Background and Objectives ETS1 is the founder member of ETS transcription factors' family which control the transcription of various immune related genes. The aim of this study was to identify if polymorphism ETS1 Rs11221332, described in Caucasian subjects, plays any role in rheumatoid arthritis (RA) susceptibility.
Materials and Methods We genotyped this polymorphism in 136 unrelated patients with RA and 147 healthy individuals with no history of autoimmune diseases. Genotyping was performed with polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay and the data were analysed by the SPSS statistical software.
Results Statistical significant difference was observed in the distribution of Rs11221332 genotypes between RA patients and controls ($p = 0.041$). Comparing Rs11221332 alleles' distribution between the studied groups the difference was higher [$p = 0.039$, (OR = 1.504, CI:1.036–2.183)].
Conclusions The present study revealed, for first time, the positive association of a polymorphism in the sequence of ETS1 transcription factor with RA susceptibility. Further studies in other ethnic group of patients are needed to confirm the results of the present genetic association study related to ETS1, a widely used transcription factor in the regulation of various genes' expression.

A7.23 THE HLA LOCUS CONTAINS NOVEL FOETAL SUSCEPTIBILITY ALLELES FOR CONGENITAL HEART BLOCK WITH SIGNIFICANT PATERNAL INFLUENCE

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Objective To identify foetal susceptibility genes in Ro/SSA autoantibody-mediated congenital heart block on chromosome six.
Methods Single nucleotide polymorphism (SNP) genotyping of individuals included in the Swedish Congenital Heart Block (CHB) study population was performed. Low resolution HLA-A, -Cw and -DRB1 allele typing was carried out in 86 families of the study population comprising 339 individuals (86 Ro/SSA autoantibody positive mothers, 71 fathers, 87 CHB index cases and 95 unaffected siblings).
Results A case-control comparison between index cases and population-based out of study controls (n = 1710) revealed an association of CHB with fifteen SNPs in the 6p21.3 MHC locus at a chromosome-wide significance of $p < 2.59 \times 10^{-6}$ (OR 2.21–3.12). In a family-based analysis between SNP markers as well as distinct MHC class I and II alleles with CHB we observed associations to HLA-DRB1*04 and HLA-Cw*05 variants that were significantly more often transmitted to affected individuals ($p < 0.03$ and $p < 0.05$, respectively), and HLA-DRB1*13 and HLA-Cw*06 variants which were significantly less often transmitted to affected children ($p < 0.05$ and $p < 0.04$). We further observed a significant association of increased paternal, but not maternal, HLA-DRB1*04 transmissions to the affected offspring ($p < 0.02$).
Conclusions Our study identifies HLA-DRB1*04 and HLA-Cw*05 as novel foetal HLA-allele variants that confer susceptibility to develop CHB in response to exposure to Ro/SSA autoantibodies, while DRB1*13 and Cw*06 emerged as protective alleles. For the first time, we also demonstrate paternal contribution to foetal susceptibility to CHB.

A7.24 THE PENTANUCLEOTIDE INSERTION IN HSPA1B GENE IS ASSOCIATED WITH IDIOPATHIC INFLAMMATORY MYOPATHY

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Background and Objectives The HSPA1B gene is one of the three HSP70 genes located within the Major Histocompatibility Complex (MHC) on chromosome 6p21. The HSP70 molecules in their intracellular form have antiapoptotic function and are responsible for stabilisation of protein structure; in their extracellular form, they act as mediators of immune response. The extracellular HSPs are part of the innate and adaptive immune response and are involved in the process of antigen presentation. The aim of our study was to find out if an association between polymorphisms of MHC located HSP70 genes and subgroups of idiopathic inflammatory myopathy exists.
Materials and Methods We have analysed 177 patients suffering from idiopathic inflammatory myopathy (82 patients with dermatomyositis – DM, 71 patients with polymyositis – PM, 22 patients with cancer associated myositis, 2 patients with inclusion body myositis) and 59 healthy controls. In total, six genetic polymorphisms located within the three HSP70 genes were analysed by direct genomic DNA sequencing. The statistical analysis was done using Fisher's exact test with calculated $p < 0.05$ considered as statistically significant.
Results and Conclusions The frequency of the "INS" allele of the pentanucleotide insertion polymorphism in HSPA1B (Rs9281590) was increased in patients suffering from myositis (43.79%) in comparison with controls (32.20%; $p < 0.05$). The Odds Ratio calculated for this polymorphism was 1.64 (CI95% 1.056; 2.545). Its increased frequency was predominantly found in DM patients ($p < 0.05$); the allele distribution in PM patients did not significantly differ from controls. Presence of INS allele was strongly related to the well described HLA associated risk, the HLA-DRB1*03 allele ($p < 0.001$), found mostly in PM patients. INS allele is independent on other myositis associated HLA allele, HLA-DRB1*16, found increased in the population of our DM patients. Other polymorphisms analysed in this study did not show any relation to the myositis. Our findings support the hypothesis, that DM and PM have partially different genetic background.
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A7.25 GENE-GENE INTERACTIONS IN INTERFERON PATHWAY GENE POLYMORPHISMS IN EUROPEAN AND AMERICAN SCLERODERMA COHORTS

