protecting more strongly than C4B in both illnesses. In schizophrenia, elevated C4 copy number elevates disease risk, whereas in SLE and Sjogren’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 Sjogren’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^-2) 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 Sjogren’s, and men’s greater vulnerability in schizophrenia.

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 arteriographic index (MYBL1, METT5D1, MAN2A1, SLC1A7, SEMA6D, PLCB1, TLK1, SDC1, CBX1), inflammation (MYBL1, NDUFA8, METT5D1, SEMA6D, PLCB1, TLK1), and endothelial dysfunction (ADAMST10, GPCPD1, CACCC88A). In addition, this analysis also identified 435 DMGs including 280 hypomethylated and 155 hypermethylated in C4 T cells from IR-Pa vs non-IR-Pa patients. Between these two groups of PsA patients, CHUK, SERINC1, RUNX1, TTYH2, TXNDC11, FA1F1, BICD1, SC5D, PDE5A, FAS, NFIA and GRP753 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 ICSII (P11/01316 and RIER RD16/0102/0015) co-funded with FEDER.

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

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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 in APS patients and its relationship with the cardiovascular (CV) pathology. 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 PsA and HD 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 echodoppler and periflux 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 arteriographic index (MYBL1, METT5D1, MAN2A1, SLC1A7, SEMA6D, PLCB1, TLK1, SDC1, CBX1), inflammation (MYBL1, NDUFA8, METT5D1, SEMA6D, PLCB1, TLK1), and endothelial dysfunction (ADAMST10, GPCPD1, CACCC88A). In addition, this analysis also identified 435 DMGs including 280 hypomethylated and 155 hypermethylated in C4 T cells from IR-Pa vs non-IR-Pa patients. Between these two groups of PsA patients, CHUK, SERINC1, RUNX1, TTYH2, TXNDC11, FA1F1, BICD1, SC5D, PDE5A, FAS, NFIA and GRP753 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 ICSII (P11/01316 and RIER RD16/0102/0015) co-funded with FEDER.
Results: Genomic-wide DNA methylation analysis identified 813 DMG, including 279 hypomethylated and 534 hypermethylated. Functional classification of these genomic regions revealed signatures associated with cellular 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, aGAPPS (CCFR2, TXLN6, GLIPR), type of thrombosis (SIGLEC1, COLEC16, LRRCA1, AHA$1$, TRIL) and aPL titters (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, EPICAM, PCDC16, DLG1) as well as with CV-risk and vascular risk scores in APS. 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 these 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 CI M T. Finally, in vitro studies supported the role of aPLs as key players in the altered methylation and transcriptional 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 titters, CV risk, thrombotic recurrences, endothelial dysfunction, and early atherosclerosis. Besides, 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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VARIABILITY OF DNA METHYLATION IS A DRIVER OF LYMPHOCYTE DYSREGULATION IN EARLY RHEUMATOID ARTHRITIS.

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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 pathogenesis1-3. 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 Methylation-EPIC BeadChip, Illumina)2. 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 DMR-cate 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 subject to multiple test correction (FDR). A validated hyper-geometric 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 Δβ ≥0.05. Only following relaxation of multiple test correction was it possible to identify DMRs in either cell type, most notably encapsulating 10 CpGAs relatively hypomethylated at the promoter of the endosome protein-encoding RUFY1 gene in CD4+ lymphocytes of RA patients (Δβ = 0.076). By contrast, striking evidence for differential variation in DNA methylation was observed at 291 and 601 CpGAs of CD4+ and B lymphocytes, respectively (examples 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 OVP associations; for example, RA-specific hypervariability implicates prostaglandin signalling in CD4+ lymphocytes.

CD4+ T cells:

B cells:

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.

References:


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

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