

**Background and Objectives** Identification of biomarkers for early diagnosis and treatment response would be beneficial for patients with early rheumatoid arthritis (ERA) to prevent ongoing joint damage. MiRNAs have features of potential biomarkers and an altered expression of miRNAs was shown in established RA. Our aim was to analyse RA-associated miR-223 and miR-16 in sera from patients with ERA to find markers of early disease, clinical activity or predictors of disease outcome.

**Materials and Methods** Clinical characteristics were obtained in 34 patients with ERA at baseline and after 3 (M3) and 12 (M12) months therapy with DMARDs. Total RNA was isolated using phenol-chloroform extraction from whole sera obtained at baseline and M3. Peripheral blood mononuclear cells (PBMC) from healthy donors were treated with methotrexate (MTX, 25 µg/ml) in vitro. Expression of miR-223 and miR-16 was analysed by TaqMan Real-time PCR.

**Results** Levels of miR-223 significantly decreased following therapy ( $p = 0.002$ ). In treatment naïve patients with ERA, the expression of miR-223 positively correlated with baseline DAS28 ( $p = 0.031$ ), change in DAS28 ( $\Delta$ DAS28) from baseline to M3 ( $p = 0.014$ ), baseline CRP ( $p = 0.008$ ) and count of peripheral leukocytes ( $p = 0.007$ ). The change in expression of miR-223 in sera may be attributable to the change in the count of leukocytes between baseline and M3 concluded from the positive correlations between these variables ( $p = 0.025$ ). In addition, the expression of miR-223 in PBMC was down regulated by 15% ( $p = 0.001$ ) after treatment with MTX.

Levels of miR-16 significantly increased ( $p = 0.008$ ) after 3 months of therapy and the increase in miR-16 was associated with the decrease in DAS28 from M3 to M12 ( $p = 0.002$ ).

**Conclusions** Our data support the potential of miR-223 to serve as a marker of disease activity in patients with treatment naïve ERA. Moreover, monitoring levels of miR-16 and miR-223 may become a useful tool to predict the disease outcome in patients with ERA.

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#### A7.4 ASSOCIATION OF GALECTIN SINGLE NUCLEOTIDE POLYMORPHISMS WITH AUTOIMMUNE DISEASES

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**Background and Objectives** Galectins are potent immune regulators. Surprisingly, genetic association of galectin genes with autoimmune diseases have not yet been studied. A polymorphism in the coding region of the galectin-8 gene (Rs2737713; F19Y) and a novel galectin-1 and interleukin 2 receptor  $\beta$  haplotype were investigated for association with rheumatoid arthritis and myasthenia gravis.

**Materials and Methods** A case-control analysis and a related quantitative trait-association study were performed to investigate the association of the galectin 8 gene polymorphism in patients (myasthenia gravis 149, rheumatoid arthritis 214 and 134 as primary and repetitive cohorts, respectively) and 365 ethnically matched (Caucasian) healthy controls. Distribution was also investigated in patients grouped according to their antibody status and age at disease onset. Comparative testing for lectin activity was carried out in ELISA/ELLA-based binding tests with both wild-type

and F19Y mutant galectin-8 from peripheral blood mononuclear cell lysates of healthy individuals with different genotypes as well as with recombinant wild-type and F19Y mutant galectin-8 proteins. Furthermore, we evaluated the association of regulatory region polymorphisms of the LGALS1 (Rs4820293, Rs4820294) and IL2R $\beta$  (Rs743777, Rs228941) genes in 146 Caucasian myasthenia gravis patients compared to 291 ethnically matched controls.

**Results** We found a strong association of the F19Y galectin 8 gene polymorphism with rheumatoid arthritis, and a mild one with myasthenia gravis. Moreover, the polymorphism also correlated with age at disease onset in the case of rheumatoid arthritis. The F19Y substitution did not appear to affect carbohydrate binding in solid-phase assays markedly. Also, a significant difference was found in the distribution of the Rs4820293/Rs743777 polymorphism haplotypes ( $p < 0.01$ ) in patients with myasthenia gravis and controls but not in rheumatoid arthritis. The Rs4820293 polymorphism of LGALS1, previously not described to be associated with any disease, did not affect LGALS1 expression in peripheral mononuclear cells and skeletal muscle.

**Conclusions** This is the first study of an association between a galectin-based polymorphisms leading to a mutant protein and autoimmune diseases, with evidence for antagonistic pleiotropy.

#### A7.5 COMBINED ANALYSIS OF EPIGENETIC AND TRANSCRIPTIONAL PROFILES IN DIFFERENT IMMUNE CELLS IDENTIFIES HOT SPOTS OF GENE REGULATION BY DNA METHYLATION

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**Background and Objectives** Methylation of DNA may contribute to the regulation of gene expression. Chip technology enables to analyse for methylation of CpG sites but requires a pre-selection of potential hot spots. Such a selection of sites is represented on the HumanMethylation450 array (Illumina). In order to test these CpG sites for possible functional effects, gene expression and DNA methylation were investigated between different immune cell types.

**Materials and Methods** Cells from 4 healthy donors were sorted by FACS technology for naïve and memory T-cells (CD4m, CD4n, CD8m, CD8n), B-cells (CD19m, CD19n), NK-cells (CD56), monocytes (CD14), and granulocytes (CD15). Genome-wide DNA methylation was assessed using the Illumina HumanMethylation450 BeadChip platform. Analysis of data was performed using GenomeStudio (Illumina). Gene expression data were collected from Affymetrix HG-U133P transcriptomes analysed in the BioRetis database. Mapping of CpG sites with genes was performed using the ensemble genome assembly GRCh37 genomic location map.

**Results** The number of differentially expressed genes or methylated CpG sites were highest between very different cell types like CD14 monocytes and CD4 T-cells (4624 genes; 19261 sites) and lower between naïve and memory cells of the same lymphocyte subtype (CD4: 638 genes; 9412 sites). There was a tendency towards more methylation in naïve (CD4n: 5433 sites  $\approx$ 2694 genes) compared to memory cells (CD4m: 3979 sites  $\approx$ 2258 genes). Overlap of differential expression with corresponding changes in methylation was found in only 629 (279) of 1951 increased (2673 decreased) expressed genes for CD14 versus CD4 comparison and 57 (53) of 332 (306) genes for CD4m versus CD4n cells. Of all CpG sites annotated to these identified genes, only about 10% were concordant with expression. These CpG site were within or immediately upstream of the annotated start of the gene with a maximum

distance of  $\approx 1500$  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

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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 file-maker 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.

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

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**Background and Objectives** The idiopathic inflammatory myopathies (IIM) are systemic connective tissue diseases in which auto-immune 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 radio-active 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.

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

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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.