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Background/Purpose Type-I interferon (IFN), a central mediator of innate immunity, has been shown to be the hallmark peripheral blood gene expression pattern in lupus (SLE) and a similar type-I IFN signature has been noted in systemic sclerosis (SSc). Interferon regulatory factor 5 and 7(*IRF5/IRF7*) and tyrosine kinase 2(*TYK2*) are important genes involved in this signalling cascade. The purpose of this work was to investigate the association and interaction of *IRF5, IRF7, TYK2* polymorphisms with SSc.

Methods We performed SNP genotyping for *IRF5*(Rs20024640), *IRF7(*Rs1061502), *TYK2*(Rs2304256) genes using the Taqman Assay in 4 large cohorts comprising of North-American Caucasian, Dutch, Italian and Spanish samples totaling to 2,091 SSc patients and 1,434 race-matched controls. All SSc patients fulfilled ACR criteria or had at least 3 of the 5 CREST features. HWE testing, chi-square, logistic regression(LR) were used for statistical comparisons. Mesoscale assays were used for cytokine detection.

Results LR analysis after controlling for gender and cohorts the association was confirmed in all cohorts {*IRF5*: P < 0.0001, OR(CI)–0.68(0.6–0.8); *IRF7*: P = 0.006, OR(CI)–0.80(0.7–0.9); *TYK2*:P = 0.05, OR(CI)–0.85(0.7–0.99)}. The association was stronger with anti-centromere subset {*IRF5*:P = 0.0002, OR(CI–0.59(0.5–0.8); *IRF7*:P = 0.0008, OR(CI)–0.69(0.6–0.9); *TYK2*:P = 0.04, OR(CI)–0.79(0.6–0.9)}.

The data was modelled based on mode of inheritance for the 3 SNPs and LR analysis performed controlling for gender and cohorts and revealed an extremely protective effect for the combination of mutations versus the wildtype {$IRF5^{M}/IRF7^{M}/TYK2^{M}$ versus $IRF5^{WT}/IRF7^{WT}/TYK2^{WT}$:P < 0.0001; OR(CI)–0.39(0.3–0.6)}.

In the $IRF5^{WT}/IRF7^{WT}/TYK2^{WT}$ group, the SSc patients had increased levels of TNF-α and IL-6 as compared to controls and there was no difference amongst the patients and controls in the $IRF5^{M}/IRF7^{M}/TYK2^{M}$ group.

Conclusions We demonstrate association of *IRF5, IRF7* and *TYK2* SNPs with SSc.

We demonstrate a gene-gene interaction in SSc between three non-linked loci- *IRF5, IRF7* and *TYK2*.

The 3 gene-SNPs have a protective effect in SSc patients and the presence of the 3 mutations simultaneously has the most protective effect.

Plasma TNF-α and IL-6 levels were increased in the SSc patients wildtype for the 3 SNPs versus controls, whereas there was no difference in TNF-α and IL-6 levels in the SSc patients having mutations for the 3 SNPs versus controls.

In summary, IRF5, IRF7, TYK2 SNPs have a protective effect in SSc which is stronger when there are polymorphisms on all of the genes as compared to each of them alone.

This suggests an important role of interferon pathway polymorphisms in susceptibility to SSc and the exact role of these interactions and their function in SSc susceptibility needs to be elucidated experimentally.

