies of individuals who developed RA after follow up display higher expression of genes involved in several immune response-related pathways (e.g. T cell and B cell receptor pathways, cytokine and chemokine signalling and antigen processing and presentation) compared with biopsies of individuals who did not develop RA. In contrast, lower expression was observed for genes involved in e.g. extracellular matrix receptor interaction, Wnt-mediated signal transduction and lipid metabolism. Subsequently, the expression level of a selection of 27 differentially expressed genes was validated by quantitative real-time PCR in 61 RA-risk individuals. Two-way hierarchical cluster analysis classified the individuals into two groups, where those individuals who developed RA (n=16) showed a preference to cluster together in the left arm of the dendogram (Chi2 p=0.03).
Immunohistochemistry analyses (n=54) showed an abundant expression of CXCL12 and CXCR4 already in most RA-risk individuals. Synovial biopsies from RA-risk individuals who developed arthritis were more likely to show a positive gp38 staining and lower lipid staining.
Conclusions: This study clearly shows molecular changes appearing in synovial tissue before onset of arthritis in the absence of overt synovitis. Preclinical synovial alterations in immune response genes and lipid metabolism were associated with development of arthritis.

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OP0267 THE PADI4 GENE PROMOTER METHYLATION LEVEL IS ASSOCIATED WITH ANTI-PADI4 ANTIBODIES LEVEL AND RA ACTIVITY

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Background: Rheumatoid arthritis (RA) is a chronic, autoimmune, inflammatory disease that predominantly affects the synovial membrane leading to joint destruction. Anti-citrullinated protein antibodies (ACPA) are important markers of RA. They recognise post-translationally modified auto-antigens generated by enzymes peptidylarginine deiminases (PADIs) mostly type 4 (PADI4), which transform arginine to new amino acid citrulline in various proteins. There were also identified anti-PADI4 antibodies (anti-PADI4) which are a specific marker of RA. DNA methylation plays a key role in the control of gene expression. The process concerns CpG islands in promoter regions of about 75% of gens and leads to gene silencing when over-expressed. It is possible that PAD4 production is also regulated via methylation.
Objectives: We aimed to identify if there is an association between PADI4 gene promoter methylation degree, anti-PADI4 antibodies level and RA activity.
Methods: A total of 155 unrelated patients, 125 with RA, 83,2% female, aged 52.2±12.3 years (mean ±SD) and 30 healthy controls (HC), 76.7% female, aged 53.2±8,1 years, were enrolled. RA patients were divided according DAS28 score into 4 groups as shown in table 1. Whole blood and serum samples were collected and stored at −80°C until analysis. DNA was extracted from whole blood and stored at −80°C until analysis. Two single-nucleotide polimorphisms (SNPs) of the PADI4 gene (PADI-94, rs2240340 and PADI-104, rs1748033) were determined by TaqMan genotyping. Quantitative real-time methylation-specific PCR (qMSP) was used to analyse the methylation status of promoter region in PADI4 gene. Anti-PAD4 antibodies were evaluated in serum by ELISA.
Results: We found the differences in anti-PADI4 level between RA severe and HC group (p<10–5), RA moderate and HC (p<10–5), RA low activity and HC (p<0.05) and in PADI4 methylation group between RA severe and RA remission (p<0.05), RA moderate and RA remission (p<10–3) and RA moderate and HC (p<10–2). What is interesting is that methylation level in the RA remission group is higher than in the HC. The intensity of PADI4 methylation correlates with anti-PADI4 antibodies level and DAS28 score with r_s=-0.2 and −0.36 respectively (both p-values<0,05) and anti-PADI4 level is associated with DAS28 score (r_s=0.38).

Abstract OP0267 – Table 1

	RA Severe DAS28>5.1; 27.2%	RA Moderate DAS28>3.2–5.1; 36.8%	RA Low (DAS28>2.6–3.2; 15.2%)	RA Remission (DAS28≤2.6; 20.8%).	Healthy Control (HC)
PADI4 Metylation [U/ml]	1.32 [0.79–2.27]	1.14 [0.69–1.82]	1.98 [1.37–2.48]	2.12 [1.73–2.94]	1.86 [1.62–3.49]
Anti-PADI4 [U/ml]	731.03 [477.98–1288.1]	716.11 [400.85–1250.8]	589.57 [306.08–2380.2]	455.54 [271.26–1104.9]	293.25 [234.39–389.12

Data are given by median [interquartile range]

Conclusions: We demonstrate the novel finding that elevated methylation of PADI4 gene promoter is associated with lower RA activity and lower level of anti-PADI4 antibodies and might play a role in patophisiology of RA or be used as future therapeutic target. The data suggest that PADI4 enzyme synthesis is epigenetically regulated by its gene promoter methylation.

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