AB0153

FUNCTIONAL CHARACTERIZATION OF THE SJÖGREN'S SYNDROME-ASSOCIATED LOCUS DDX6-CXCR5

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Background: Sjögren's syndrome (SS) is a chronic, heterogeneous disease with hallmark features of auto-inflammation and autoantibody production. We previously identified association between the *DDX6-CXCR5* locus and SS surpassing genome-wide significance.

Objectives: This study aims to determine the mechanism by which this association contributes to disease.

Methods: Fine mapping and imputation allowed enrichment of existing genetic datasets from a total of 1916 SS cases and 3684 controls with 971 testable variants in the *DDX6-CXCR5* interval. Candidate variants were prioritized using statistical and bioinformatics approaches. Electromobility shift assays (EMSAs) and pull-downs (PDs) followed by mass spectrometry (MS) were used to determine allelic-specific differences in binding using lysates from HSB-2 (T), Jurkat (T), Reh (B), Ramos (B), Daudi (B), THP-1 (monocyte), and HEK 293T (epithelial) cells.

Results: Bioinformatic analysis of the top associated variants after imputation (rs7125066 and rs7119038) in the *DDX6-CXCR5* region did not yield evidence of regional functionality. However, 46 other candidates that span the region of association were identified through imputation. Chromatin methylation pattern data from the Roadmap database showed several variants in this region were within transcription start sites or enhancer elements depending on the cell type and state. Using RegulomeDB, Haploreg, and other databases, rs4938572, rs12365699, rs57494551, and rs10892294 showed strong evidence affecting binding and/or expression of one or more target genes in the region and were selected for further study. Using EMSAs, statistically significant increase in binding for the risk allele as compared to the non-risk allele was found for rs4938572 ($p < 0.01$) for cell lysates from HSB-2, Reh, THP-1, Ramos, Jurkat, and Daudi but not HEK 293T cells. While decreased binding of risk allele for rs10892294 and rs12365699 were found for Ramos cells only, rs57494551 showed decreased binding for both Ramos and THP-1 cell lysate. The risk allele of rs57494551 also decreases binding using THP-1 cell lysate. Preliminary MS analysis showed several immune related transcription factors likely binding this region, including: IRF8, GTF1, RFX5, IKZF3, PAX5, and IKZF1. Cell type-specific expression of the genes in the region shows the expression of *DDX6* spans many immune cells subsets while the *CXCR5* expression is more restricted.

Conclusions: The genetic variants in the *DDX6-CXCR5* locus that were selected based on bioinformatics data resulted in a difference in the direction of binding, with the risk allele all residing on the same risk haplotype. The change in binding of protein to each associated variant likely would alter expression of both protein coding genes in a cell/context specific manner. Ongoing studies will assess the regulatory role of these sequences using luciferase assays and CRISPR/Cas9 based genetic modification of target SNPs in various cell types.

Disclosure of Interest: None declared

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AB0154

NOTCH3 IS UPREGULATED IN LUPUS NEPHRITIS AND ITS DEFICIENCY ACCELERATES LUPUS PROGRESSION

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Background: Systemic lupus erythematosus (SLE) is a chronic inflammatory disease that affects the kidneys in about 50% of patients. Lupus nephritis (LN) is a major risk factor for overall morbidity and mortality in SLE patients. Notch signaling constitutes an evolutionarily conserved pathway that determines major decisions in cell proliferation, survival, and differentiation.

Objectives: To address the role of Notch-3 signalling in the disease development of SLE and particularly LN.

Methods: We measured renal expression of Notch-3 in kidneys of both, LN patients and lupus-prone MRL.lpr mice. Notch-3^{-/-} and B6. Fas^{lpr/lpr} mice were

crossbred to generate a B6. Fas^{lpr/lpr} Notch-3^{-/-} genotype and we analyzed the spontaneous disease manifestations in 40 week-old mice. Furthermore, we administered antisense oligodeoxynucleotides (ODNs) targeting Notch-3 or scrambled controls in the more aggressive lupus-prone mice strain (MRL. Fas^{lpr/lpr}) mice starting at week 12 for 3 weeks and analyzed these mice at the age of 16 weeks.

