shown that the largest factor influencing disease susceptibility is genetics. Genome-wide association studies have successfully characterized genetic variants that are associated with RA, reflecting 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=17) and low disease activity (DAS28<3.2, n=33). All samples were sequenced 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), we captured 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 suggest that there might be different biological pathways contributing to disease in RA patients with inactive disease compared to patients with high disease activity.

Disclosure of Interests: None declared


FR10006

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

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Background: PCSK9 is a serine protease that regulates plasma LDL-C levels by inhibiting the activity of the proprotein convertase subtilisin/kexin type 9 (PCPSK9), a protease responsible for the degradation of low-density lipoprotein receptor (LDLR). PCSK9 is involved in the homeostasis of cholesterol, and a traditional CV risk factor related to RA and atherosclerosis.1. RA patients show increased PCSK9 expression and PCSK9 polymorphisms can both increase and decrease the risk of CV disease in patients with atherosclerosis.2,3,4,5,6 Moreover, PCSK9 levels have been also related with CV risk.7 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 genes influence PCSK9 mRNA and protein levels.

Methods: PCSK9 rs2479409, rs11583680, rs2483205, rs2495477 and rs652556 were polymorphisms in 1.169 Spanish RA patients, who met the 1987 ACR and the 2010 ACR/EULAR criteria for RA and 528 healthy controls. Associations were estimated using odds ratios (OR) and 95% confidence intervals (CI). The potential association between PCSK9 polymorphisms and classical CV risk factors was examined by logistic regression analysis.

Results: Significantly higher 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 subclinical atherosclerosis in RA patients. Furthermore, rs2495477 decreased PCSK9 serum levels in RA that may be crucial to control the disease.

References:

Disclosure of Interests:
Acyklim support was supported by ERDF and P115/00525 (ISCIII). Personal fees: M. S. 2006; 1500€; M. S. 2011; 1500€; M. S. 2012; 1500€; M. S. 2013; 1500€; M. S. 2014; 1500€; M. S. 2015; 1500€; M. S. 2016; 1500€; M. S. 2017; 1500€; M. S. 2018; 1500€; M. S. 2019; 1500€. Speakers bureau: Consultation fees/participation in company sponsored speaker’s bureau from Pfizer, Lilly, Solli, Celegene, Novartis, Roche and Sanofi.


FR10007

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 increased 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 plasma 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 miRNA 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 Q-PCR with LNA™ microRNA qPCR assays and analyzed with 2^(-ΔΔCt) method. KruksKal-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 80 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 β = 0.029mm; p > 0.007 and β = 0.035mm; 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), CRP (r=0.23; p=0.001), CRP (r =0.15; p = 0.016) and fibrinogen (r=0.28; p =0.001). 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-451 as potentially miRNAs involved in the CVD risk observed in RA patients.

Acknowledgement: The authors thank the patients and healthy controls for their participation in the study.

Disclosure of Interests: SILVIA PAREDES Speakers bureau: Bristol, Roche, Argen, Pfizer, Abbvie, Lilly, UCBB, Delta Taverner Speakers bureau: amgen, pfiser, Bristol, Lilly, Roche, Raimon Ferre: None declared. Josep Maria Allegr Speakers bureau: Daichii, Lluis Masana Consultant for: amgen, daichii, sanofi, Speakers bureau: AMGEN, SANOFI, MYLAN, Joan Carles Vilardell: None declared.


References:


FR10008

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 environmental and inherited 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 Eli-Immunofinity assay. Genes and CpG sites quantified with the MethylationEPIC array (both Illumina). Gene expression levels were measured using Illumina 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.

REFERENCES:
