Background: The autoimmune regulator gene (*AIRE*) has very important role in self-tolerance. Estrogen induced hypermethylation of *AIRE* promoter was put forward as one of the reasons for susceptibility to autoimmunity in females. [1] Sulphasalazine is a commonly used drug in rheumatology and is known to be responsible for various autoimmune side effects.

Objectives: We aimed to investigate the effects of commonly used treatments on the methylation status of *AIRE* and levels of interleukin-16 (IL16), interleukin-1 β (IL1 β), and interferon- γ (IFN γ), in lipopolysaccharides (LPS)-induced RAW264.7 macrophage cells which mimic inflammatory state in rheumatoid arthritis in vitro.[2]

Methods: RAW264.7 cells were stimulated by LPS (1 μ /mL). Cell viability test was performed to determine drug concentrations. Following drug treatments, cell media was isolated for the determination of IL16, IL1 β , and IFN γ levels by ELISA. Also, the cells detached by trypsin-EDTA were used for DNA isolation and bisulfite modification. Then, the promoter methylation status of *AIRE* was analyzed with methylation-specific PCR and agarose gel electrophoresis.

Results: Our results demonstrated that the *AIRE* promoter is highly methylated in absence of any inflammatory stimulus. LPS treatment changed methylation status to unmethylated form. Leflunomide (LEF), sulfasalazine (SLZ) and methotrexate (MTX) significantly altered methylation status as the methylated ($p=0.041, p<0.001, p<0.001$, respectively). Surprisingly, SLZ treatment caused significantly more methylation in *AIRE* promoter than other drugs ($p<0.001$, Fig 1A). Analysis of IL16, IL1 β and IFN γ levels demonstrated that LPS caused potent increase in all three cytokines ($p<0.001$, Fig 1B-D). LEF and SLZ significantly prevented LPS-induced increase in these cytokines ($p=0.025, p<0.001$ for IL-16, $p<0.001$ for IL-1 β and IFN γ , Fig 1B-D). Although MTX exacerbated LPS-induced increase in IL16 levels, it inhibited LPS induced increase in IL1 β and IFN γ levels ($p<0.001$, Fig 1B-D).

Figure 1. PCR gel image. All data expressed as mean $\pm$ SD. *** $p<0.001$ versus control, ## $p<0.001$, # $p<0.05$ versus LPS, +++ $p<0.001$ versus sulfasalazine.

Conclusion: Our results suggest that although LEF, SLZ and MTX potently suppresses LPS-induced inflammatory cytokines, they affect *AIRE* methylation differently. Changes in the methylation status of *AIRE* are important for autoimmunity. Relative increase in methylation of *AIRE* promoter by SLZ usage can be responsible for its autoimmune side effects, like drug induced lupus or hypersensitivity reactions, which are not common in MTX and LEF usage.

IL16 is a key regulator of biological properties of CD4 $^{+}$ T cells, it also regulates migration of CD4 $^{+}$ CD25 $^{+}$ Treg cells.[3] MTX treatment was shown to increase Treg cells in early rheumatoid arthritis patients. LEF and SLZ's effect on Treg cells was shown to be different from that of MTX.[4] Our results demonstrated that MTX exacerbated LPS-induced increase in IL16 levels in contrary to LEF and SLZ. This difference may also be responsible for the different effect of these medications on Treg functions.

The different effect of commonly used disease modifying drugs on IL16 levels and methylation of *AIRE* promoter is interesting and deserves attention.

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AB0013

ASSOCIATION BETWEEN STAT4 POLYMORPHISM AND MANIFESTATIONS OF SLE

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Background: SNP rs7574865, located within the third intron of STAT4 gene at chromosome 2, has been associated with susceptibility to SLE among different ethnic groups.^{1,2} Interestingly, we recently have documented an association between this gene and susceptibility to systemic lupus erythematosus (SLE) in Indian population.³

Objectives: To determine whether the STAT4 (rs7574865) SNP is associated with clinical and immunological manifestations in SLE.

Methods: The study was carried out on 100 unrelated SLE (SLICC criteria 2012) patients from North-East India. Genotyping of STAT4 rs7574865 SNP was done using Taqman probe and Real-Time Polymerase chain reaction. An association study was performed between the alleles and genotypes of STAT4 rs7574865 with the clinical and immunological manifestations included in the SLE SLICC classification criteria. For all analysis, the statistical significance was fixed at 5% level of significance ($p < 0.05$).

Results: The mean duration of illness was 2.69 $\pm$ 2.55 years. Cases and Controls remained in Hardy-Weinberg equilibrium. The occurrence of Photosensitivity and hyperpigmentation was significantly higher in TT genotype group (97.22% and 77.77%, respectively) with $p < 0.001$ in each case. SLE patients with nephritis (Albuminuria >500mg/24 hours) and elevated serum creatinine were both significantly higher in TT genotype group as compared to GT and GG ($p < 0.001$ and $p = 0.001$ respectively). The Anti-dsDNA antibody was significantly associated with TT genotype ($p < 0.001$).

