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Fig. 1. Dual-luciferase reporter assay with the regular 3'UTR of PRTN3 cloned into the pmir-GLO vector compared to empty vector (NTC). Significant effect for miR-184 (17.5 %), miR-708 no effect and let-7f small but not significant reduction in luciferase activity (12.3 %). Data represent 3 independent experiments with triplicate measurements. miR-184 was tested 6 times. * $P < 0.05$; ns = not significant; error bars display standard deviation.

Fig. 2. Dual-luciferase reporter assay with the alternative 3'UTR of PRTN3. Significant effects of let-7f (29.2 %) and miR-708 mimic (23.6 %) but no significant effects of miR-184 of luciferase activity. 3 independent experiments with triplicate measurements. * $P < 0.05$

Acknowledgments: Vasculitis foundation for funding

Disclosure of Interests: Nick Reichard: None declared, Anja Kerstein-Staehle: None declared, Antje Müller: None declared, Gabriela Riemekasten Consultant of: Cell Trend GmbH, Janssen, Actelion, Boehringer Ingelheim, Speakers bureau: Actelion, Novartis, Janssen, Roche, GlaxoSmithKline, Boehringer Ingelheim, Pfizer, Peter Lamprecht: None declared, Susanne Schinke Speakers bureau: Pfizer
DOI: 10.1136/annrheumdis-2020-eular.4700

THU0026 CLONAL HAEMATOPOIESIS ASSOCIATED SOMATIC MUTATIONS IN RHEUMATOID ARTHRITIS

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Background: Clonal haematopoiesis of indeterminate potential (CHIP) occurs when somatic mutations arise in myeloid neoplasia driver genes of haematopoietic progenitor cells, in the absence of overt cytopenia or dysplasia. The prevalence of CHIP increases with age. The most common genes affected by CHIP mutations in unselected populations are DNMT3A, ASXL1, and TET2. The presence of CHIP is linked to increased basal level of inflammation and a high risk of cardiovascular disease and all-cause mortality. Rheumatoid arthritis (RA) is one of the most common and debilitating multi-system autoimmune disorders, affecting up to 1% of adults in developed countries. The role of somatic mutations in the pathogenesis of autoimmune diseases is an unexplored area; therefore, we aimed to test the hypothesis that clonal haematopoiesis (CH) is associated with the incidence and severity of RA.

Objectives: To evaluate the association of CH somatic mutation with severity of RA.

Methods: 163 RA patients were recruited from the following cohorts: (i) Early RA/ treatment naive (n=31), (ii) Refractory RA - non-responders to Disease-Modifying Anti-Rheumatic Drugs (DMARDs) and biologics (n=48), (ii) Flare (n=41) vs Remission patients (n=43) –patients treated with DMARDs and withdrawn from treatment on achieving remission. Six months later, 50% relapse and 50% sustain remission. Single molecule molecular inversion probes (smMIPs) were used to screen for somatic mutations in 40 loci known to carry clonal haematopoiesis driver mutations (CHDMs). Whole exome sequencing was also performed on Flare/Remission patients (n = 84) to screen for CHDMs and other somatic mutations. In-house bioinformatics pipelines were used to call mutations from both the datasets.

Results: We identified CH in RA with an overall prevalence of 14%. Twenty-four unique variants with a variant allele frequency (VAF) of 2-35% were found in ten genes including ASXL1, CBL, DNMT3A, GNAS, GNB1, PTPN11, PTPN12, SF3B1, TET2, and TP53. The number of unique patients carrying mutations in these genes are follows: **refractory:** n=12/48, **flare:** n=6/41, **remission:** n=4/43 and **early RA:** n=2/31. The majority of the mutations occurred in DNMT3A (n=6) followed by TP53 (N=4) and TET2 (n=3). Two variants with VAF of 15% were identified in two patients under the age of 30, both with clinically severe disease. In patients between the ages of 50-59 yrs., 60-69 yrs., and 70-79 yrs., CH was observed at 11% (4/35), 23% (11/46) and 17%(7/41), respectively.

Conclusion: We here report the prevalence of CH in RA, affecting more patients with clinically advanced/refractory disease compared to those with early/less severe disease.

Further study will be conducted to confirm the results.

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Acknowledgments: National Institute for Health Research, United Kingdom

Disclosure of Interests: Fareeha Tariq: None declared, Bilal Alobaidi: None declared, Miguel Xavier: None declared, Michael McCorkindale: None declared, Joris Veltman: None declared, John Isaacs Consultant of: AbbVie, Bristol-Myers Squibb, Eli Lilly, Gilead, Janssen, Merck, Pfizer, Roche, Arthur Pratt Grant/ research support from: Pfizer, GSK, Amy Anderson: None declared, Matthew Collin: None declared
DOI: 10.1136/annrheumdis-2020-eular.5568

THU0027

THE ASSOCIATION OF THE RS35677470 DNASE1L3 GENE POLYMORPHISM WITH SLE, RA AND SSC: STRUCTURAL/BIOLOGICAL INSIGHTS

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Background: Genome-wide association studies (GWAS) have identified hundreds of autoimmune diseases-associated loci so far but much of the heritability of these diseases remains unknown. In an attempt to identify potential causal variants, various studies revealed that the missense variant rs35677470 at DNASE1L3 is associated with the development of systemic lupus erythematosus

(SLE), rheumatoid arthritis (RA) and systemic sclerosis (SSc), thus exhibiting a pleiotropic effect. Deoxyribonuclease I-like 3 (DNase1L3) is a member of human DNase I family, representing a nuclease that cleaves double-stranded DNA during apoptosis and is involved in the development of autoimmune diseases [1].

