shown that the largest factor influencing disease susceptibility is genetics. Genome-wide association studies have successfully characterized genetic variants that are associated with RA, with the vast majority of them mapping to non-coding regulatory elements. Understanding the mechanisms by which this phenomenon leads to disease is essential to translate results from genetic association studies to the clinic.

Objectives: There is evidence showing that autoimmune diseases are the consequence of erroneous wiring of the regulatory circuitry between enhancers and their target genes. The aim of this study is to characterize non-coding regions containing RA-associated variants, in order to determine the genes and pathways by which these regions act to increase the risk of disease.

Methods: We isolated CD4⁺ T-cells from blood obtained from RA patients. We stratified patients in two subgroups, high disease activity (DAS28>5.1, n=18) and low disease activity (DAS28<3.2, n=33). All samples were genotyped using Illumina Infinium Exome-24 v1.0 BeadChip arrays. RNA-Seq Libraries were generated for matching RNA samples using the Lexogen QuantSeq Library Prep kit and sequenced on an Illumina NextSeq500. For a subset of 6 samples (3 high disease activity and 3 low disease activity patients), capture Hi-C was performed to characterize chromatin interactions between all RA associated loci and their potential target genes.

Results: We observed numerous chromatin interactions between RA variants and potential causal genes. Preliminary results show that a number of disease-associated SNPs interact with compelling candidate genes situated several megabases away. Whilst some of these chromatin interactions are common to both patients groups, subsets of them are specific to each disease subgroup, which are correlated with differential gene expression.

Conclusion: These results suggests that there might be different biological pathways contributing to disease in RA patients with inactive disease compared to patients with high disease activity.

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FRI0006

PROTECTIVE ROLE OF THE PROPROTEIN CONVERTASE SUBTILISIN/KEXIN TYPE 9 (PCSK9) RS2495477 POLYMORPHISM IN PATIENTS WITH RHEUMATOID ARTHRITIS AND SUBCLINICAL ATHEROSCLEROSIS

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Background: RA is associated with the development of cardiovascular (CV) disease and subclinical atherosclerosis¹. The presence of carotid plaques assessed by ultrasonography studies is a surrogate marker for subclinical atherosclerosis². Proprotein convertase subtilisin/kexin type 9 (PCSK9) is involved in homeostasis of cholesterol, a traditional CV risk factor related to RA and atherosclerosis^{1,3}. PCSK9 polymorphisms can both increase and decrease the risk of CV disease in patients with atherosclerosis³. Moreover, PCSK9 levels have been also related with CV risk⁴. However, there is little information on PCSK9 in RA.

Objectives: To assess the role of several PCSK9 polymorphisms in RA and subclinical atherosclerosis in RA as well as to determine if these ones may influence on PCSK9 mRNA and protein levels.

Methods: PCSK9 rs2479409, rs11583680, rs2483205, rs2495477 and rs562556 polymorphisms were genotyped in 1,169 Spanish RA patients, who met the 1987 ACR and the 2010 ACR/EULAR criteria for RA⁵⁻⁶, and 528 healthy controls. Associations were estimated using odds ratios (OR) and 95% confidence intervals (CI). The potential association between PCSK9 polymorphisms in both RA and controls and the presence/absence of carotid plaques in RA was evaluated by logistic regression. PCSK9 mRNA expression and PCSK9 serum levels were

determined by qPCR and ELISA, respectively. All results were adjusted by sex, age and traditional CV risk factors.

Results: Significant differences in the allele frequencies of PCSK9 rs2495477 between RA patients and controls were found (minor allele: OR=0.55, 95% CI=0.34-0.89, p=0.01). A significant association between minor allele of rs2495477 and carotid plaques was also disclosed in RA patients (OR=0.72, 95% CI=0.56-0.92, p=0.01). PCSK9 levels were significantly decreased in RA patients carrying rs2495477 minor allele compared to controls (97.8 ± 104.9 vs 235.8 ± 93.5 ng/mL, p=0.001). None of the five PCSK9 polymorphisms influenced on its expression.

