distance of ≈1500 nucleotides, indicating that overlap with the promoter site is most likely. A common sequence motif around these CpG sites was not immediately detectable but requires more detailed analysis.

Conclusions Microarray based comparative analysis of transcriptional and epigenetic differences suggests a detailed picture of methylation associated gene regulation and enables to generate an epigenetic map of relevant CpG site for genes expressed and regulated in immune cell types. As many of the microarray based suspected CpG sites of a defined gene did not match with differential gene expression, epigenetic profiling with microarrays has to be interpreted carefully.

A7.6 COMPARATIVE TRANSCRIPTOME ANALYSIS OF HUMAN AND MOUSE SYNOVIAL FIBROBLAST RESPONSES TO TNF

doi:10.1136/annrheumdis-2013-203221.6

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Background and Objectives Animal models are essential for development of new therapeutics to test their effectiveness and to control for side effects. Targeted therapies with biologics reveal differences in responsiveness and suggest substantial differences in the molecular pathomechanisms of human as well as murine arthritis. In order to characterise such differences, we started a pilot investigation based on published transcriptomes to develop bioinformatic strategies of interspecies gene expression comparison.

Materials and Methods A GEO repository search for compatible data sets on arthritis in human and mouse revealed the Affymetrix based transcriptomes of synovial fibroblasts from rheumatoid arthritis (RA) and osteoarthritis (OA) patients (GEO-accession: GSE13837) and mouse BALBc wt (GSE17160) with similar stimulation (TNF-alpha) and time point after stimulation (12 hours). MAS5 algorithms of signal extraction and pairwise comparison were performed. The BioRetis database was used for group comparisons, candidate gene selection and data sharing. Differentially expressed genes were exported for interspecies comparison in a filemaker database. Affymetrix annotation files were used for mapping of orthologous genes.

Results The number of differentially expressed genes induced by TNF in synovial fibroblast from RA, OA and BALBc were 1584, 1476 and 503 respectively. Comparison of genes overexpressed in RA and OA revealed up to 70% (1034 genes) overlap. Murine and human response was similar for 197 genes in all three groups, 45 only between BALBc and RA and 24 only between BALBc and OA. Ranking the genes by dominance in change call and/or fold change, 7 genes were overlapping between the top 10 of both human cells, OA and RA, and 13 out of the top 20. All overlapping genes in human revealed a high correlation (R = 0.795). In contrast, overlap between RA and BALBc SFbl responses revealed only 4 genes out of the top 10 and 5 out of the top 20 in each and a weaker correlation (0.392). Pathway tools including IPA may identify TNF as one potential upstream regulator. However, the reference list of expected gene candidates for scoring in IPA seemed to be about 2.5 fold longer in human and to include less than 60% of the murine reference candidates.

Conclusions Interspecies comparison in this defined experiment revealed first insight in common responses but also demonstrates that differences can be observed even in most dominant candidate genes. Therefore, systematic investigations are needed to provide a reliable basis for comparative mapping of the molecular pathomechanisms in mouse models and human disease.

Acknowledgement BTCure IMI grant agreement no. 115142

A7.7 DIFFERENT GENETIC BACKGROUND OF DERMATOMYOSITIS AND POLYMYOSITIS IN A SINGLE CENTRE COHORT

doi:10.1136/annrheumdis-2013-203221.7

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Background and Objectives The idiopathic inflammatory myopathies (IIM) are systemic connective tissue diseases in which autoimmune pathology is responsible for promotion of chronic muscle inflammation and weakness. As in many other autoimmune diseases, the development of IIM is also associated with genes of HLA complex. The aim of this study was to determine the basic relation between alleles of HLA genes and IIM.

Materials and Methods We have performed low to high resolution genotyping to characterise the allelic profiles of HLA-DRB1, -DQB1 and -DQA1 loci in a large group of single centre cohort of patients suffering from IIM (n = 269). The genomic DNA was prepared by standard DNA extraction methods and the HLA typing was done using the commercial LABType® SSO kit (One Lambda, USA). Statistical evaluation of results was done with chi-2 test and Fisher exact test. Autoantibody profiles were analysed with radioactive immunoprecipitation.

