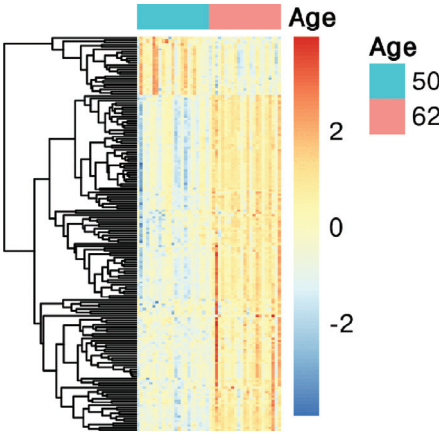
ageing mechanisms, diagnosis/prognosis of age-related disease and even novel treatment targets.
Objectives: To quantify relationships between circulating microRNA expression and biological ageing and determine whether microRNAs may be a molecular clock.
Methods: This pilot work is a nested substudy within a prospective, longitudinal birth cohort from May/June 1947 (the Newcastle Thousand Families Study NTFS). Serum samples from 23 subjects taken at ages 50 and 62 years were extracted from the biobank. HTG EdgeSeq microRNA whole transcriptome assay was performed, measuring expression of 2083 human microRNA transcripts using an array followed by next generation sequencing. Global microRNA expression profiles were generated and analysed using this technology, profiling all known microRNAs from a small volume of serum (<15 µL). NIHR funding has been secured for whole cohort analysis.
Results: Resulting data has shown very strong associations (up to $p<10–1^2$) for biological ageing, with 84 microRNAs meeting p-values<0.05 (see heat map). Analysing the whole cohort will independently validate and extend the findings, in order to identify an ageing signature; the molecular clock.



Conclusions: This study suggests very strong changes in microRNAs in individuals between 50 and 62, suggesting microRNA signature is a molecular clock. These observations need to be confirmed and extended to validate serum microRNAs as biomarkers for ageing, for early detection of age-related disease and as tools to monitor ageing trajectory.

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THU0020 TOCILIZUMAB DECREASES THE PRO-INFLAMMATORY ROLE OF PLATELETS IN RHEUMATOID ARTHRITIS: IDENTIFICATION OF A NEW MECHANISM OF ACTION ASSOCIATED WITH POSITIVE RESPONSE?

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Background: Tocilizumab (TCZ), a humanised monoclonal antibody directed against IL-6 receptor, is an efficient treatment for rheumatoid arthritis (RA) but its mechanisms of action are not yet well understood.
Objectives: To identify new mechanisms of action of TCZ, by the study of gene expression fluctuations, between baseline (BL) and 3 months of treatment (T_{3m}), in RA patients.
Methods: TOSCA study has been realised to evaluate efficiency and tolerance of TCZ administrated subcutaneously in active RA patients. Among the 125 patients of TOSCA study, 38 were ranked according to their treatment response after 3 months: 29 good responders (GR), 19 of which were also treated by methotrexate (MTX) and 10 non responders (NR), 7 of which were treated by MTX (GR: DAS 28-ESR_{3m} <3,2 and Delta DAS28-$ESR_{(BL-3m)}\geq$3; NR: DAS 28-ESR>3,2 and Delta DAS28-$ESR_{(BL-3m)}$<3). A transcriptomic analysis was performed using a 44K whole human genomic DNA microarray (Agilent) on whole blood cells collected at BL and at T_{3m}. We identified genes with statistically significant expression fluctuations between BL and T_{3m} specifically in GR group (and not in NR group), treated by TCZ in monotherapy (excluding genes which fluctuated only with the association TCZ-MTX). Functional bio-informatics analysis was applied to this set of transcripts, by interrogation of Gene Ontology database, using Single Experiment Analysis tool and Natural Language Processing algorithms.
Results: Overall, 1089 transcripts significantly dysregulated were identified only in GR group at T_{3m} (t test, p<0.05). This first set of transcripts was reduced to 783 by exclusion of transcripts that were fluctuated specifically when MTX was associated with TCZ in GR group. The functional analysis with these 783 genes dysregulated under TCZ in monotherapy enabled the identification of 8 transcripts (*CLU, F13A1, ITGA2B, ITGB3, SELP, SNCA, SPARC, TREML1*) whose relative abundances were significantly reduced at $T_{3m.}$ These genes were enriched in "platelet alpha granule" GO functional category. Proteins encode by these genes, either released in blood circulation or expressed at the cell membrane in case of platelet activation, have a pro-inflammatory activity through an interaction between platelets and immune cells.
Conclusions: This transcriptomic analysis suggests a new mechanism of action of TCZ in RA and the importance of platelets activation in RA pathophysiology. Indeed, genes linked with the pro-inflammatory role of platelets were down regulated. Further functional studies will be necessary to validate the direct effect of TCZ on platelets in RA.
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Disclosure of Interest: C. Prum Delepine: None declared, C. Derambure: None declared, F. Vidal: None declared, A. Pinta Employee of: Roche Laboratory, D. Pau Employee of: Roche Laboratory, E. Conde da Silva Fraga Employee of: Roche Laboratory, O. Boyer: None declared, E. Senbel: None declared, P. Gaudin: None declared, O. Vittecoq: None declared, T. Lequerre Grant/research support from: Roche Laboratory
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THU0021 DIFFERENTIAL EFFECTS OF TR14 VERSUS DICLOFENAC ON COX/LOX PATHWAYS REVEALED BY RNASEQ