Results: In 20-week old MRL.lpr mice, receptor Notch-3 and its target gene *Hes2* were significantly up-regulated in kidneys in comparison to 8 and 12 week-old littermates. *De novo* renal Notch-3 expression in lupus mice and SLE patients was especially pronounced in the glomerular compartment. B6. Fas^{lpr/lpr} Notch-3^{-/-} mice exhibited an aggravated lupus phenotype with earlier mortality, increased lymph nodes/spleen size, enhanced glomerular collagen IV deposition and increased immune cell infiltration in the kidneys. In line with this, application of anti-sense Notch-3 ODNs in MRL.lpr mice at the time of the transition from clinically inapparent to a manifested disease state (starting at week 12), resulted in accelerated disease progression as evidenced by enhanced lymphadenopathy, splenomegaly and an increase of inflammatory markers.

Conclusions: Overall, these results demonstrate that Notch-3 exerts protective effects in the course of SLE, and its deficiency or inhibition accelerates the development of SLE manifestations.

Disclosure of Interest: None declared

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AB0155

1,25 DIHYDROXYVITAMIN D SUPPLEMENTATION REDUCES HIND PAW EDEMA IN PRISTANE-INDUCED LUPUS MODEL

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Background: Systemic lupus erythematosus (SLE) is a multifactorial, autoimmune inflammatory disease with pleomorphic clinical manifestations involving different organs and tissues. Vitamin D (VITD) deficiency is highly prevalent and evidence is mounting that it contributes to the morbidity and mortality in SLE. VITD is an essential steroid hormone with well-established effects on mineral metabolism, skeletal health, and more recently established profound effects on immune system health.

Objectives: To evaluate the development of SLE after VITD supplementation in pristane-induced lupus model.

Methods: Female BALB/c mice divided into 3 groups: healthy animals (CO), SLE animals (PIL) and SLE animals + vitamin D supplementation (PIL+VITD). PIL +VITD group received a subcutaneous injection of Calcijex® [2 µg/kg] in PBS-Tween 20 buffer every second day during 180 days. Animals were monitored every 2 months for body weight, free exploratory locomotion, grip strength, endurance exercise performance and edema size. Interleukin 2 (IL-2), IL-4, IL-6, IFN-γ and TNF-α were measured by Luminex technology. The histological and immunofluorescence parameters (IgG, IgM and C3) of the kidney and the joints are under analysis. Data was analyzed with ANOVA Two-Way followed by Bonferroni and independent sample t-test. $p < 0.05$ was considered significant. All data are represented as Mean±SEM.

Results: When compared with CO group (5.12±1.23 g), both PIL (7.53±1.09 g) and PIL+VIT D (8.08±2.28 g) groups exhibited a greater body weight gain by day 180 of the experiment ($p < 0.05$). There was no statistically significant difference in free exploratory locomotion and grip strength between the groups, but both the PIL (29.39±6.75 min) and the PIL+VITD (25.75±12.48 min) groups showed increased fatigue compared to the CO group (42.08±3.47 min). Still, VITD supplementation reduced mean paw swelling to compared PIL group (0.207±0.29 vs 0.24±0.051, $p < 0.05$).

Conclusions: The supplementation of VITD improves the clinical severity of PIL-manifestations, such as the reduction of hind paw edema. Further analysis of the histology of the joints and the kidney will complement the evaluation of the vitamin D modulating effects on the immune system.

Disclosure of Interest: None declared

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AB0156

POLYSACCHARIDES FROM DENDROBIUM OFFICINALE AMELIORATE THE DEVELOPMENT OF MURINE SJÖGREN'S SYNDROME VIA INDUCING IL-10-PRODUCING B CELLS

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Background: Primary Sjögren's syndrome (SS) is a chronic autoimmune disease with salivary gland (SG) hypofunction and inflammation. Currently, effective