Conclusion: Our study showed for the first time that PCSK9 rs2495477 confers protection against RA susceptibility and the development of sub-clinical atherosclerosis in RA patients. Furthermore, rs2495477 decreased PCSK9 serum levels in RA that may be crucial to control the disease.

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FRI0007

IDENTIFICATION AND VALIDATION OF PLASMA MICRO-RNA 425-5P AND -451A AS MICRO-RNAs ASSOCIATED WITH CARDIOVASCULAR DISEASE RISK OBSERVED IN RHEUMATOID ARTHRITIS PATIENTS

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Background: Cardiovascular disease (CVD) risk is increased in Rheumatoid arthritis (RA) patients, and therefore, improved approaches for its early detection are needed. An accelerated atherosclerosis is considered the cause of the increase CVD risk. As microRNAs (miRNAs) are increasingly recognized as critical regulators in atherosclerosis and possess excellent stability in plasma, this study focused on using miRNAs as noninvasive CVD risk biomarkers in RA patients.

Objectives: To identify plasmatic miRNAs in RA patients that can facilitate earlier diagnosis of CVD and provide insight regarding the increase risk for CVD observed in these patients.

Methods: A discovery and validation studies were performed. To discover miRNAs candidates, we first compared plasmatic profiles of 754 miRNAs in 7 RA patients without CVD, in 7 patients with acute myocardial infarction (AMI) but without RA, and in 7 healthy controls matched for age and for classical CV risk factors. miRNAs commonly expressed in the two group of patients but differentially expressed from the controls were selected as miRNA candidates for validation. Selected miRNAs were validated in independent serum samples from 214 RA patients (validation cohort) by studying its association with subclinical atherosclerosis measured by carotid intima-media thickness (cIMT). Plasma profile of miRNAs in the discovery study was analyzed using validated TaqMan Open Array miRNA panels which enables the quantification of 754 human miRNAs.

Differential expression analysis was performed with Expression Suite software and selected miRNAs candidates were validated in the validation study by real-time PCR with LNA™ microRNA qPCR assays and analyzed with 2^{-DCt} method. Kruskal-Wallis test, Dunns post-test and linear regression were used for statistical analyses.

Results: In the discovery study we were able to measure 379 (50%) of the miRNAs represented in the array. We observed that 10 miRNAs (miRNA-Let-7a, miRNA-96, miRNA-381, miRNA-451a, miRNA-518d, miRNA-425-5p, miRNA-572, miRNA-190b, miRNA-708, and miRNA-1180) were expressed at the same level in RA and AMI patients but were significantly downregulated compared with controls. These 10 miRNAs were selected as potentially miRNAs associated with the increase risk of CVD in RA patients. Four of those miRNAs were expressed at very low level and were discarded for the validation study. In the validation study with 214 plasma samples from RA patients, we observed that two of the six candidate miRNAs (miRNA-425-5p and miRNA-451a) were significantly associated with cIMT. Thus, adjusted multivariable linear regression analysis showed that miRNA-425-5p and miRNA-451a independently explained 1.4% of the cIMT variability. Furthermore, adjusted regression estimates of the effect of miRNA-425-5p and miRNA-451a on cIMT were $\beta = 0.029\text{mm}$; $p = 0.037$ and $\beta = -0.035\text{ mm}$; $p = 0.039$, respectively. No other miRNA candidate exhibited association with cIMT values. Furthermore we observed that miRNA-425-5p was significantly correlated with ESR ($r = -0.136$; $p = 0.024$) and miRNA-451a with DAS28 ($r = 0.19$; $p = 0.003$), ESR ($r = 0.23$; $p = 0.0001$), CRP ($r = 0.15$; $p = 0.016$) and fibrinogen ($r = 0.28$; $p = 0.0001$). miRNAs concentrations were not affected by any of the AR treatments. No association was observed between the presence of carotid plaque and the expression level of the microRNAs tested.

Conclusion: In the present study, we have identified miRNA-425-5p and miRNA-451a as potentially miRNAs involved in the CVD risk observed in RA patients.