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Background: Anti-inflammatory agents are used widely in treating numerous pain and inflammatory conditions. With a focus on the COX/LOX pathways in cutaneous wound repair in mice, the therapeutic activities of Tr14 (Traumeel), a multicomponent/multitarget natural product, and diclofenac (NSAID), a non-selective cyclooxygenase (COX) inhibitor were compared. The COX enzymes convert arachidonic acid into prostaglandins and thromboxanes, while the lipoxygenase (LOX) pathway generates more pro-inflammatory leukotrienes. Differential effects were identified via transcriptome analysis (RNAseq).
Objectives: To compare the transcriptomic changes after administration of Tr14 or diclofenac in a mouse cutaneous wound healing model, with particular emphasis on the COX/LOX pathway.
Methods: After abrasive wounding, the wounds were treated with topical Tr14 (34 mg/ml) in combination with subcutaneous Tr14 injections (9.5 mg/ml), or with subcutaneous Tr14 injections only, or topical diclofenac at clinically relevant doses (2 mg/ml). Skin samples were analysed for RNA transcript profiling by RNAseq at specific times (12 hour, 24 hour, 36 hour, 72 hour, 96 hour, 120 hour, 192 hour) after injury. Differentially expressed genes (DEGs) were computed at each time point between diclofenac vs control or Tr14 vs control, using EdgeR.
Results: At early time points (12–36 hour), both control and Tr14-treated wounds showed marked increase in the inducible COX2 enzyme mRNA, while diclofenac-treated wounds did not, likely due to blocking the PGE2 necessary for the feedback induction. Tr14, in contrast, had a striking inhibitory effect on mRNA levels

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for leukotriene A4 hydrolase, which converts LTA4 to LTB4; microsomal glutathione S-transferase, which converts LTA4 to LTC4; and gamma-glutamyltransferase (LTC4 >LTD4). In contrast, Tr14, but not diclofenac strongly induced Nrf2 mRNA at 12–36 hours.

Conclusions: Tr14 and diclofenac had very different effects on the COX/LOX synthetic pathway after cutaneous wounding. Tr14 allowed normal autoinduction of COX2 mRNA by PGE2, but suppressed mRNA levels for the key enzymes in the leukotriene synthetic pathway. A likely explanation for these effects is that Tr14 strongly induced Nrf2 mRNA, which is known to co-repress the leukotriene enzymes via transcription factor Bach1.

Disclosure of Interest: None declared

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THU0022 ANALYSIS OF 47 NON-MHC ANKYLOSING SPONDYLITIS SUSCEPTIBILITY LOCI REVEALS SHARED ASSOCIATED VARIANTS ACROSS CAUCASIANS AND CHINESE HAN

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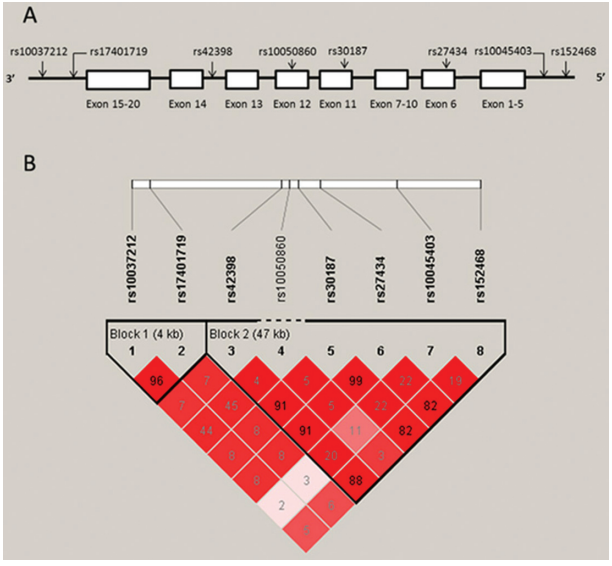
Background: Genetic factors play a prominent role in AS pathogenesis. So far over 40 non-MHC Ankylosing Spondylitis(AS) susceptibility loci with genome-wide or suggestive significance have been initially reported in Caucasians, however, lack of association evidence of most loci was seen in Chinese Han and some results seemed controversial.

Objectives: Here, we present a systematic evaluation of 47 non-MHC AS susceptibility loci using GWAS datasets in Chinese Han.

Methods: Totally 1853 AS cases and 4048 newly matched controls in 4 cohorts were obtained, after imputation meta-analysis results of 93 589 variants within 47 reported loci were extracted. Best-guess genotype data were used for interaction analysis and weighted genetic risk score model construction which was then assessed by receiver operator characteristic analysis. Functional annotation was conducted using HaploReg, RegulomeDB and rVarBase Database.

