OP0286

GENOTYPIC EFFECTS OF ANKYLOSING SPONDYLITIS ASSOCIATED IL7R POLYMORPHISMS ARE MEDIATED THROUGH MONOCYTES IN INFLAMMATION

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Background: Interleukin 7 (IL-7) plays a key role in T cell survival and proliferation. Both cell-surface expressed and soluble forms of the IL-7 receptor (sIL7R) are recognised. sIL7R has been shown to prolong IL-7 activity in inflammation.¹ IL7R polymorphisms are associated with multiple inflammatory diseases including ankylosing spondylitis (AS). Higher levels of circulating sIL7R are present in those carrying the risk variant but the cellular sources of soluble IL7R are unclear. Expression quantitative trait loci (eQTL) studies have shown genetically regulated IL7R gene upregulation in monocytes after innate immune stimuli.²

Objectives: This study aims to characterise the genotypic effects of IL7R polymorphism on monocyte protein surface expression and sIL7R release.

Methods: Monocyte cell surface IL7R expression was measured by flow cytometry in the presence or absence of LPS or TNF in a cohort of volunteers recruited from the Oxford biobank. Soluble IL7R was quantified by ELISA in purified monocyte cultures stimulated with LPS. RNA sequencing was performed for 8 paired samples of control and recombinant IL-7 exposed stimulated monocytes.

Results: Surface IL7R expression was induced after 24 hours of LPS stimulation both in isolated monocytes (n=84, p<8.9e-19) and CD14 +cells in whole PBMC cultures (n=103, p<3.9e-31). We find the key genotypic regulator of this response to be rs6897932, previously associated with AS predisposition, both in isolated monocytes (n=85, p=9e-7) and CD14 +cells in whole PBMC cultures (n=103, p=9.4e-5). There was no genotypic effect seen in unstimulated monocytes. Notably IL7R positivity of CD4+, CD8 +and CD56+cells measured within the PBMC culture both before or after LPS stimulation showed no association with rs6897932. The addition of anti-TNF (Infliximab) abrogated the genotypic effect. In a second independent cohort, genotype-specific surface IL7R induction was also observed after stimulation with recombinant human TNF (n=62, p=0.0007). In a third cohort, release of soluble IL7R was observed to be under genetic control after stimulation with LPS (n=99, p<3.1e-11). RNA sequencing of isolated monocytes stimulated with LPS and recombinant IL-7 showed differential expression of 3240 transcripts (FDR<0.05) compared with LPS alone (n=8). Direct ex-vivo staining of monocytes from inflamed synovial fluid of SpA showed significant upregulation of IL7R compared to paired PBMC monocytes (n=4, p<0.015).

Conclusions: Monocytes upregulate IL7R expression and soluble IL7R secretion after LPS treatment in a functional, genotype- and TNFalpha-dependant manner. SpA synovial monocytes express IL7R suggesting preactivation. These data draw attention to an unappreciated key myeloid role for AS risk variants at IL7R.

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OP0287

GENE ENHANCERS ASSOCIATED WITH AN INCREASED RISK OF DEVELOPING JIA FAIL TO DOWN REGULATE RUNX1 AFTER CELL STIMULATION

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Background: In recent years there have been tremendous strides in determining the genetic component of complex diseases, not least in Juvenile Idiopathic Arthritis (JIA). We now have 17 genetic loci robustly associated with an increased risk for developing JIA.¹ In JIA, like all complex diseases, the regions of the genome harbouring genetic changes are found outside protein coding DNA, in regions of the genome shown to control gene expression, often sited some distance from the closest gene. One of these JIA associated regions, on chromosome 21q22, is labelled with the *RUNX1* gene, although the associated variants are situated over 450 kb from the gene. We have previously demonstrated, through Capture HiC analysis, that the genomic region containing the JIA associated variants makes a physical contact with the *RUNX1* promoter. The next challenge in this region, therefore, is to determine how a risk genetic background effects the expression of the gene, contributing to the susceptibility to JIA.

Objectives: Determine the function and mechanism of how the gene enhancer region, situated over 450 kb upstream of the *RUNX1* gene, works to increase the risk of developing JIA.

