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-1β (IL1β), and interferon-γ (IFNγ) in lipopolysaccharides (LPS)-induced RAW264.7 macrophage cells which mimic inflammatory state in rheumatoid arthritis in vivo.[2] 

Methods: RAW264.7 cells were stimulated by LPS (1μg/mL). Cell viability test was performed to determine drug concentrations. Following drug treatments, cell media was isolated for the determination of IL16, IL1β, and IFNγ 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<0.001, Fig 1B-D). Although MTX exacerbated LPS-induced increase in IL16 levels, it inhibited LPS induced increase in IL1β and IFNγ levels (p<0.001, Fig 1B-D).

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 an 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±2.55 years. Cases and Controls remained in Hardy-Weinberg equilibrium. The occurrence of photosensitivity and hyperpigmentation was significantly higher in TT genotype group (p=0.001) 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.

REFERENCES:

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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, pharmacoepidemiology, and methotrexate 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).

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ABC transporters are involved in the efflux of MTX from cells. An increased expression and function of these transporters should decrease MTX concentration in target cells, resulting in less therapeutic response. ABCG1 3435 C/T (rs1045662) 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.

References:


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

**AB0016**

THE IMPACT OF IL-17A THERAPY ON IGG SIALYLATION 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 α-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 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-IL-17A treatment can inhibit pro-inflammatory IgG glycosylation patterns in human RA.

**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-TNFα 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 agarose and IgG glycosylation was 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 for secukinumab for at least six months. Furthermore, in a longitudinal approach, IgG1, IgG2, and IgG4 glycosylation was analyzed from samples, isolated before the beginning of anti-IL-17 blockade and at least after six months of therapy (n=16).

**Results:** Cross-sectional comparison of cohorts treated with Ustekinumab, Secukinumab, and anti-TNFα therapy did not show any significant differences in sialylation, galactosylation, or fucosylation of IgG1 and IgG2. The results 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).

**Conclusions:** IL-17 blockade specifically affects IgG sialylation, while otherFc-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