Results: Among the 117 patients with RP, 5 (4.3%) and 6 (5.1%) patients had GD and HT, respectively. Patients with RP were more likely to be complicated with GD (p = 0.047; OR: 2.95, 95% CI 1.24–6.89) compared with prevalence in general Japanese population (0.62% and 5.9%, respectively). RP patients with GD showed a trend to have nasal involvement (100% vs 45.5%, p = 0.023; OR: 2.58, 95% CI 0.99–6.58). There were no association of HLA in the complication of HT among patients with RP.

Conclusion: Patients with RP have high co-occurrence ratio of GD. Patients with the two diseases may be characterized by nasal involvement and HLA-DPB1*02.02.

References:


DOI: 10.1136/annrheumdis-2020-eular.5349

THU0025 METHYLATION ANALYSIS OF VITAMIN D SIGNALING PATHWAY GENES IN RHEUMATOID ARTHRITIS PATIENTS

E. Puncieviciene1, 2, 3, I. Gaizevskas4, L. Venceviciene5,6, D. Vukus5, 6, J. Jarmalaite5, 6, J. Butrimien6, 7, Centre of Rheumatology, Vilnius University Hospital Santaros Klinikos, Vilnius, Lithuania; 2State Research Institute Centre for Innovative Medicine, Vilnius, Lithuania; 3Clinic of Rheumatology, Traumatology Orthopaedics and Reconstructive Surgery, Institute of Clinical Medicine of the Faculty of Medicine, Vilnius University, Vilnius, Lithuania; 4Clinical Center of Rheumatology, Traumatology and Oncology, Institute of Clinical Medicine, Vilnius University, Vilnius, Lithuania; 5Centre of Family Medicine, Vilnius University Hospital Santaros Klinikos, Vilnius, Lithuania; 6Vilnius University, Faculty of Medicine, Vilnius University, Vilnius, Lithuania; 7Centre of Laboratory Medicine, Vilnius University, Vilnius, Lithuania.

Background: Vitamin D is known for its immunomodulatory and epigenome interacting effects. Vitamin D deficiency is frequently observed in rheumatoid arthritis (RA) patients compared to healthy controls, is also named as a potential risk factor in RA ethiopatogenesis and may alter DNA methylation of certain genes [1, 2]. Still, causality of vitamin D deficiency in RA patients needs to be elucidated.

Objectives: The aim of the study was to evaluate relationship between DNA methylation status of vitamin D related genes (VDR, CYP24A1, CYP2R1), miRNA-155 expression, vitamin D level and its association with RA.

Methods: CpG islands in promoter region of the VDR, CYP24A1, CYP2R1 genes were chosen for DNA methylation analysis by means of pyrosequencing. DNA from blood mononuclear cells of 31 RA patients and 31 age and sex matched healthy controls was assessed for methylation pattern after informed consent was obtained in Vilnius university Hospital Santaros klinikos Centre of Rheumatology. For miRNA analysis quantitative reverse transcription PCR was used. Chimeriluminescent microplate immunoassay was used to assay 25(OH)D serum levels.

Results: 25(OH)D concentrations varied from deficiency (<50 nmol/l), insufficient (50-75 nmol/l) to normal range (75-100 nmol/l) in RA (mean 47.49 nmol/l; SD ± 27.93) and healthy controls (mean 57.38 nmol/l; SD ± 29.93), CYP24A1 methylation level was significantly higher in comparison to VDR (p<0.0001) and CYP2R1 (p<0.0001) genes in both groups. CYP24A1 hypermethylation was also observed in older subjects (p=0.012). The study demonstrated a significant positive correlation between vitamin D concentration and VDR and CYP2R1-genes methylation intensity (r²=0.31, p<0.014; r²=0.25, p=0.042, respectively). However, gene methylation frequency and methylation intensity showed no significant difference between RA patients and healthy controls (VDR – 2.4 vs 2.6 %, CYP24A1 – 16.6 vs 15.3 %, CYP2R1 – 2.6 vs 2.6 %) (p>0.05). To note, miRNA-155 expression negatively correlated with CYP24A1 methylation intensity (r²=0.43, p=0.009).