Methods: B-lymphocyte cell lines, containing either a risk or protective JIA genetic background at the 'RUNX1' loci were interrogated for potential causative functional mechanism. Allele specific interactions were investigated using chromosome conformation capture (3C), enhancer activity (H3K4me1) with chromatin immunoprecipitation (ChIP), and the resultant expression of the *RUNX1* gene analysed by qPCR in both stimulated (IL4/anti CD40) and unstimulated cell lines.

Results: The enhancer region associated with JIA interacts with the *RUNX1* promoter in B-cell lines. A stimulation specific effect is observed in B-cell lines harbouring the protective alleles. In this protective context, prior to stimulation *RUNX1* expression is higher than in the risk genotype with enhancer enrichment detected near the proximal promoter. After stimulation the interaction between the enhancer and *RUNX1* promoter is much stronger, and whilst no enhancer enrichment was detected, this increased interaction frequency after stimulation corresponds to a sharp downregulation of *RUNX1* expression. In contrast, the risk genotype showed no stimulation dependant change, maintaining both interaction and expression levels.

Conclusions: 3C and ChIP experiments were performed to investigate genotype and stimulation specific DNA-DNA and DNA-protein interactions and identify correlation with gene expression. These results suggest that the ability for B-cells to switch from relatively high expression of the *RUNX1* transcript to low expression upon stimulation is crucial to an appropriate immune response.

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OP0288

TRANSCRIPTIONAL PERTURBATION OF RA-RISK ENHANCER BY CRISPR-DEADCA9 REGULATES LONG RANGE GENE TARGETS

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Background: Findings from genome wide association studies in complex diseases indicate over 90% of genetic variants associated with risk of developing disease are found outside protein coding regions, suggesting regulation of gene expression is key to disease susceptibility. For rheumatoid arthritis (RA) it has been demonstrated that risk variants are found in gene regulation regions, and are significantly enriched in T-cell specific enhancers. In addition, a significant proportion of associated variants lay some distance from the nearest gene and enhancers may not necessarily regulate the closest gene, effectively 'skipping' genes. Using chromatin conformation technology (HiC) we have demonstrated that an enhancer region intronic of the *COG6* gene, containing variants associated with RA, make robust physical contact with the promoter of *FOXO1*, almost 1 Mb away on the linear chromosome. *COG6* is not an obvious candidate risk gene for RA, whilst *FOXO1* is involved in T-cell development and shown to be over expressed in RA synovium. The challenge now is to provide empirical evidence that the enhancer found within *COG6* does regulate *FOXO1* expression, and how an RA risk genetic background affects this regulation.

Objectives: Use CRISPR-Cas9, to perturb the COG6 intronic enhancer region and measure the downstream effect on the expression of FOXO1.

Methods: We utilised a modified form of the Cas9 enzyme, dead Cas9 (dCas9), that can precisely target DNA, but will not induce a cut. Using the dCas9 attached to either enhancers (p300) or repressors (KRAB) of expression we investigated how perturbation of the enhancer intronic of COG6 changed the expression of FOXO1.

We designed 3 guides across the COG6 enhancer, and transduced a cell line (HEK293) using a lentiviral dCas9 CRISPR system, with either dCas9-KRAB or dCas9-p300 and each of the three guides. We cultured the cells until 70%–80% confluent, GFP sorted the cells and then extracted RNA. A quantitative PCR was performed (QuantStudio) for both COG6 and FOXO1 gene transcript expression and normalised to housekeeping genes.

Results: Up to 90% of HEK cells were transduced with the dCas9 enzyme and guide, and these were sorted by FACS using GFP to sort the top 60%. The 3 guides gave consistently increased levels of FOXO1 expression with the dCas9-p300, compared to both control and dCas9-KRAB ($p=0.02$). This was particularly evident for guide 3, with a 40% increase (p300) and 10% decrease (KRAB) of FOXO1 expression observed. Expression of COG6 was also perturbed, but in a less consistent manner, with both increase and decrease expression for KRAB and p300.

Conclusions: Over 90% of HEK cells were transduced with the dCas9 enzyme and guide, and these were sorted by FACS using GFP to sort the top 60%. The 3 guides gave consistently increased levels of FOXO1 expression with the dCas9-p300, compared to both control and dCas9-KRAB ($p=0.02$). This was particularly evident for guide 3, with a 40% increase (p300) and 10% decrease (KRAB) of FOXO1 expression observed. Expression of COG6 was also perturbed, but in a less consistent manner, with both increase and decrease expression for KRAB and p300.