Results The frequencies of HLA-DRB1*03:01 and -DRB1*16:01 alleles were increased in IIM patients and the difference reached statistical significance when compared to healthy controls ($P < 0.01$ for DRB1*03:01; $P < 0.05$ for DRB1*16:01). Different alleles were associated with dermatomyositis (DM) or polymyositis (PM). The frequency of DRB1*16:01 was significantly higher in DM patients ($P < 0.01$), whereas the frequency of DRB1*03:01 was higher in patients suffering from PM ($P < 0.01$), when compared to controls.

Presence of HLA-DRB1*03:01 allele was associated with anti-Jo-1, anti-Ro52, or anti-Pm-Scl positivity in all IIM patients ($P < 0.05$). Interestingly, the DRB1*03:01 allele was also associated with the negativity for anti-p155/140 in our patients ($P < 0.01$).

The DRB1*16:01 allele was associated with negativity of all studied autoantibodies, particularly in subgroup of DM patients ($P < 0.05$).

Conclusions This study identifies different genetic background between patients with dermatomyositis and polymyositis in a homogenous population of patients from a single centre.

Acknowledgement This work is supported by Internal Grant Agency of Ministry of Health of the Czech Republic NT/12438–4 and NT/13699.

A7.8 DNA DEMETHYLATION IN SALIVARY GLAND EPITHELIAL CELLS FROM PATIENTS WITH PRIMARY SJÖGREN'S SYNDROME MAY BE ASCRIBED TO INFILTRATING B CELLS

doi:10.1136/annrheumdis-2013-203221.8

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Background and Objectives Sjögren's syndrome (SS) is an autoimmune exocrinopathy characterised by an epithelium injury surrounded by dense lymphocytic infiltrates composed of activated T and B cells. Present at the interface of genetic and environmental risk factors, epigenetic modifications are suspected to play a key role in SS. Accordingly, we decided to further characterise DNA methylation in SS.

Materials and Methods We tested, using a 5 methyl cytosine (5MeCyt) ELISA, global DNA methylation in long-term cultured salivary gland epithelial cells (SGEC), peripheral T cells and B cells from eight SS patients. DNA methylation/demethylation partners were assessed by real time quantitative PCR (DNA methyl transferase (DNMT)1, DNMT3a/b, PCNA, UHRF1, MBD2, MBD4, and Gadd45-alpha). Immunofluorescence was conducted on labial salivary gland biopsy. Co-culture experiments were performed associating the human salivary gland cell line (HSG) and B cells.

Results Global DNA methylation was reduced in SGEC from SS patients (5MeCyt: 36.3 ± 3.2% in SS *versus* 43.1 ± 3.3% in controls, $P = 0.01$), while no difference was observed in T and B cells. SGEC demethylation in SS patients was associated with a 7-fold decrease of DNMT1 and a 1.8-fold increase of Gadd45-alpha expression. The other DNA methylation/demethylation partners tested were not differently expressed when compared to controls. Interestingly, SGEC demethylation may be attributed to the B cell infiltrate as DNA methylation increased in salivary gland biopsy after rituximab (anti-CD20 antibody) treatment. Such hypothesis was confirmed using co-culture experiments (HSG cells and B cells) revealing an alteration of the PKC-delta/ERK/DNMT1 pathway. Finally, DNA methylation was associated with the overexpression of several SGEC genes such as ICAM-1 and human endogenous retrovirus (HERV).

Conclusions SGEC dysfunction in SS may be linked to epigenetic modifications and this tissue specific defect may be ascribed in part to infiltrating B cells. This observation opens new therapeutic perspectives in SS.