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FRI0008

ADDRESSING THE DIAGNOSTIC GAP IN RHEUMATOID ARTHRITIS BY COMPLEMENTING THE SEROLOGY WITH GENETIC INFORMATION

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Background: Rheumatoid arthritis (RA) is caused by an interaction of inherited and environmental factors. The genetic component was revealed in different twin studies and the shared epitope was identified as main contributor. During several genome-wide association studies (GWAS) different genes have been highlighted as relevant for developing RA but so far, none of these have been used for a diagnostic approach to address the diagnostic gap for RA.

Objectives: The aim of this study was to identify genes within pathways relevant for developing RA and to combine these genetic risk factors with serologic data to improve the diagnosis of RA, especially in regards to CCP negative RA patients.

Methods: The cohort consists of 804 RA patients, 159 Disease controls and 495 Healthy individuals. Serology data were obtained using the EliA™ CCP IgG, ELIA RF IgM and ELIA RF IgA (Phadia AB, Uppsala, Sweden) tests. The genetic measurements were performed using the Next-Generation-Sequencing technology AmpliSeq™ on the Ion GeneStudio™ instruments (Thermo Fisher Scientific, Carlsbad, USA). The combined dataset was analysed by machine learning focusing on multivariate supervised models and discrete models.

Results: The Disease Research Area database (Thermo Fisher Scientific, Carlsbad, USA) uses a proprietary algorithm to create connections between genes and a specific disease highlighting relevant genes and pathways using the available datasets from NCBI, DisGeNet, ClinVar and further databases. Based on this information we were able to identify genes and variants within relevant pathways for RA and designed a sequencing panel specific for diagnosis of RA. The results from the

targeted sequencing approach and the serologic data of the patients were analysed by an algorithm resulting in an improved diagnosis.

Each variant within the algorithm has a specific weighting score resulting in a combination of protective and destructive variants, which are used together with the serology to calculate a RA risk score.

In this study the combination of genetic and serology leads to an improved sensitivity of over 10% compared to CCP alone, with a comparable specificity. This increase in sensitivity results in the identification of >20% CCP-negative RA patients. Additionally, a genetic pattern was identified distinguishing between CCP-negative and CCP-positive RA patients.

Conclusion: Usage of genetic variants in combination with serologic data improves the diagnosis of RA within the measured cohort of Caucasian RA patients. Furthermore, a genetic pattern specific for CCP negative RA patients was accentuated and could be used to improve the diagnosis of CCP negative RA patients in the future. The combination of serology and genetic information for the diagnosis of RA patients could lead to an earlier diagnosis, especially for CCP negative RA patients.

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FRI0009

MOLECULAR PROFILING OF CIRCULATING B-LYMPHOCYTES REVEALS THE SUPERIOR PERFORMANCE OF METHYLOME OVER TRANSCRIPTOME DATA FOR DISCRIMINATING RHEUMATOID ARTHRITIS PATIENTS IN AN EARLY ARTHRITIS CLINIC: IMPLICATIONS FOR TRANSLATING "BIG DATA" INTO CLINICALLY USEFUL TOOLS

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Background: Defining optimal strategies for translating "big datasets" of transcriptome and methylome data into clinically valuable tools in RA will benefit from comparisons where potential confounders – including therapeutic background, disease phase and cell substrate heterogeneity – are controlled as far as possible.

Objectives: We have obtained paired B- and CD4+ T-lymphocyte whole genome expression and methylation data from drug-naïve early arthritis clinic patients at a single centre. Focussing on B-lymphocyte data we here ask which of these datasets has most value in discriminating early RA – and the additive value of combining them.

Methods: CD19+ B-lymphocytes were isolated by positive selection from fresh peripheral blood of 90 drug-naïve patients attending the Newcastle Early Arthritis Clinic (NEAC), comprising 36 RA patients and 54 disease controls matched, so far as possible, for age, sex and acute phase response. Paired RNA and DNA extracted. Gene expression was profiled using the Human HT12 v4 BeadChip, and DNA methylation at >850,000 CpG sites quantified with the MethylationEPIC array (both Illumina). Gene expression and/or DNA methylation classifiers for RA prediction were developed based on a combined approach of classification algorithm