protecting more strongly than *C4B* in both illnesses. In schizophrenia, elevated *C4* copy number elevates disease risk, whereas in SLE and Sjögren's lower copy numbers of *C4* genes correlate with higher disease risk. In all three illnesses, *C4* alleles acted more strongly in men than in women: common combinations of *C4A* and *C4B* generated 14-fold variation in risk for lupus and 31-fold variation in risk for Sjögren's syndrome in men (versus 6-fold and 15-fold among women respectively) and affected schizophrenia risk about twice as strongly in men as in women. At a protein level, both *C4* and its effector (*C3*) were present at greater levels in men than women in cerebrospinal fluid ($p < 10^{-5}$ for both *C4* and *C3*) and plasma among adults ages 20-50, corresponding to the ages of differential disease vulnerability. Sex differences in complement protein levels may help explain the larger effects of *C4* alleles in men, women's greater risk of SLE and Sjögren's, and men's greater vulnerability in schizophrenia.

Figure 2. Common class II association after removing *C4* signal

Conclusion: These results nominate the complement system as a source of sexual dimorphism in vulnerability to diverse illnesses.

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THU0003

ALTERED DNA METHYLATION AND DIFFERENTIAL EXPRESSION OF GENES INFLUENCING CARDIOVASCULAR RISK AND IMMUNITY IN CD4+ T CELLS FROM SUBJECTS WITH PSORIATIC ARTHRITIS.

I. Arias de la Rosa¹, M. D. López Montilla¹, J. Rodríguez², E. Ballester², C. Torres-Granados¹, C. Perez-Sanchez¹, M. D. C. Abalos-Aguilera¹, G. C. Ignacio¹, D. Ruiz¹, A. M. Patiño-Trives¹, M. Luque-Tévar¹, E. Collantes-Estévez¹, C. Lopez-Pedraza¹, A. Escudero Contreras¹, N. Barbarroja Puerto¹.
¹IMIBIC/Reina Sofia Hospital/University of Cordoba, Cordoba, Spain; ²Josep Carreras Institute (IJC), Badalona, Spain

Background: Cardiovascular risk factors are increased in Psoriatic Arthritis (PsA). In fact, around 60% out of PsA patients display insulin resistance (IR), a hallmark of metabolic syndrome, which might significantly contribute to the cardiovascular disease. Latest studies suggested that inflammatory and metabolic disorders may be under epigenetic control, including DNA methylation. DNA methylation is an unexplored area in the field of PsA.

Objectives: To study the alterations in the genome-wide DNA methylation profile of CD4⁺ T cells from PsA patients and its relationship with its pathology and the risk of cardiovascular comorbidity.

Methods: Twenty healthy controls (HC) and 20 PsA patients were included in the study. PsA patients were classified into insulin resistant and non-insulin resistant according to HOMA-IR index. CD4⁺ T lymphocytes were isolated from peripheral blood by positive immunomagnetic selection. The Illumina Infinium Methylation EPIC Beadchip was used to obtain DNA methylation profiles across approximately 850,000 CpGs (TSS1500, TSS200, 5'UTR, 3'UTR, first exon, gene body). Beta values (β) estimating methylation levels were obtained at each CpG site, and differentially methylated genes (DMG) between PsA and HC were identified. Functional classification of these genes was carried out through gene ontology analysis (PANTHER database). Gene expression analysis of the selected genes was also evaluated by RT-PCR. Vascular parameters including carotid

intima-media thickness (cIMT) and endothelial function was analyzed by eco-doppler and perfused respectively.

Results: The genome-wide methylation analysis identified 112 DMGs including 41 hypomethylated and 71 hypermethylated. These differentially methylated genes were enriched with several signaling pathways and disease categories including immune response, metabolic processes, oxidative stress, vascular and inflammatory pathways. The altered gene expression of selected genes with altered methylation levels in PsA was also validated. Correlation and association analysis of these DMGs with clinical and analytical variables, cardiovascular risk factors and endothelial microvascular function revealed that the degree of methylation of these genes was significantly associated with cIMT (IGF1R, NDRG3, SMYD3, HLA-DRB1, WDR70), arterial pressure (METT5D1, NRDG3, ADAM17, SMYD3, WNK1, CBX1), insulin resistance (AKAP13, SEMA6D, PLCB1), altered lipid profile and atherogenic index (MYBL1, METT5D1, MAN2A1, SLC1A7, SEMA6D, PLCB1, TLK1, SDK1, CBX1), inflammation (MYBL1, NDUFA5, METT5D1, SEMA6D, PLCB1, TLK1), and endothelial dysfunction (ADAMT10, GPCPD1, CCDC88A). In addition, this analysis also identified 435 DMGs including 280 hypomethylated and 155 hypermethylated in CD4⁺ T cells from IR-PsA vs non IR-PsA patients. Between these two groups of PsA patients, CHUK, SERINC1, RUNX1, TTYH2, TXNDC11, FAF1, BICD1, SCD5, PDE5A, FAS, NFIA and GRP75 displayed the most significantly altered methylation, suggesting the role of these genes in the metabolic complications associated with PsA.