Results: We revealed 14 AS-associated variants with nominal evidence in Chinese Han, including rs10865331(p=2.96E-9), rs10050860 (p=1.84E-4) and rs8070463(p=2.81E-4) and found potential associated variants within these loci. We then extracted variants in ERAP1 as well as HLA-B27 tag snp rs13202464 for HLA-B27-ERAP1 interaction analysis (figure 1). Epistatic association between ERAP1 (rs30187, rs10045403) and HLA-B27 (rs13202464) was confirmed. Among those 14 variants, rs30187 showed weaker risk effect in Chinese while rs10050860 and rs12504282 seemed to attribute more risk (Table 1). Genetic prediction model combining 14 variants in 11 loci with HLA-B27 achieved better discrimination ability(AUC=0.884, 95%CI=0.873~0.895) than HLA-B27 alone (p=2.17E-6). We also identified some likely functional variants at these loci.



Abstract THU0022 – Figure 1

Abstract THU0022 – Table 1. Comparison of 14 shared associated non-MHC SNPs across European and Chinese

Locus	SNP	Nearby Gene(s)	Risk allele	RAF% (CEU/CHN)	OR (CEU/CHN)	PARP% (CEU/CHN)
1 p36	rs6600247	RUNX3	C	0.50/0.69	1.16/1.15	7.41%/9.42%
2 p15	rs10865331	Intergenic	A	0.38/0.49	1.34/1.27	11.44%/11.60%
3 p24	rs10510607	CMC1	C	0.83/0.54	1.15/1.14	11.07%/7.03%
4q21	rs12504282	ANTXR2	T	0.54/0.91	1.14/1.20	7.03%/15.38%
5 p13	rs11742270	IL7R	G	0.73/0.84	1.11/1.14	7.43%/10.53%
5q15	rs30187	ERAP1	T	0.34/0.53	1.32/1.11	9.81%/5.47%
5q15	rs10045403	ERAP1	A	0.73/0.82	1.20/1.18	12.74%/12.92%
5q15	rs10050860	ERAP1	C	0.78/0.95	1.18/1.45	12.31%/30.01%
6q15	rs639575	BACH2	T	0.61/0.49	1.08/1.10	4.65%/4.69%
14q13	rs8006884	PPP2R3C	C	0.35/0.40	1.11/1.09	3.71%/3.44%
17q11	rs2297518	NOS2	A	0.19/0.16	1.13/1.11	2.41%/1.72%
17q21	rs9901869	NPEPPS	A	0.52/0.62	1.15/1.14	7.24%/7.95%
17q21	rs8070463	TBKBP1	C	0.51/0.44	1.14/1.16	6.66%/6.64%
21q22	rs2836883	Intergenic	G	0.74/0.83	1.19/1.16	12.33%/11.71%

Conclusions: Our results provided a detailed spectrum of non-MHC AS susceptibility loci in Chinese Han and highlighted 2 p15, ERAP1 and TBKBP1 may play a critical role in AS pathogenesis.

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THURSDAY, 14 JUNE 2018:

Adaptive immunity (T cells and B cells) in rheumatic diseases

THU0023 COMPLEX IMMUNOPHENOTYPING STRATIFIES PATIENTS WITH PRIMARY SJÖGREN'S SYNDROME, SYSTEMIC LUPUS ERYTHEMATOSUS AND SECONDARY SJÖGREN'S SYNDROME ASSOCIATED WITH SYSTEMIC LUPUS ERYTHEMATOSUS INTO DISTINCT CLINICALLY RELEVANT GROUPS WITH POTENTIAL THERAPEUTIC IMPLICATIONS

N. Thompson[1], A. Gandhi[2], R. Radmmore[2], V. Gupta[2], G. Robinson[1], L. Martin-Gutierrez[1], D. Isenberg[2], E. Jury[1], C. Ciurtin[2]. [1]*Inflammation*; [2]*Rheumatology, University College London, London, UK*

Background: Similarities in the clinical and laboratory features of patients with primary Sjögren's syndrome (pSS) and systemic lupus erythematosus (SLE) have led to attempts to treat pSS and SLE patients with similar biologic therapeutics. However, the results of many clinical trials are disappointing and no effective treatments are available for pSS and few for SLE patients with refractory disease.

Objectives: To identify novel patient endotypes using in depth immune phenotyping that facilitates the selection of biological therapies for patients regardless of diagnostic labels.

Methods: Peripheral blood was collected from patients with pSS (n=55), SLE (n=38), SS/SLE (n=15) and age/sex-matched healthy controls (HCs) (n=34). In-depth phenotyping of peripheral B and T-cell subsets by flow-cytometry, followed by unsupervised cluster analysis were performed. ROC analysis identified immune signatures characteristic for every cluster (endotype).

Results: Patients with pSS, SLE and SS/SLE had both unique and shared defects in immune cell phenotype. Hierarchical clustering of $CD19^+$ B-cells, $CD4^+$ and $CD8^+$ T-cells across the three disease groups identified five distinct endotypes spanning diagnostic boundaries. Three of the endotypes had distinct immune signatures, characterised by predominantly B-cell, T-cell memory or $CD4^+/CD8^+$ T-cell subset fingerprints respectively, while two clusters had no distinct immune profiles. Notably, clinical and disease features were not significantly different between clusters.

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