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OP0289

INTEGRATION OF CHROMATIN CONFORMATION, TRANSCRIPTOME AND GENOME-WIDE LANDSCAPE OF BRD2 AND BRD4 BINDING MOTIFS IDENTIFIES MECHANISMS OF BET INHIBITOR ACTION IN RHEUMATOID ARTHRITIS SYNOVIAL FIBROBLASTS

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Background: Rheumatoid arthritis (RA) is a chronic autoimmune disease characterised by infiltration of immune cells into the synovium and hyperplasia of the synovial lining, resulting in the formation of pannus that degrades cartilage and bone. Fibroblast-like synoviocytes (FLS) are the main cell types of the rheumatoid synovium and possess phenotypic and molecular characteristics of transformed cells. JQ1, an inhibitor of the bromodomain and extra terminal domain (BET) family that includes BRD2, BRD3, BRD4 and BRDT has shown efficacy *in vitro* on RA-FLS proliferation and *in vivo* in a murine model of arthritis.

Objectives: We sought to elucidate the mechanism of action of BET proteins in FLS biology and determine the potential therapeutic utility of targeting BRD2/BRD4 for RA disease treatment and interception.

Methods: To understand the mechanism of JQ1 action, we subjected JQ1-treated RA-FLS to transcriptional profiling by RNA-Seq and determined BRD2 and BRD4 cistromes by identifying global BRD2/BRD4 chromatin binding sites by ChIP-Seq. In addition, Assay for Transposase Accessible Chromatin by high throughput Sequencing (ATAC-Seq) was employed to identify open and closed regions of chromatin in JQ1-treated RA-FLS.

Results: We demonstrate that the active isomer of JQ1 but not its inactive isomer inhibits IL-1b-induced RA-FLS activation and proliferation. Through an integrated analysis of RNA-Seq, ATAC-Seq to profile changes in chromatin accessibility and ChIP-Seq of BRD2/4 and Pol2 proteins, we found that JQ1 inhibited multiple key inflammatory pathways, and altered the genome wide occupancy of crucial transcription factors involved in inflammatory signalling. Specifically, JQ1 treatment resulted in reduced occupancy of both BRD2 and BRD4 in approximately 2000 regions genome-wide, and a loss of Pol2 occupancy in approximately 600 genomic regions. Collectively we found that 105 genes had altered occupancy in all three proteins (BRD2, BRD4 and Pol2) and were also differentially expressed. Most prominently, JQ1 resulted in down regulation of IL6, IL8, p38 MAP kinase and HMGB1/TLR4 signalling pathways. In addition, we have identified BRD2/BRD4 super-enhancer genes and demonstrate that JQ1 altered BRD2/BRD4 occupancy in the IL6 and IL8 super-enhancer regions, and significantly down-regulated IL6, IL8, TLR4 and IL1b expression.

Conclusions: Our results suggest pleiotropic effects of JQ1 on pathways that have been individually targeted and shown to be efficacious for the treatment of RA. These studies provide a strong rationale for targeting of BRD2/BRD4 in RA.

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FRIDAY, 15 JUNE 2018

PARE abstract session

OP0290-PARE KEEP AN EYE OUT FOR INVISIBLE PAINS – A DANISH CAMPAIGN

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Background: How does it feel to live with a RMD and have chronic pain? How do you explain pain caused by RMD to your friends and family? How do you make others understand how pain affects your daily life? The communication department of the Danish Rheumatism Association needed to convey living with invisible pains, because it is of such importance to people with RMDs. Based on interviews with the users (people with RMDs) we found the best way of making others understand was to create a film, which illustrated living with invisible pain.

Objectives: We wanted to increase the general understanding of RMDs and living with invisible pain in society. The secondary objective was to brand awareness of the Danish Rheumatism Association and to generate leads for further processing, e.g. signing up for membership.

Methods: Based on a few interviews with users and several posts from Social Media we focused on making invisible pain visible. By choosing the media of a film we could show what the users found difficult putting into words. The film had to be short to make it possible to share, e.g. on Facebook, and by choosing a film without speak we avoided language barriers. Through adverts we retargeted visitors to our website and generated more members of the association.

Results: We launched the film on World Arthritis Day 2017 In 3½ weeks the film reached 1 million users and more than 1000 comments on Facebook, and the number of followers went up by 2000. The total impact of the film will be presented in June 2018.