8. Bone/cartilage biology

A8.1 ALARMINS S100A8/A9 CAUSE OSTEOPHYTE FORMATION IN EXPERIMENTAL OSTEOARTHRITIS WITH HIGH SYNOVIAL INVOLVEMENT

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Background and Objectives Osteophyte formation is an important hallmark of osteoarthritis (OA) causing limited joint movement and pain. There is increasing belief that synovial activation contributes to OA pathology. As shown recently in our lab, alarmins S100A8 and S100A9 (major products of synovial macrophages) are involved in cartilage degradation and synovial activation during human and murine OA.

In the current study, we explored the involvement of S100A8/A9 in osteophyte formation in experimental OA.

Methods Experimental OA was elicited in C57Bl/6 (WT) mice and $S100A9^{-/-}$ mice, which also lack functional S100A8. Collagenase induced OA (CIOA) was induced by two times intra-articular injection of 1U collagenase, DMM was induced by transsection of the medial anterior meniscotibial ligament leading to destabilisation of the medial meniscus (DMM). Osteophyte size was assessed by a blind observer using Leica Application Suite (LAS) imaging software. Chondrogenesis was induced by bringing human foetal mesenchymal stem cells (MSCs) in pellet and stimulating for 5 days with BMP-2 and TGFβ1, with or without human recombinant S100A8. Proteoglycan content was quantified using the LAS imaging software on SafO stained sections.

Results First, we measured osteophyte size in $S100A9^{-/-}$ mice at day 42 of CIOA. Synovial activation is high in CIOA and this is significantly reduced in $S100A9^{-/-}$ mice. Osteophyte size was dramatically reduced in the $S100A9^{-/-}$ compared to WT in the medial collateral ligament (92.5% reduction) but also significantly at the medial side of both tibia and femur (68.2% and 64.6% reduction) (n = 10).

One explanation for the reduced osteophyte size in $S100A9^{-/-}$ mice may be a direct effect of S100-proteins on chondrogenesis. To investigate this, we stimulated MSCs in pellet culture with BMP-2 and TGFβ1, supplemented with 1 and 5 µg/ml S100A8. Proteoglycan deposition as measured by redness in SafO staining was increased 27% and 71% respectively, indicating that S100A8 stimulates chondrogenesis.

Finally, we determined osteophyte size in the DMM model, in which synovial involvement is very low. At day 56, we observed no significant differences in osteophyte size between the $S100A9^{-/-}$ and WT at the medial femur and tibia (105% and 136% of WT, n = 8). This confirms the importance of the synovium in the S100-effect on osteophyte development.

Conclusions S100A8/S100A9 play a crucial role in osteophyte formation in an OA model that shows clear synovial involvement, probably by stimulating chondrogenesis.

Considering also the deleterious effect of S100A8/A9 on joint destruction in OA, targeting these alarmins during OA may be very promising.

A8.2 ANTI CITRULLINATED PROTEIN ANTIBODIES FROM SYNOVIAL FLUID OF RHEUMATOID ARTHRITIS PATIENTS ENHANCE OSTEOCLASTOGENESIS

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Background/Purpose Presence of anti CCP2 antibodies identifies a subgroup of RA patients that are more prone to develop bone erosions. We hypothesised that anti CCP2 IgG might have a direct effect on bone, and thus investigated the effect of anti CCP2 IgG isolated from synovial fluid (SF) of RA patients on osteoclastogenesis and bone destruction in an in vitro system.

Methods IgG were isolated on Protein G columns from SF of 26 RA patients and applied on CCP2 affinity columns. Pools of the purified anti-CCP2 and flow through IgG fractions were tested for the ability to influence osteoclastogenesis (TRAP positive multinucleated cells) and bone destruction (% of resorption area on osteologic discs). To do this immature dendritic cells derived from CD14+ cells from peripheral blood of healthy individuals were cultured in the presence of RANKL and M-CSF, with or without CCP2 IgG or flow through IgG (at a final concentration of 100 ng/ml).

Results The CCP2 IgG pool induced a significant mean ± SEM of 1.5 ± 0.1 fold increase in the number of osteoclasts formed from immature dendritic cells in the presence of RANKL, while no such effect was observed with flow through IgG fractions. Osteoclasts cultured in the presence of the CCP2 IgG induced a significant mean ± SEM of 3.4 ± 1.3 fold increase of bone resorption while no such effect was observed for the flow through fractions.