Conclusion: Our study provides evidence regarding the association between STAT4 rs7574865 gene polymorphism is risk factor for cutaneous manifestations, Lupus nephritis and Anti ds-DNA positivity in SLE. So, our findings reinforce the need for further association studies including prospective studies with larger subjects in order to replicate such findings.

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AB0014

METHOTREXATE - IMPLICATIONS OF PHARMACOGENETICS IN THE TREATMENT OF PATIENTS WITH RHEUMATOID ARTHRITIS

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Background: Methotrexate (MTX) is an anti-folate drug with anti-proliferative and anti-inflammatory effects. MTX proved to be the most highly effective, fast-acting disease modifying anti-rheumatic drug (DMARD), being widely used for the treatment of rheumatoid arthritis (RA) (1).

Objectives: This review aims to describe the main genetic variants identified concerning proteins that play a role in methotrexate's kinetics and efficiency profile.

Methods: A literature review was conducted since January of 2000 until December 2020, by searching the PubMed and Embase bibliographic databases, employing the following MeSH terms: methotrexate, pharmacogenetics, pharmacokinetics and rheumatoid arthritis. The search was limited to articles in English language. Two independent reviewers screened the titles and abstracts followed by a full-text review to assess papers regarding their eligibility. A total of 48 articles matched the research criteria and were analysed.

Results: Genetic variants of four main proteins, with different functions, have been consistently described.

Reduced folate carrier 1 (RFC1), a constitutively expressed folate transport protein that has high affinity for MTX is responsible, almost exclusively, for the transport of folate and MTX into the cell. The most commonly studied variant of the gene is the 80G > A variant (rs1051266), mapped within exon 2, on chromosome 21. It seems to improve RA responses to MTX, clinical efficacy with long disease remission (2).

ABC transporters are involved in the efflux of MTX from cells. An increased expression and function of these transporters should decrease MTX concentrations in target cells, resulting in lack of therapeutic response. *ABCB1* 3435 C/T (rs1045642) is a high frequency polymorphism, significantly associated with RA good responses, symptom remission and reduced adverse events, due to MTX treatment (3).

Thymidylate synthase (TYMS) is involved in thymidine synthesis. MTX decreases TYMS activity by inhibition and decreasing the access to tetrahydrofolate (THF) cofactors (1). The most common genetic variant of the TYMS gene consists of a 28 bp tandem repeat (rs34743033), with double and triple number of repeats (2R and 3R). The 3R allele genotype was associated with decreased efficacy and increased toxicity (4).

The 5,10-methylenetetrahydrofolate reductase (MTHFR) enzyme is indirectly inhibited by MTX. The most common SNPs of the MTHFR gene are C677T (rs1801133) and A1298C (rs1801131). Both are associated with a decreased efficacy and an increased toxicity of MTX (5).

Conclusion: MTX response is affected by many gene variants; the effect of each variant separately is likely to be small. Additionally, gene-gene interaction, enhancing the potential role of linkage disequilibrium. This shows the emerging need for a better gene characterization and to improve the knowledge about variants distribution according to ethnicity, to explain different responses to MTX at an individual level.

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AB0015

STUDY OF VDR AND VDBP GENES AS CANDIDATE SUSCEPTIBILITY GENES FOR THE DEVELOPMENT OF IMMUNE-MEDIATED DISEASES IN THE PARAGUAYAN POPULATION

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Background: Immune Mediated Inflammatory Diseases (IMIDs) are complex diseases that are believed to have a strong interaction between the genome and the environment as part of their aetiology. In studies using the candidate gene strategy, genetic variation in a gene where functionality has been associated with the pathophysiology of the disease under study is being analyzed. In the last decade, polymorphisms of the vitamin D receptor (VDR) and VDBP genes have been more emphatically studied in IMIDs in different populations, but the results reported have not yet been conclusive.

Objectives: To identify an association between vitamin D receptor (VDR) and vitamin D-binding protein (VDBP) gene polymorphisms, and IMIDs in Paraguayan patients.

Methods: Association study of VDR (SNPs rs731236, rs7975232, rs2228570) and VDBP (rs4588) gene polymorphisms with susceptibility to IMIDs in Paraguayan population. A total of 399 patients with IMIDs (i.e. Systemic Lupus Erythematosus (SLE), Scleroderma (ES), Rheumatoid Arthritis (RA), and Cutaneous Psoriasis (CPS) and 100 hypernormal controls (HC) from the same population were included in this study. Genotyping was performed using Tagman real-time PCR-based technology (Life Technologies, USA). Statistical analysis was performed using Rv3.0.1 statistical language software (www.R-project.org). A p value ≤ 0.05 was used for statistical significance.

Results: A total of 399 individuals, 100 controls and 299 patients (99 RA, 100 SLE, 50 ES, and 50 PSO) were included. Seventy-six percent were female and 24% were male. The mean age was 43.7±14 years. Four SNPs were genotyped: rs731236, rs7975232, rs2228570, rs4588. The HWE test was not statistically

significant for any of the 4 SNPs considered (P>0.05), confirming the quality of genotyping and the absence of technical bias. (Table 1).

