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.