Poster Presentations

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 hypomethylation[2].

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:
[1] Verstappen SMM et al. (2012) Int. J. Clin. Rheu. 7(5):559–567.
[2] Kim Y, et al. (1996) J. Lab.Clin.Med. 128:165–172.

Disclosure of Interest: None declared

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 (*SLC2A4*, *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-κB 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.

Disclosure of Interest: None declared

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,