(SYBR Green technology). 2-ΔΔCT method was used for analysis. 10 healthy donors were used as controls.

Results: The expression levels of miR-124–3 p were upregulated in the plasma of 33 (97.6%) of the patients compared to controls. Receiver operating characteristic curve analysis was conducted in order to evaluate the diagnostic accuracy of the expression levels of the studied miRNA in the plasma. Area under the curve for miR-124–3 p was 0.879 (95% CI=0.740–1.00), p=0.303 × 10–3. When the relative quantification (RQ) cut value was 2.89, the sensitivity was 91.2% and the specificity was 80%. Levels of miR-124–3 p in plasma correlated with the diagnosis (p=0.106 × 10–3), with markers of inflammation – ESR (p=0.0497) and CRP (p=0.047) as well as with the immunological activity – the presence of RF (p=0.007), RF IgM (p=0.004), RF IgG (p=0.004), RF IgA (p=0.005) and anti-CCP antibodies in the serum (p=0.025).

Conclusions: In contrary to the literature data that report levels of miR-124 to be decreased in RA synovial fibroblasts we found increased expression of miR-124–3 p in plasma of RA patients which might reflect the pathophysiological response to the inflammation, the effect of the treatment regimen or the presence of miR-124a gene promoter hypermethylation in the synovial tissue which might downregulate miR-124 locally. To our knowledge this is the first study to evaluate the diagnostic accuracy of plasma levels of miR-124 in RA patients as well as the possibility of using miR-124 as biomarker for disease activity but larger set is needed to confirm these results in the clinical practice.

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AB0015 THE EFFECT OF RARE CODING VARIANTS ON RESPONSE OF TNF INHIBITORS TREATMENT IN RHEUMATOID ARTHRITIS

S. Bang1,Y. Park2, H.-H. Kwon1, H. Yoo1, H.-S. Lee1, S.-C. Bae2,3. 1Rheumatology, Hanyang University Hospital for Rheumatic Diseases, Guri; 2Rheumatology, Hanyang University Hospital for Rheumatic Diseases, Seoul, Korea, Republic of Ireland

Background: Although pharmaco genetic studies of TNF inhibitors (TNFi) response presented the estimates of high heritability, only few loci with suggestive weak common association as biomarkers for TNFi response have been identified.

Objectives: We aimed to identify novel functional rare variants associated with response to etanercept using targeted exon sequencing in Korea.

Methods: Disease activity scores were assessed at baseline and after 6 months in 156 Korean RA patients who started etanercept due to moderate or high disease activity. We analysed targeted exon sequencing data of 399 genes selected from a multi-marker approach. We conducted a single-marker association test (MAF >1%) and a gene-based analysis (optimal sequence kernel association test [SKAT-O]) of rare variants (MAF <1%). In addition, we performed gene set analyses of TNF pathway genes.

Results: We identified that clinical factors seem to influence the therapeutic good response of etanercept including male, high disease activity score at baseline, BMI. After stringent quality control, we analysed 14 024 variants of 399 genes in 156 RA patients. We identified two novel significant functional SNPs [rs16942564, rs61734378 (exon of AKAP13)] associated with response to etanercept, surpassing study-wide significant threshold (p=3.0 × 10−5) in single variant association tests. Using a gene-based approach, we found two genes with nominal burden signals (p<0.001) which did not reach study-wide significance. In the gene set enrichment test, we found no evidence for enrichment of association at genes involved in the TNF pathway.

Conclusions: We were unable to identify rare coding variants with large effect of 399 targeted genes. Our study suggests that rare coding variants of RA risk associated genes do not contribute to heritability of response to etanercept therapy.

Disclosure of Interest: None declared


AB0016 CHROMATIN LOCALIZATION OF SURVIVIN IN CD4+ T-CELLS OF PATIENTS WITH RHEUMATOID ARTHRITIS

S. Andres1, K.M. Anderson2, A. Dandimopoulos2, G. Katona1, M.I. Bokarewa3,4. 1Department of Chemistry and Molecular Biology; 2Department of Rheumatology and Inflammation Research, University of Gothenburg, Gothenburg; 3Department of Biosciences and Nutrition, Karolinska Institutet, Solna; 4Sahlgrenska University Hospital, Gothenburg, Sweden

Background: Oncoprotein survivin emerged as an important player in the pathogenesis of rheumatoid arthritis (RA). Results of genome-wide study suggest that survivin may take part in transcription stimulation of the RA-specific genes.

Objectives: To identify and describe survivin-dependent differences in transcription pattern between CD4+ T-cells of RA patients and healthy subjects focusing in particular on a subset of genes involved in maturation of Th1 and Th17 cells.

Methods: CD4+ T-cells were isolated from PBMC of 3 RA patients and 5 non-smoking and 2 smoking healthy controls using a positive selection and activated with Pam3cys+Concanavalin A+LPS. Chromatin immunoprecipitation (ChIP) was done using rabbit polyclonal anti-Survivin, purified DNA was prepared into libraries using TruPLEX (Rubicon) and sequenced using Hiseq 2000 (Illumina).

Results: Resulting fastq sequencing files were mapped to the human reference genome (hg38) using the STAR aligner. Peaks were associated with the closest transcription start site. Enriched peak regions (p<10−5) were identified in survivin-ChIP samples above background (“input”) using the Homer software. The peaks were analysed using gene ontology (GO) technique as implemented in GOrilla and GSEA software. The genes, scored high in RA and not present/low in any controls or vice versa were enriched. The enriched GO groups were searched for presence of Th1/Th17 regulating genes.

Results: We identified 11 145 survivin-bound chromatin sequencies. Out of them, GO technique indicated 770 genes in RA samples (7.3%) and 786 genes in healthy controls (19.5%) which were annotated and enriched (q<0.05) in GO.