Poster Presentations

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Genomics, genetic basis of disease and HLA / T cell recognition

THU0001 DIFFERENTIAL METHYLATION AS A POTENTIAL BIOMARKER OF METHOTREXATE RESPONSE IN PATIENTS WITH RHEUMATOID ARTHRITIS

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Background: Methotrexate (MTX) is the first-line disease modifying anti-rheumatic drug for the treatment of rheumatoid arthritis (RA). However, many patients do not respond adequately or experience adverse effects^{1,2}; therefore, identifying blood-based biomarkers that predict treatment response is a research priority. DNA methylation is an epigenetic marker that modifies but does not alter DNA sequence, and it is thought that MTX may act, at least in part, by inhibiting intracellular methyl donor transfer leading to DNA hypomethylation².

Objectives: We aimed to identify differential DNA methylation signatures in whole blood, which may act as biomarkers predictive of response to MTX in patients with RA.

Methods: DNA methylation was measured using the HumanMethylation450 BeadChip in DNA samples from individuals recruited to the Rheumatoid Arthritis Medication Study (RAMS), a one year observational study in the UK including patients with RA starting MTX for the first time. In RAMS, demographic and clinical data are collected prior MTX start (baseline) and at 6 months after commencing MTX. DNA was extracted from whole blood samples collected baseline and at 4 weeks from patients who, at 6 months, had a EULAR good response (n=36) or EULAR poor response (n=36) to MTX. Differentially methylated positions (DMPs) between the baseline and 4 weeks, and between good and poor response were identified using linear regression, adjusting for gender, age, cell composition, baseline disease activity score (DAS28), and smoking status. Analyses also compared methylation with changes in DAS28 and the individual DAS28 components over 6 months. DMPs that showed significant differences in the test cohort were selected for replication by pyrosequencing in an independent group of 100 patients with both baseline and 4 week samples.

Results: Based on percentage change in methylation between pre-treatment and following 4 weeks of therapy, two DMPs were significantly associated with response status in samples taken at 4 weeks (p-value <10⁻⁵). Three additional DMPs were associated with change in tender joint count, whilst three other DMPs were associated with change in swollen joint count, and a further four DMPs associated with change in C-reactive protein. Of the four DMPs tested to date, hypermethylation at cg23700278 at baseline suggests replicated association with improvement in swollen joint count by 6 months. The nearest gene to the cg23700278 locus is adrenoceptor alpha 2C (*ADRA2C*), involved in neurotransmission.

Conclusions: These preliminary results suggest DNA methylation may provide a biomarker of MTX response but requires replication in other data sets.

References:

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- Kim Y, et al. (1996) J. Lab.Clin.Med. 128:165–172.

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THU0002 ETANERCEPT AND ADALIMUMAB EXHIBIT HETEROGENEOUS EARLY SIGNATURES OF RESPONSE IN RHEUMATOID ARTHRITIS THERAPY

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Background: Up to 40% of rheumatoid arthritis (RA) patients exhibit insufficient response to TNF inhibitor (TNFi) therapy, which has an adverse effect on long term outcomes. Without reliable biomarkers to direct treatment decisions, many non-responder (NR) patients experience a delay in switching to an alternative therapy. Ideally, blood-based biomarkers would be measured before treatment (baseline) and throughout treatment in order to select and monitor therapeutic response to maximise the chances of responding to the first biologic therapy.

Objectives: To compare transcriptomic changes between patients administered etanercept and adalimumab therapy, and to identify biomarkers to predict or monitor response.

Methods: From the Biologics in RA Genetics and Genomics Study Syndicate (BRAGGSS) cohort, 37 EULAR good-responders in clinical remission (GR) and 18 NR to etanercept, and 50 GR and 20 NR to adalimumab were selected. Total RNA was isolated from Tempus™-stabilised whole blood samples collected at baseline and following 3-months (3M) of therapy using the MagMAX™ RNA extraction kit. RNA was amplified and converted into biotinylated sense-strand DNA using the Affymetrix WT PLUS kit for hybridisation onto Affymetrix GeneChip® Human Transcriptome arrays. Quality control and differential expression/splice analysis were assessed using the Affymetrix Expression and Transcriptome Analysis Console™ and appropriate Bioconductor packages. Differential transcript expression was adjusted for baseline DAS, age, gender and concurrent DMARD therapy. Pathway analysis was performed using the Database for Annotation, Visualization and Integrated Discovery (DAVID) and Ingenuity Pathway Analysis (IPA) tools.