Conclusion: Our study identified significant associations between the VDR and CYP2R1 promoter methylation and vitamin D concentration. However, no significant differences in DNA methylation pattern between RA patients and healthy controls were detected. MrIR-155 expression was associated with CYP24A1 methylation level, confirming its possible involvement in vitamin D metabolism. The data of our study suggests that epigenetic phenomena are significantly involved in vitamin D metabolism and may have an indirect effect on RA ethiopathogenesis.

References:

Acknowledgments: This project has received funding from the Research Council of Lithuania (LMTL), agreement No. S-MIP-17-12.

Disclosure of Interests: None declared.

DOI: 10.1136/annrheumdis-2020-eular.2593

THU0026 MICRO-RNA DIFFERENTIALLY REGULATE THE ALTERNATIVE PRTN3-MRNA IN GRANULOMATOSIS WITH POLYANGIITIS

N. Reichard1, A. Kerstein-Staehle1, A. Muller1, G. Riemekasten1, P. Lamprecht1, S. Schinke1, 1University Lübeck, Rheumatology and Clinical Immunology, Lübeck, Germany.

Background: Micro-RNAs (miRNAs) are short non-coding RNAs that regulate inflammation mostly by translational repression. Previously, we screened 847 miRNAs in nasal tissue from GPA patients and found a disease associated alteration of miRNA expression compared to healthy controls and chronic rhinosinusitis. MR-184 was most over expressed in nasal tissue from GPA (13.4x). The dual-luciferase reporter assay confirmed a significant reduction of Proteinase-3 (PRTN3) expression by miR-184 (1). PRTN3 transcripts with an alternative 3’ untranslated region (UTR) have been described in GPA (2). The pathophysiological relevance of this alternative transcript remains unclarified.

Objectives: To identify new miRNA targets of potential pathophysiological relevance in GPA, we validated the effect of the 21 most dysregulated miRNAs on the mRNA of PRTN3. Further, we included the alternative PRTN3 mRNA in our screen to look for new regulatory differences.

Methods: The inhibitory capacity of miRNAs on Proteinase-3 mRNA was estimated by a dual-luciferase reporter system. The sequences of the alternative (132bp longer) and the regular 3’UTR-PRTN3 were cloned and inserted into the pmirGLO vector and co-transfected with 21 miRNA mimics into HeLa cells. Co-transfection with Caenorhabditis elegans mir-67 mimic (cel-mir-67) was used as negative control. Statistical significance was evaluated by students t-test adjusted for multiple comparisons (Holm-Sidak).

Results: For 18 of 21 investigated miRNAs no effects could be observed on the alternative and the regular 3’UTR-PRTN3. But there were remarkable differential effects of let-7l, mir-184 and mir-708. Let-7l (-29.2%) and mir-708 (-23.6%) both showed a suppression of the alternative 3’UTR-PRTN3 but no effect on the regular 3’UTR-PRTN3 while mir-184 only suppressed the regular 3’UTR (-17.5 %) and not the alternative variant (fig. 1-2).

Conclusion: Disease specific miRNA signatures together with an increased PRTN3 level and in alternative PRTN3 mRNA in GPA suggest a dysregulation of PRTN3 expression in GPA. To our knowledge this is the first analysis in GPA showing that miRNAs can differentially regulate the expected and the alternative 3’UTR variants of PRTN3-mRNA. As miR-184 is markedly upregulated in GPA, a repression of PRTN3 is to be anticipated, possibly as a reaction to previous neutrophil activation with PRTN3 overexpression. Our findings also strengthen the potential pathophysiological role of the alternative PRTN3 mRNA.

References: