

a-modvim abs in 37 pts (31.4%). All pts except one had a-sialMCV ab concentrations above cut-off level (n=117, 99.2%) and none had a-vim abs above cut-off. The mean DAS28 after 3 months of treatment was 3.68 ± 1.23 , with 78 pts (66.1%) not achieving remission or low disease activity. In simple logistic regression models, poor response to therapy was significantly associated with smoking (OR 4.0, 95%CI 1.1–14.4) and a positive test for a-modMCV abs (OR 3.0, 1.4–6.6), but not with positivity for the other ACPAs, age, sex or disease duration. The combination of smoking and positivity for abs against a-modMCV or a-MCV, significantly predicted poor response to the TNF-inhibitor (OR 14.7, 1.8–120.0 and OR 7.0, 1.5–32.0, respectively) compared with not having these factors. Similar patterns, although not significant, were also seen for a-CCP abs and a-modvim abs in combination with smoking.

Conclusion: Antibodies against modified MCV, or smoking in combination with a-MCV abs or a-modMCV abs, respectively, significantly predicted a poor response to TNF inhibitor after 3 months of therapy. Thus, the individual ACPA status could represent a negative predictive factor for response to TNF inhibitors, particularly in conjunction with smoking habits.

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Protein Array Screening Reveals Autoantigenicity Patterns Predicting Anti-TNF Alpha Therapy Response in Rheumatoid Arthritis Patients.

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Background/Purpose: One third of rheumatoid arthritis patients treated with biologicals targeting TNF α are therapy non-responders. We investigated the differences in seroreactivity of patients responding and not responding to anti-TNF alpha therapies prior and after therapy to deduce diagnostically applicable autoantigenicity patterns

Methods: Screening with patient sera were conducted on protein microarrays consisting of 37,830 unique putative expression clones. Response patterns of different immunoglobulin classes were recorded and bioinformatically evaluated enabling us to deduce a set of proteins, which allow to distinguish between therapy responders and non-responders. Next, selected candidates were expressed recombinantly in *E. coli*, purified and further stratified with larger patient cohort in ELISA.

Results: Comparative analysis of microarray results with sera from responders and non-responders to anti TNF drug revealed a more than 30-fold higher number of autoantigens targeted by high titers of IgA autoantibodies in non-responders compared to responders (221 versus 6). More detailed analyses suggest that with 5 autoantigens found to be common in all individual non-responders to anti-TNF α treatment, a reduced number of antigens might be sufficient to predict non-responsiveness. Pretreatment sera from patients with diagnosis of RA based on the ACR classification criteria who were initiated on therapy with TNF inhibitors were analyzed with three markers from the biomarker set of highest priority (RAB11B, PPP2R1A, KPNB1) using an ELISAs assay. In total, analyses of 69 patients were carried out, of which 13 were clearly defined as responder and 8 were clearly defined as non-responder. Of these, already 5 (62.5%) Non-responders could clearly be identified with already three markers from biomarker set of highest priority (RAB11B, PPP2R1A, KPNB1). None of the Responder or Intermediate Responder gave any signal on said markers on IgA-level. The remaining 48 patient samples are derived prior treatment with anti-TNF α inhibitors and were blinded. According to published studies, 20–25% of RA patients treated with TNF α inhibitors are Non-responders: Hence we expect ~10 patients to be non-responder. Within this set, 5 patients (50%) showed clear IgA response to three markers from biomarker set of highest priority (RAB11B, PPP2R1A, KPNB1).

Conclusion: These data suggest that non-response to anti-TNF α biologicals might be predicted based on frequency and magnitude of autoantibodies of the IgA class. Furthermore, 5 IgA autoantigens common in all individual non-responders may be sufficient to predict non-responsiveness.

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Gene-Gene Interactions in Folate Pathway Contribute to Methotrexate Adverse Events in Rheumatoid Arthritis.

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Background/Purpose: To determine whether gene-gene interactions (epistasis) in folate, purine and pyrimidine gene pathways contribute to adverse events following methotrexate (MTX) therapy in rheumatoid arthritis (RA).

Methods: The discovery cohort consisted of 186 patients (93% Caucasians) with early RA enrolled in the monotherapy arm of the BeSt study. MTX therapy was started at 7.5 mg/week and increased to 25 mg/week to control disease activity. MTX related adverse events were evaluated after 6-months therapy. A total of 12 SNPs in folate (RFC-1, MTHFR, MTHFD1, MS, GGH, SHMT1), purine (ATIC, AMPD1, ITPA) and pyrimidine (TYMS) pathways were measured using standard molecular methods. Haplotypes in Methylenetetrahydrofolate Reductase (MTHFR 677C/T-1298A/C) were inferred using expectation-maximization algorithms. Data analysis consisted of multifactor dimensionality reduction technique (MDR). MDR detects non-linear gene-gene interactions by combining predisposing genotypes of adverse events (predisposing genetic attribute) into two separate groups depending on whether they are more common in patients presenting with adverse events or not. The robustness and significance of the model was tested through internal cross validation consistency (CVC, 10-fold) and 1000-fold permutation testing. The predictive value of the predisposing genetic attribute of adverse events was further tested independently in a validation cohort consisting of 47 RA patients from the United States (92% Caucasians) starting MTX (7.5 mg/week) with adverse events evaluated after 4-months MTX therapy.

Results: After 6-months MTX therapy, the incidence of MTX related adverse events was 29% in patients enrolled in the BeSt study (median MTX dose received was 25 mg/week). MDR analysis revealed a non linear pattern of interactions between MTHFR 677C/T-1298A/C haplotypes and variants in GGH [C16T] and MTHFD1 [G1958A] (Table I). The stepwise addition of the three genetic components increased the testing accuracy from 0.49 to 0.64. The constructed predisposing genetic attribute pooling higher and lower likelihood of adverse events in two separate groups revealed a 6.1 fold (CI 95%: 2.9–12.9, $p < 0.001$) greater likelihood of adverse events in carriers (50%) versus non carriers ($p < 0.001$). Sensitivity was 79.6% and specificity was 60.1%. In the validation cohort, the incidence of MTX adverse event was 51% after 4 months therapy (median MTX dose received was 15 mg/week). A total 66% patients carried the genetic attribute predisposing to MTX adverse events. These patients were 5.4 fold (CI 95%: 1.4–21.3, $p < 0.001$) more likely to develop adverse events than those without the predisposing genetic attribute. Sensitivity was 83% and specificity was 52%.

Table. Multifactor dimensionality reduction analysis

Model	Training accuracy	Testing accuracy	CVC	P value
GGH C16T	0.553	0.490	6/10	0.838
GGH C16T + MTHFR 677C/T-1298A/C haplotype	0.621	0.610	10/10	0.081
GGH C16T + MTHFR 677C/T-1298A/C haplotype + MTHFD1 G1958A	0.706	0.639	10/10	0.016

CVC: cross validation consistency; GGH: Gamma-glutamyl-Hydrolase; MTHFD1: Methylene tetrahydrofolate dehydrogenase.

Conclusion: These hypothesis generating data indicate that gene-gene interactions contribute to MTX-induced adverse events in RA.

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Patterns of Interaction Between Genetic and Non-Genetic Attributes and Methotrexate Efficacy in Rheumatoid Arthritis.

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Background/Purpose: The contribution of low-penetrance single nucleotide polymorphisms to methotrexate (MTX) efficacy in rheumatoid arthritis (RA) is inconsistent between studies. We sought to elucidate an architecture of MTX response in three cohorts of RA patients treated with MTX.

Methods: SNP frequencies in genes from folate, purine and pyrimi-