Results: In adalimumab GR, 636 genes were downregulated and 253 upregulated at 3M (FDR $p < 0.05$, fold-change > 1.2). There was significant upregulation of immune cell components, most notably HLA genes including *HLA-DRB1*, other RA susceptibility genes (*SLC24A4*, *PADI4* and *CD28*) and many B and T cell signalling genes. Etanercept GR exhibited a milder transcriptomic change overall, showing little overlap with adalimumab GR; 395 genes were downregulated and 27 upregulated at 3M (FDR $p < 0.05$, fold-change > 1.2). Downregulated genes included downstream TNF components such as mitogen activated protein (MAP) kinases, as well as genes involved in NOD-like receptor, Toll-like receptor and NF- κ B signalling. Such significant changes were absent in NR to adalimumab and etanercept. Furthermore, alternative splice changes in RA-relevant genes such as *MMP9* were apparent in adalimumab GR at 3M but not etanercept GR.

Conclusions: The heterogeneity in the blood-based transcriptomic profiles of etanercept and adalimumab response observed herein suggests that different TNFi therapies function by alternative mechanisms that impact patient outcomes. It also calls into question the reliability of response studies that consider TNFi therapies as a homogenous group. The candidate biomarkers identified require replication in independent datasets but may provide early and objective response biomarkers to inform timely therapeutic switching in patients who are not responding to their current TNFi drug.

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THU0003 CD4+ AND B LYMPHOCYTE EXPRESSION QUANTITATIVE TRAITS AT RHEUMATOID ARTHRITIS RISK LOCI IN UNTREATED EARLY ARTHRITIS: IMPLICATIONS FOR CAUSAL GENE IDENTIFICATION?

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Background: Rheumatoid arthritis (RA) is a genetically complex disease of immune dysregulation. Genome-wide association scans (GWAS) have confirmed its association with variants at >100 genetic loci. Outside of the HLA region,

accumulating data now highlight an overlap between these risk loci and cell-specific enhancer elements that is maximal in CD4+ lymphocytes, followed by B lymphocytes

Objectives: Seeking insight into genetic risk mechanisms, we conducted and compared expression quantitative trait locus (eQTL) analyses of risk loci in CD4+ T cells and B cells from carefully phenotyped early arthritis patients naïve to therapeutic immunomodulation.

Methods: 254 patients donated RNA and DNA from purified B and/or CD4+ T-cells within 4 hours of blood draw. Genotyping and global gene expression measurement were carried out using the Illumina Human CoreExome array and either HT12v4 or WG6v3 BeadChip arrays respectively. Variants in linkage disequilibrium (LD) with 101 confirmed non-HLA RA- SNPs ($r^2>0.8$) were analysed, seeking evidence of *cis*- or *trans*- eQTLs according to whether associated probes were or were not within 4MB of these LD blocks.

Results: Genes subject to *cis* eQTL effects common to both CD4+ and B-lymphocytes at RA risk loci were *FADS1*, *FADS2*, *BLK*, *FCRL3*, *ORMDL3* and *GSDMB*. At the 8p23 *BLK-FAM167A* locus, we found adjacent genes subject to eQTLs whose activity differed markedly between cell types, the *FAM167A* effect displaying striking B-lymphocyte specificity. By contrast, *cis* eQTLs acting on *METTL21B*, *IKZF3*, and *PADI4* were unique to CD4+ lymphocytes, the latter two of these being identified for the first time in this cell subset. No *trans* eQTLs approached experiment-wide significance, and linear modelling did not identify a significant influence of biological co-variables (diagnosis, systemic inflammation, age) upon eQTL effect sizes.

Conclusions: Our findings refine understanding of candidate causal genes in RA pathogenesis, providing an important platform from which downstream functional studies may be prioritised and directed towards particular cell types.

References:

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THU0004

CROSS PHENOTYPE ASSOCIATION MAPPING OF THE MHC IDENTIFIES GENETIC VARIANTS THAT DIFFERENTIATE PSORIATIC ARTHRITIS FROM PSORIASIS

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Background: The identification of genetic variants that differentiate PsA from psoriasis has the potential to help us understand the underlying biological pathways that lead to the development of PsA. Associations to genetic variants within the major histocompatibility complex (MHC), in particular to *HLA-C*0602*, increase risk of both PsA and psoriasis when compared to control populations. However direct comparisons of PsA to psoriasis have led to paradoxical associations where *HLA-C*0602* has been reported to be protective of PsA. In addition *HLA-C*0602* has been reported to be associated with age of onset of psoriasis. A more recent study has reported the amino acid at position 45 of the HLA-B protein as the most important factor for differentiating PsA from psoriasis.

Objectives: Here we perform a cross phenotype association analysis in an attempt to identify genetic variants in the MHC that differentiate PsA from psoriasis in a large collection of PsA patients and psoriasis patients screened for the absence of PsA.