Table 1. Genotyping of SNPs of the VDR and VDBP gene in Paraguayan population with IMIDs.

SNP	IMID	Minor Allele	Major Allele	MAF Control	MAF Case	OR	IC.L	IC.H	p allelic	P.Genetic
rs731236	SLE	G	A	0.5	0.4	0.64	0.42	0.97	0.035	0.08
rs731236	RA	G	A	0.5	0.41	0.69	0.46	1.05	0.071	0.12
rs731236	SS	G	A	0.5	0.42	0.71	0.42	1.18	0.18	0.37
rs731236	CPS	G	A	0.5	0.38	0.6	0.36	1.01	0.049	0.042
rs2228570	SLE	A	G	0.36	0.38	1.14	0.74	1.74	0.6	0.45
rs2228570	RA	A	G	0.36	0.31	0.83	0.53	1.28	0.4	0.56
rs2228570	SS	A	G	0.36	0.36	1.02	0.6	1.73	1	0.057
rs2228570	CPS	A	G	0.36	0.39	1.16	0.68	1.96	0.61	0.83
rs7975232	SLE	C	A	0.36	0.32	0.82	0.53	1.26	0.4	0.072
rs7975232	RA	C	A	0.36	0.29	0.72	0.46	1.12	0.14	0.064
rs7975232	SS	C	A	0.36	0.22	0.49	0.27	0.88	0.012	0.0064
rs7975232	CPS	C	A	0.36	0.41	1.21	0.72	2.03	0.45	0.016
rs4588	SLE	T	G	0.23	0.27	1.24	0.77	2	0.42	0.48
rs4588	RA	T	G	0.23	0.22	0.93	0.56	1.53	0.81	0.84
rs4588	SS	T	G	0.23	0.21	0.89	0.47	1.65	0.77	0.76
rs4588	CPS	T	G	0.23	0.29	1.37	0.76	2.43	0.26	0.53

Conclusion: There is evidence of nominal association between VDR SNPs: rs731236 (in SLE and CPS), and rs7975232 (in SS and CPS) and the presence of IMIDs disease in Paraguayan patients.

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Adaptive immunity (T cells and B cells) in rheumatic diseases

AB0016

THE IMPACT OF IL-17A THERAPY ON IGG SYALYLATION IN HUMANS

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Background: Rheumatoid arthritis (RA) is characterized by autoreactive B- and T cells. Autoantibodies are a hallmark of RA and contribute to synovial inflammation. We have recently demonstrated that Th17 cells suppress the enzyme ST6 a-galactoside b-2,6-sialyltransferase (ST6GAL1) in developing plasma cells. Thereby, Th17 cells regulate the degree of autoantibody sialylation leading to the increased inflammatory activity of autoantibodies. These events correlate with the onset of RA, arguing for a crucial role of the IL-23/Th17 axis during the transition of asymptomatic autoimmunity into active RA. Therefore, treatment against the IL-23/Th17-axis might present an attractive therapeutic approach to halt or delay RA's onset. However, the effects of Th17 cytokines like IL-17 on IgG glycosylation in humans are so far poorly studied. **Objectives:** To explore whether anti-IL17A treatment can inhibit pro-inflammatory IgG glycosylation patterns in humans.

Methods: Total IgG from patient cohorts suffering from psoriatic arthritis (PsA) treated with Secukinumab (anti-IL-17 blockade, n=26) or Ustekinumab (anti-IL12/23 blockade, n=14) was compared with patients treated with anti-TNFa blockade as a control (n=20). The cohorts were age- and sex-matched and included patients being on therapy for at least six months. Total IgG was isolated using Protein G columns, and IgG glycopeptides of IgG1, IgG2, and IgG4 were analyzed using the LC-MS technique. The effect of IL-17 depletion on IgG glycosylation was analyzed in psoriatic arthritis patients who have been treated with secukinumab for at least six months. Furthermore, in a longitudinal approach, IgG1, IgG2, and IgG4 glycosylation were analyzed from samples, isolated before the beginning of anti-IL-17 blockade and after at least six months of therapy (n=16).

Results: Cross-sectional comparison of cohorts treated with Ustekinumab, Secukinumab, and anti-TNFa therapy did not show any significant differences in sialylation, galactosylation, or fucosylation of IgG1 and IgG2. IgG4 from anti-TNFa treated patients displayed a small increase of sialylation when compared to the Ustekinumab treated cohort.

Longitudinal analyses, however, showed that IL-17A blockade during Secukinumab therapy caused a significant increase of sialic acid-rich IgG glycoforms on IgG1, IgG2 IgG4 patients, while the galactosylation, fucosylation remained unaffected.

Conclusion: This data indicates that IL-17A blockade specifically affects IgG sialylation, while other Fc-glycan modifications remain unaltered. This data confirms our recent findings in mice, where cytokines of the IL-23/Th17 axis specifically induce the production of hypo-sialylated, proinflammatory autoantibodies in