Poster Presentations

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Genomics, genetic basis of disease and functional genomics

THU0001

GENOME-WIDE ASSOCIATION STUDY ON JOINT EROSIONS IN RHEUMATOID ARTHRITIS SUPPORTS DIFFERENTIAL PATHOLOGICAL MECHANISMS ACCORDING TO ANTI-CCP STATUS

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Background: Joint damage is the pathological hallmark of rheumatoid arthritis (RA). To identify the genetic variation associated with a higher level of erosions has proven elusive.

Objectives: The objective of the present study was to perform a genome-wide association study on joint damage in a cohort of RA patients of the Spanish population. Our aims were to provide independent validation of previously reported variants and to identify new candidate risk loci. A stratified analysis was performed based on positivity to ACPA status.

Methods: A total of 1,135 patients diagnosed with RA using the ACR-EULAR criteria recruited by the IMID Consortium were genotyped using a 550,000 single-nucleotide polymorphism array. Additional SNPs were imputed using the 1KG genome data. Joint damage was performed using the S-score, a simplified radiographic erosion score that has a high correlation with the Sharp-van der Heijde score (1). Association testing of SNPs with joint damage was performed via linear regression with the addition of the years of evolution as covariate. The two main components of genetic variation were also added to adjust for potential population stratification. A total of 50 SNPs representing previously reported loci associated with joint damage were selected. Genetic association was also performed at the pathway level using Pascal.

Results: 45 out of 50 SNPs representing 31 previously reported loci for joint damage could be satisfactorily imputed. Association testing of the whole patient cohort replicated the association with *IL2RA* and *TRAF1*. Of relevance, after stratifying for anti-CCP five new loci were replicated: *KIF5A* and *SOST* in ACPA-positive RA and *CD40*, *DKK1* and *TNF* in ACPA-negative RA. *IL2RA* was only significant in the ACPA-positive group and *TRAF1* was not significant in either strata. GWAS on the ACPA-positive cohort and on the ACPA-negative group identified $n=7$ and $n=18$ loci with P -values $< 1 \times 10^{-5}$, respectively. From these, however, only 1 SNP showed nominal significant association in the other patient group. Based on this evidence, we performed a pathway-based analysis to understand the biological mechanisms underlying this difference. Pathway analysis showed 52 biological processes associated with joint damage in ACPA-negative RA and 32 pathways in the ACPA-positive group, with only two shared biological processes between the two groups. Fc Gamma receptor mediated phagocytosis was the topmost biological process associated with erosions specifically in ACPA-negative RA and Signalling by Fibroblast Growth Factor mutants was the top process specific for ACPA-positive patients.

Conclusion: The results from our study provide suggestive evidence that the genetic basis for joint damage is different according to the presence of ACPA. Replication of the new candidate loci in an independent patient cohort is underway.

References:

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THU0002

SOLVING THE COMPLEX MHC ASSOCIATIONS IN SLE IDENTIFIES SEX-RELATED GENE EFFECTS

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Background: Genome-wide association analyses reveal that the Major Histocompatibility Complex (MHC) is the site of the strongest association signals in SLE and Sjögren's syndrome. This associations in lupus and Sjögren's syndrome are linked to *HLA* alleles: *HLA-DRB1*03:01* and *HLA-DRB1*15:01* (in Europeans). The *DRB1*03:01* allele resides on an extended MHC haplotype which includes loss of the complement *C4A* gene. Whether *C4* makes a genetic contribution to SLE/Sjögren's risk has been a long standing issue of contention¹. In comparison, it has been shown that elevated copy number of *C4* is a genetic risk factor for schizophrenia².

Objectives: To define the causal MHC genes in SLE/Sjögren's accommodating both structural and highly polymorphic variation.

Methods: Use NG sequencing data from across the MHC to generate a panel of variants that inform class III structural variation involving the candidate genes coding complement *C4A* and *C4B* as described². To further improve the resolution of the association using transancestral mapping approach in SLE: examining cohorts of European ancestry (from ImmunoChip) and data from the MHC region of an African-American GWAS in SLE.

Results: Comparing European and African data, we have shown that the association signals in SLE can be best explained by signals arising from 1) copy number variation of the complement component 4 (*C4*) genes in the MHC locus (Fig. 1) and 2) by a shared region in the class II region on the *HLA-DRB1*15:01* (in Europeans) and *HLA-DRB1*15:03* (in Africans) that likely operates to elevated *HLA* class II gene expression (Fig. 2). The *C4* locus generates a 7-fold variation in risk for lupus (95% CI: 5.88-8.61; $p < 10^{-117}$ in total) and 16-fold variation in risk for Sjögren's syndrome (95% CI: 8.59-30.89; $p < 10^{-23}$ in total), with *C4A*

Figure 1. Loss of *C4* is risk in African and European ancestry cohorts. A = *C4A*, B = *C4B*, A-B = *C4A* + *C4B(L)* = Long form (with HERV), (S) = short form

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.

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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, 5UTR, 3UTR, 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 (ADAMST10, 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

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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 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, 5UTR, 3UTR, 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.