A7.9 DOES TELOMERE SHORTENING IN WOMEN WITH RHEUMATOID ARTHRITIS PREDICT X CHROMOSOME INACTIVATION BIAS?

doi:10.1136/annrheumdis-2013-203221.9

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Background Rheumatoid Arthritis (RA), like most auto-immune diseases, is a female predominant disease. As a possible explanation for gender bias, we have previously shown that women with RA have non-random X chromosome inactivation (XCI) that could trigger autoimmunity (article in preparation). Intriguingly, this bias in XCI correlates with presence of the shared epitope (SE) and with disease duration.

Also associated with presence of the SE, premature immunosenescence, characterised by shorter telomere length, has been described in peripheral blood cells from patients with RA [1]. Moreover, telomeric non coding RNAs have been reported to be enriched near the inactive X chromosome in mammals [2] indicating a potential link between telomere length and XCI.

Objectives In this context, we propose to test whether women with RA have shortened telomere length and whether that could influence the epigenetic mechanism of XCI.

Methods A total of 73 women with RA and 48 healthy women with no history of autoimmune diseases, who had previously been tested for XCI and HLA-genotyped, were evaluated for telomere length. The relative telomere length was estimated by real-time PCR as originally described by Cawthon [3] with the $2^{-\Delta\Delta Ct}$ method.

Results Preliminary results show that women with RA have smaller telomere length than healthy women, although the difference is modest ($p = 0.07$) and has to be adjusted for age on a larger cohort. Contrary to expectations, shorter telomere length is not correlated with skewed XCI status, disease duration or the presence of shared epitope in our small cohort.

Conclusions This preliminary study seems to confirm that women with RA have shorter telomeres than healthy women. Further telomere length measurements have to be done on a larger group of patients with RA and healthy controls, as well as HLA-genotyping them and evaluating their XCI status. This will be a step forward in understanding the relationship between immune senescence, female predisposition and genetic risk (SE) in RA.

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A7.10 GENETIC VARIANTS IN THE *IL-4* AND *IL-4* RECEPTOR GENES IN ASSOCIATION WITH THE SEVERITY OF JOINT DAMAGE IN RHEUMATOID ARTHRITIS: A STUDY IN SEVEN COHORTS

doi:10.1136/annrheumdis-2013-203221.10

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Objective The progression of joint destruction in rheumatoid arthritis (RA) is determined by genetic factors. Changes in *IL-4* and *IL-4R* genes have been associated with RA severity but not replicated. We studied the association between *IL-4* and *IL-4R* tagging SNPs and progression rate of joint damage in RA in a multi-cohort candidate gene study.

Methods *IL-4* and *IL-4R* tagging SNPs (8 and 39, respectively) were genotyped in 600 RA-patients of whom 2,846 sets of hands and feet X-rays were collected during 7 years follow-up. Subsequently, significantly associated SNPs were genotyped and studied in relation to 3,415 X-rays of 1,953 RA-patients; these included data-sets from Groningen (NL), Lund (SE), Sheffield (UK), NARAC (USA), Wichita (USA) and NDB (USA). The relative increase in progression rate per year in the presence of a genotype was determined in each cohort. An inverse variance weighting meta-analysis was done on the six datasets that together formed the replication-phase.

Results In the discovery-phase none of the *IL-4* SNPs and seven of the *IL-4R* SNPs were significantly associated with joint damage progression rate. In the replication-phase, two SNPs in *IL-4R* gene were significantly associated with joint damage progression rate (Rs1805011, $p = 0.02$ and Rs1119132, $p = 0.001$).

Conclusions Genetic variants in *IL-4R* were identified and independently replicated to associate with progression rate of joint damage in RA.

A7.11 GENETIC VARIATION IN PROMOTER SEQUENCE OF B-CELL-ACTIVATING FACTOR OF THE TNF FAMILY (BAFF) IN PATIENTS WITH IDIOPATHIC INFLAMMATORY MYOPATHIES (IIM)

doi:10.1136/annrheumdis-2013-203221.11

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