Conclusion: These findings help our understanding of the pathogenesis of PsA and advance epigenetic studies in regards to this disease and the cardiometabolic comorbidities associated. Funded by ISCIII (PI17/01316 and RIER RD16/0012/0015) co-funded with FEDER.

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THU0004

GENOME-WIDE DNA METHYLATION PROFILING IN MONOCYTES FROM PRIMARY ANTIPHOSPHOLIPID SYNDROME PATIENTS IDENTIFIES AN ABERRANT METHYLATION SIGNATURE ASSOCIATED WITH THEIR ATHEROTHROMBOTIC PHENOTYPE

C. Perez-Sanchez¹, A. M. Patiño-Trives¹, M. A. Aguirre¹, P. S. Laura¹, M. Luque-Tévar¹, I. Arias de la Rosa¹, C. Torres-Granados¹, M. D. C. Abalos-Aguilera¹, P. Seguí Azpilcueta¹, J. Rodríguez², E. Ballester², N. Barbarroja Puerto¹, E. Collantes Estevez¹, C. Lopez-Pedraza¹.
¹IMIBIC/Reina Sofia Hospital/University of Cordoba, Cordoba, Spain; ²Epigenetics and Immune Disease Group, Josep Carreras Research Institute (IJC), Barcelona, Spain

Background: Recent studies underlined the crucial role of DNA methylation in several autoimmune diseases by altering gene expression profiles, thus influencing disease severity. Yet, aberrant methylation patterns in monocytes, key players in the pathogenesis of APS patients, has not been evaluated.

Objectives: To analyze the genome-wide DNA methylation profile of monocytes from APS patients and its relationship with the cardiovascular (CV) pathology. 2. To evaluate the role of antiphospholipid antibodies (aPL) in the regulation of this process.

Methods: Thirty-three APS patients and 15 healthy donors (HD) were included in the study. Monocytes were isolated from peripheral blood by positive immunomagnetic selection. The Illumina Infinium Methylation EPIC Beadchip was used to obtain DNA methylation profiles across approximately 850,000 CpGs (TSS1500, TSS200, 5'UTR, 3'UTR, first exon, intergenic, gene body). Beta values (β) estimating methylation levels were obtained at each CpG site, and differentially methylated genes (DMG) between APS and HD were identified. Functional classification of that genes was carried out by gene ontology analysis (PANTHER database). Gene expression of selected DMG genes was evaluated by RT-PCR. CV-risk parameters, including carotid intima-media thickness (cIMT) and microvascular endothelial function were further assessed, and correlation/association studies were developed with clinical and analytical variables. The effects of aPLs were also evaluated by *in vitro* studies.

Results: Genome-wide DNA methylation analysis identified 813 DMG, including 279 hypomethylated and 534 hypermethylated. Functional classification of these genes revealed signatures associated with biological processes and pathways related to their clinical profile, including immune response, adhesion, oxidative stress and vascular signaling. Correlation and association studies showed that the methylation levels of genes related to immune response were associated with the CV-risk score, aGAPSS (CCR2, TXLNB, GLIPR), type of thrombosis (SIGLEC11, COLEC11, LRR16A, AHS1, TRIL) and aPL titers (CLEC4G, RGS4, HLA-DPA1, GBP6, RAET1E, HLA-G, HLA-DPA1, HLA-H, TXLNB). Besides, methylation levels of DMG related to vascular signaling and adhesion processes were associated with the presence of thrombotic recurrences (VEGFA, MAPK14, ITGA8, EPCAM, PCDHA6, DLG1) as well as with traditional CV-risk factor such as hypertension and dyslipidemia (ITGA11, DSCAM, CLEC4F, CDH4, LTBP2, PCDHB14). In addition, methylation levels of DMG genes related to oxidative stress (GP2, PGD, ADH1) were associated with microvascular endothelial dysfunction. An altered mRNA expression of some of those genes with aberrant methylation and related to increased CV-risk and thrombotic recurrences in APS was also identified. Both, abnormal methylation and transcription levels of several genes were further associated with a pathological increase of the CIMT. Finally, in vitro studies supported the role of aPLs as key players in the altered methylation and transcriptomic profiles of APS patients.

Conclusion: APS patients showed an impaired methylation profile in monocytes of genes associated with clinical features of the disease, including aPL titers, CV risk, thrombotic recurrences, endothelial dysfunction and early atherosclerosis. These results offered a map to the monocytes methylome and shed light on the pathophysiology of APS, paving the way for the development of new, more effective biomarkers and therapeutics.

