infliximab treatment. In reviewing the literature we find that anti TNF paradoxically brings about an immediate response in erythema nodosum patients, however provokes erythema nodosum and other skin manifestations in patients with either rheumatic pathology or inflammatory bowel disease. [1, 2]

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7. Genetics and epigenetics of rheumatic diseases

A7.1 A GENETIC VARIANT IN THE REGION OF MMP-9 IS ASSOCIATED WITH SERUM LEVELS AND PROGRESSION OF JOINT DAMAGE IN RHEUMATOID ARTHRITIS

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Background and Objectives The severity of joint destruction is highly variable between Rheumatoid Arthritis (RA) patients. We aimed to identify new genetic risk factors by studying genetic susceptibility loci of several auto-immune diseases.

Patients and Methods In phase-1, 646 Dutch RA-patients with yearly X-rays of hands and feet over 7 years follow-up were genotyped for 148,880 SNPs by Immunochip which contains 186 loci previously associated with autoimmune diseases. Association of SNPs with MAF > 0.01 (130,841 SNPs) with joint destruction was analysed using a marginal regression model. Correction for multiple testing was done by Bonferroni correction for the number of uncorrelated SNPs (threshold $p < 1.1 \times 10^{-6}$). In phase-2, 686 North American RA-patients with repeated hands X-rays over 15 years follow-up, for which Immunochip genotyping data were also available, were studied. SNPs that were significantly associated in phase-1 were selected and evaluated. All X-rays were scored by Sharp van der Heijde score (ICC 0.91 and 0.98 for phase-1 and 2 respectively). MMP-9 levels were measured in baseline serum by ELISA (Ebioscience) in 120 RA-patients that were selected on the Rs11908352-genotype.

Results In phase-1, 109 SNPs were significantly associated with joint destruction ($p < 1.1 \times 10^{-6}$). Of these, 76 variants were on the HLA region. The 33 non-HLA genetic variants, though several were in high LD, were studied in the North-American RA-patients. Here, after correction for the number of uncorrelated SNPs (threshold $p < 0.0036$), two variants were associated with the severity of joint destruction: Rs451066 on chromosome 14 ($p_{\text{uncorrected}} = 0.002$, MAF = 0.20) and Rs11908352 on chromosome 20 ($p_{\text{uncorrected}} = 0.002$, MAF = 0.21). The region of Rs451066 on chromosome 14 has previously been linked to type-1 diabetes susceptibility. Presence of a risk allele was associated with a 3.7% higher rate of joint destruction per year; this equaled 29% over 7-years. Rs11908352 is located at the MMP-9 region on chromosome 20. Patients with a risk allele had a 2.7% higher radiological progression rate per year, which equaled 20% more joint destruction over a 7-years period. Furthermore, the minor genotype was associated with significantly higher levels of MMP-9 compared to the common genotype ($p = 0.007$).

Conclusions Two new risk loci for progressive joint destruction in RA were identified (Rs451066 and Rs11908352). The risk allele in Rs11908352 also associated with higher serum MMP-9 levels, indicating to a role for MMP-9 in progression of joint destruction in RA. **Acknowledgements** RACI study group, Dutch Arthritis Foundation, Netherlands organisation for scientific research.

A7.2 ALLOGRAFT INFLAMMATORY FACTOR 1 (AIF1) POLYMORPHISMS IN FRENCH CAUCASIANS WITH RHEUMATOID ARTHRITIS

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Background Allograft inflammatory factor 1 (AIF1) is a cytoplasmic inflammatory protein encoded within the HLA class III genomic region on chromosome 6 (6p21.3). Although several risk loci for Rheumatoid Arthritis (RA) have been identified by Genome Wide Association Studies (GWAS), none of them involved AIF1 polymorphisms. However, two studies on small cohorts have shown that AIF1 single nucleotide polymorphism (SNP) Rs2269475 (C/T), causing a non-synonymous change of amino acid, is associated with RA (Harney, SM *et al*, 2008; Pawlik A *et al*, 2008). Moreover, AIF1 overexpression in inflammatory synovial tissues and macrophages isolated from synovial fluids of patients with RA, confirms its potential role in RA.

Objective We propose to examine the association of the seven most described AIF1 SNPs in our French RA patients.

Methods We have tested 99 Anti-Citrullinated Protein Antibody (ACPA) positive Caucasian RA patients who fulfilled ACR/EULAR criteria and 104 healthy Caucasians. We designed AIF1 primers to specifically amplify the AIF1 gene region containing the 7 SNPs: Rs2844475, Rs4711274, Rs2736182, Rs2736181, Rs2259571, Rs2269475 and Rs13195276. PCR products were sequenced (Cogenics Beckman Coulter) and chromatogram results analysed for the 7 SNPs positions in patients and controls. Patients and controls were genotyped for HLA-DRB1.

Results Two SNPs out of the 7 were associated with RA: Rs4711274 (G/A) and Rs2269475 (C/T). Regarding Rs4711274, G/A and A/A genotypes were increased when compared with controls ($p = 0.0005$). The minor A allele was strongly associated with RA ($p = 0.0005$). Regarding Rs2269475, in linkage disequilibrium with the former, we found a similar pattern with increased T/T and C/T genotypes ($p = 0.0009$) and increased minor T allele frequency ($p = 0.0008$) in patients with RA. Interestingly, patients carrying the minor associated AIF1 allele expressed HLA-DRB*04 more often than the patient's group carrying the C/C or G/G genotype (63.8% versus 44.4%), although the difference was marginal ($p = 0.06$).

Conclusions In this study of French Caucasians with RA, we confirmed Rs2269475 association already described in British and Polish Caucasians. Additionally, we find an association with Rs4711274 in linkage disequilibrium with Rs2269475. Intriguingly, such associations have never been found in GWAS.

A7.3 ASSOCIATION OF CIRCULATING MIR-223 AND MIR-16 WITH DISEASE ACTIVITY IN PATIENTS WITH EARLY RHEUMATOID ARTHRITIS

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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 (Δ 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 β 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 β (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 sites were within or immediately upstream of the annotated start of the gene with a maximum