Objectives: To investigate the role of the rs35677470 polymorphism at *DNASE1L3* gene leading to the R206C mutation in SLE, RA and SSc [2-3] and the mechanism that may affect the loss of function in the protein structure.

Methods: The *DNASE1L3* evolution was investigated to define conservation elements in the protein sequence using, BLASTP extended searches [4], TCOFFEE [5] multiple sequence alignments, and MEGAX [6] for phylogenetics analysis. Three-dimensional (3D) homology modeling was used to localize the polymorphism under study. The mutant was constructed by molecular modeling using the structures of homologous DNases (PDB entries 1atn, 4awn, 3d3w; [7-9]). Molecular mechanics/dynamics studies were applied to validate structural/functional changes caused by the R206C substitution. All figures depicting 3D models were generated using the PyMOL molecular-graphics system V.2.2 (Schrodinger, LLC).

Results: The evolutionary analysis shows heavily conserved sequence elements among species indicating structural/functional importance. Structural analysis revealed that the rs35677470 SNP codes for a nonconservative amino acid variation, R206C, disrupts the conserved electrostatic network holding protein secondary structure elements to place. Specifically, the R206 to E170 interaction, part of a salt bridge network stabilizing two α -helices, is being interrupted, thereby affecting the molecular architecture (Fig. 1). Indeed, previous studies on the effect of this SNP in Caucasian populations resulting in a lower level of DNase1L3 activity are consistent with this observation [10].

Figure 1. Ribbon representation of the DNase1L3 homology model showing the position of the stabilizing salt bridge network (E170- R206, R208- D219). Insert figure shows the R206C mutation. Positively charged R (in blue), negatively charged D,E (in red) and C (in yellow) are shown. Distances are in Angstroms.

Conclusion: This study represents a comprehensive evaluation of the shared autoimmune loci of *DNASE1L3* (rs35677470), reported to produce an inactive form of DNase1L3 [10]. The structural analysis, explains the potential role of the produced mutation by modifying the placement of structural elements and consequently introducing disorder in the protein folding and affecting biological function. Altogether, this study contributes to the delineation of the genetic architecture of SLE, RA and SSc.

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Disclosure of Interests: None declared

DOI: 10.1136/annrheumdis-2020-eular.1150

THU0028

AN EXPLANATION FOR HOW VIRAL INFECTION MAY TRIGGER SPONDYLOARTHRITIS BASED ON TLR9 DRIVEN TNF RESPONSES FROM ENTHESEAL DERIVED PLASMACYTOID DENDRITIC CELLS

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Background: It is well known that viral infections may trigger psoriatic arthritis (PsA), a disease that typically has extensive pre-clinical enthesal abnormalities. Skin resident plasmacytoid dendritic cells (pDCs) produce IFN α that contribute to T cell expansion and the development of experimental psoriasis [1, 2]. IFN pathway SNPs have been reported in both PsA and psoriasis and we previously reported the presence of pDCs at the human enthesis [3].

Objectives: To investigate whether the TLR9 agonist ODN that replicates viral infection activate a wide array of enthesal derived pDCs molecular cascades including the TNF pathway that might provide a link between viral infection and PsA.

Methods: pDCs were sorted from enthesis and blood and stimulated with ODN as previously described (n=16) [3, 4]. IFN α protein pre and post stimulation were detected by ELISA. Intracellular flow cytometry (IFC) of enthesal pDCs was used to detect TNF protein. RNA was extracted post-stimulation. The mRNA were hybridised and tagged by probes then measured on the nCounter platform. Data was analysed using nSolver 4.0. Log2 fold change >1 and P-value <0.05 were considered statistically significant. The gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) of differentially expressed genes (DEGs) were analyzed using DAVID. Protein-protein interaction (PPI) network was drawn by STRING.

Results: Stimulated enthesal pDCs showed a strong DEGs pattern pointing towards increased TNF expression. There were 11 genes significantly upregulated including TNF. RIPK3 is involved in TNF signalling pathway. TNF, RIPK3 and ZBP1 are involved in necroptosis. TNF and ITGB2 are involved in IL-4 and IL-13 signaling pathway. TNF, HLA-DOA, ITGB2/TLR7 are involved in virus infection. Together it highlights extremely activated TNF pathway genes.

IFN protein was induced in sorted enthesal pDCs following stimulation (n=8). TNF protein was detected by IFC on stimulated enthesal pDCs (CD45+HLA-DR+CD123+CD303+ CD11c-) (n=3). We also compared enthesal and matched peripheral blood pDCs (n=8) following stimulation where no major differences in the TNF pathway were present between groups.

The KEGG analysis was mapped in Figure 1. GO analysis showed the most significant change in biological processes was enriched in the positive regulation of DNA binding transcription factor activity. The change in molecular function was mainly enriched in p53 binding.

Figure 1. DEGs between stimulated and unstimulated enthesal pDCs. (A) Volcano map showing significantly upregulated genes (red). (B) PPI network. (C) KEGG pathway enrichment.

Conclusion: Enthesal pDCs, upon viral molecule stimulation, show several markers of activation. However, TNF pathway genes were highly activated which provides a novel mechanistic link between viral infection and PsA as reported in epidemiological studies.

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Disclosure of Interests: Qiao Zhou: None declared, Jayakumar Vadakekolathu: None declared, Kassem Sharif: None declared, Tobias Russell Grant/research support from: Novartis UK Investigator Initiated non-clinical research funding