Methods: A total of 1069 patients with psoriasis and 981 patients with PsA from the UK were genotyped using either the Illumina Immunochip or the Illumina OmniExpress genotyping arrays. SNPs, amino acids and classical HLA alleles were imputed using SNP2HLA. Logistic regression was used to compare the imputed dosage of MHC markers between PsA and psoriasis. All analyses were repeated using age of psoriasis onset as an additional covariate.

Results: The most significant association when comparing PsA to psoriasis was to *HLA-C*0602* ($p=4.17\times10^{-15}$) with a protective effect for PsA (OR 0.52, CI 0.44:0.61). *HLA-C*0602* was found to be significantly associated with a younger age of psoriasis onset ($p=1.51\times10^{-60}$) where the median age of onset in years for carriage is 22 compared to 33 for non-carriage. We observed a difference in

the median age of psoriasis onset in years between the PsA and psoriasis study subgroups (34 vs. 21), highlighting the potential for bias at markers associated with age of psoriasis onset. When controlling for the age of psoriasis onset in the analyses we observed no association of PsA to *HLA-C*0602* ($p=0.07$) and the most significant association was to the amino acid at position 97 of HLA-B ($p=1.54\times10^{-9}$) where the presence of asparagine or serine residue increased risk of PsA. Asparagine at position 97 of HLA-B defines the HLA-B*2705 allele.

Conclusions: Comparing PsA to psoriasis we show *HLA-C*0602* confers no effect, either risk or protective, for PsA after correction for age of psoriasis onset. The results suggest that the previously observed protective effect of *HLA-C*0602* could be due to confounding due to a younger age of psoriasis onset in the psoriasis subgroup. When accounting for age of psoriasis onset, the primary association conferring risk for PsA in patients with psoriasis is to HLA-B amino-acid 97 where an asparagine residue defines the HLA-B*2705 allele. In addition, this amino acid has been reported as the largest genetic effect for ankylosing spondylitis thereby refining the genetic overlap between these two spondyloarthropathies.

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THU0005

ERAP POLYMORPHISMS AND ITS ASSOCIATION WITH HLA-B15 AND HLA-B27 POSITIVE SPONDYLARTHROSIS PATIENTS

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Background: Since 1973, the association of HLA-B27 and spondylarthrosis (SpA) is well known, however in Colombian population it is present in only 40% of patients and HLA-B15 is present almost in 25%. A mechanism of polygenic mechanism has been proposed as an explanation for the development of SpA. Endoplasmic reticulum aminopeptidase (ERAP) genes 1 and 2 have been implicated. ERAP1 is strongly associated with HLA-B27 positive patients and ankylosing spondylitis, but not with ERAP2

Objectives: To determine the association between ERAP polymorphisms and HLA-B27 or HLA-B15 positive SpA patients

Methods: 178 patients with SpA according to ASAS criteria were included in the study. HLA typing was performed by the PCR technique using the Biorad® HLA-SSP plates. The polymorphisms were determined by the RT-PCR technique using Roche® probes for ERAP1 rs27044, rs17482078, rs10050860, and rs30187. For ERAP2 the probes used were rs2910686, rs2248374 and rs2549782. The

Table 1. ERAP2 Haplotypes in HLA-B15 and B27 Patients

Haplotypes	HLA B15 n (AF)	HLA B27 n (AF)	OR (CI 95%)	p
TGT	0.201	0.078	2.943 (1.264–6.585)	0.009*
TGC	0.055	0.227	4.483 (1.524–13.187)	0.003*
CAT	0.021	0.119	9.014 (1.181–68.807)	0.009*
CAC	0.643	0.499	1.750 (0.968–3.162)	0.077
CGC	0.016	0.035	0.465 (0.053–4.056)	0.672
CGT	0.031	0.013	2.406 (0.332–17.45)	0.584
TAT	0.019	0.013	1.185 (0.106–13.29)	1.00
TAC	0.013	0.015	1.185 (0.106–13.29)	1.00

ERAP: endoplasmic reticulum aminopeptidase; AF: allelic frequency; OR: odds ratio.

Figure 1. Linkage disequilibrium (LD) map of the ERAP gene. The value of the LD coefficient in each cell D' [(value close to 1.0) x 100] is shown. The colour map of the haplogroups represents the LD relationships, the bright red squares represent evidence of LD (log odds [LOD] score <2 and D' = 1), the light red squares indicate a slight evidence of LD (log odds [LOD] score <2 and D' < 1), and white squares mean that there is no LD and independent segregation (log odds [LOD] score <2 and D' < 1)