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THU0005

VARIABILITY OF DNA METHYLATION IS A DRIVER OF LYMPHOCYTE DYSREGULATION IN EARLY RHEUMATOID ARTHRITIS.

A. Clark¹, N. Naamane¹, N. Nair^{2,3}, A. Anderson¹, N. Thalayasingam¹, J. Diboll¹, A. Barton^{2,3}, S. Eyre^{2,3}, J. D. Isaacs^{1,4}, L. Reynard⁵, A. Pratt^{1,4}.

¹Newcastle University Clinical and Translational Research Institute, Newcastle upon Tyne, United Kingdom; ²Versus Arthritis Centre for Genetics and Genomics, University of Manchester, Manchester, United Kingdom; ³NIHR Manchester Musculoskeletal BRC, Manchester University NHS Foundation Trust, Manchester, United Kingdom; ⁴Musculoskeletal Services Directorate, Newcastle upon Tyne Hospitals NHS Trust, Newcastle upon Tyne, United Kingdom; ⁵Newcastle University Biosciences Institute, Newcastle upon Tyne, United Kingdom

Background: DNA methylation patterns differ between leukocyte subsets and mediate the impact of environmental exposures on the molecular and functional phenotype of immune cells. Besides differences in mean methylation of CpG positions amongst patients with immune mediated diseases, recent evidence indicates variability of site-specific DNA methylation also contributes to pathogenesis^{1,2}.

Objectives: To seek evidence of altered DNA methylation patterns in RA, controlling for systemic inflammation and immunotherapy use.

Methods: Patients with confirmed clinical diagnoses were enrolled from the Northeast Early Arthritis Cohort (NEAC). CD4⁺ and CD19⁺ lymphocytes were isolated from fresh blood by positive selection prior to therapeutic immune modulation. Methylation was quantified in cell subset-specific DNA (Infinium MethylationEPIC BeadChip, Illumina)³. Differentially methylated positions and regions (DMPs, DMRs) between RA and non-RA patients were identified (linear modelling, filtering on 5% pairwise difference in mean DNA methylation, and DMRcate package). Next, to identify instances where methylation variance differed between comparator groups, Bartlett's test was performed using the iEVORA package, which accounts for outlier values⁴. Findings were controlled for technical confounders and subject to multiple test correction (FDR). A validated hypergeometric test was used to annotate enriched pathways.

Results: After sample- and probe-level quality control, CD4⁺ and B lymphocyte specific data were respectively available for 45 and 49 RA patients, and 64 and

81 disease controls matched for systemic inflammation (CRP, ESR). No DMPs were identified in either cell type at FDR < 0.05 and $\Delta\beta \geq 0.05$. Only following relaxation of multiple test correction was it possible to identify DMRs in either cell type, most notably encapsulating 10 CpGs relatively hypomethylated at the promoter of the endosome protein-encoding *RUFY1* gene in CD4⁺ lymphocytes of RA patients ($\Delta\beta = 0.076$). By contrast, striking evidence for differential variation in DNA methylation was observed at 291 and 601 CpGs of CD4⁺ and B lymphocytes, respectively (exemplars depicted in Figure 1). Only 15 of these differentially variable positions (DVPs) were common to both cell types. Pathway analysis highlighted potential functional consequences of DVP associations; for example, RA-specific hypervariability implicates prostaglandin signalling in CD4⁺ lymphocytes.

Figure 1. Plots are shown for CpGs in CD4⁺ T cells that were found to be either (A) hypomethylated or (B) hypermethylated in rheumatoid arthritis patients, with equivalent B cell plots again depicting (C) rheumatoid arthritis hypomethylated and (D) hypermethylated positions. BTq = Bartlett's q-value, a false discovery rate adjusted measure of group differences in DNA methylation variance; TTP = T-test p-value, applied to test for differences between the group means of any positions found to exhibit differential variance between cases and controls in the Bartlett's test ($q < 0.001$).

Conclusion: We highlight a role for altered variability in DNA methylation during the molecular pathogenesis of RA, and emphasise the importance of its study in relevant cell subsets.

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THU0006

ASSOCIATION BETWEEN ALTERED MICRORNA EXPRESSION AND ARTERIAL WALL REMODELING IN GIANT CELL ARTERITIS

L. Bolha¹, J. Pižem¹, M. Frank-Bertoncelj², A. Hocevar^{3,4}, M. Tomic^{3,4}, V. Jurcic¹. ¹Institute of Pathology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia; ²Department of Rheumatology, Center of Experimental Rheumatology, University Hospital Zurich, Zurich, Switzerland; ³Department