Genomics, genetic basis of disease and antigen presentation

AB0001 ASSOCIATION OF MDR1 GENE G2677T POLYMORPHISM WITH METHOTREXATE RESISTANCE IN PATIENTS WITH UZBEK RHEUMATOID ARTHRITIS

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Background: Methotrexate (MTX) is the most widely prescribed disease-modifying antirheumatic drug (DMARD) for treatment of rheumatoid arthritis (RA)[1]. According to different authors, in 25–40% [3] of cases "complete clinical remission" or "low disease activity" is not achieved, by reason of refractoriness to methotrexate and this may be related to the activity of the MDR1 (ABCB1) gene which is involved in its metabolism. According to many studies on the C3435T isoform MDR1 polymorphism CC genotype is associated with methotrexate refractoriness [2]. But a certain interest is also influenced by the other isoform of the MDR1 gene (G2677T) for the presence of resistance to methotrexate.
Objectives: The aim of this research was to study the effect of MDR1 gene polymorphisms G2677T (rs2032582) on resistance to treatment with methotrexate in Uzbek patients with RA.
Methods: The study involved 76 patients with RA of uzbek nationality and 24 healthy people. The average age of patients was 48,9 ± 15,9 years. RA was diagnosed according to the criteria of the American College of Rheumatology (ACR). 75.6% of patients had high and 24.4% moderate RA activity (DAS 28). All patients took methotrexate in monoterapy, at a dose of 7.5-15 mg for 3-6 months. All patients were genotyped by the MDR1 gene G2677T polymorphisms by using the polymerase chain reaction (PSR-Real time).
Results: Genotyping of the G2677T isoform of MDR1 gene revealed the following results: in patients with CC genotype was found in 22 patients (28.9%), GT genotype was found in 31 patients (40.7%) and TT genotype was found in 23 patients (30.2%). In patients treated with methotrexate, the following disease activity was observed: in patients with CC genotype, the disease activity was Das28 <2.6, with CT genotype Das28 3.2–4.5. Patients with the TT genotype had an activity of Das28> 5.1. Despite the increase in the dose of methotrexate, the remission was not achieved.
Conclusion: TT genotype G2677T isoform of MDR1 gene is associated with resistance to methotrexate. Patients with TT genotype are recommended to replace methotrexate with other DMARD preparations. Patients are recommended to conduct genotyping to the MDR1 gene for personal selection of drugs.

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AB0002 INCREASED SENSITIVITY TO DNA DAMAGING AGENTS IN HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS FROM PATIENTS WITH RHEUMATOID ARTHRITIS

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Background: Rheumatoid arthritis (RA) is a chronic autoimmune disorder with not fully elucidate pathogenesis. Rheumatoid arthritis patients have increased risk of developing lymphomas. One of the possible mechanisms of this predisposition is increased genomic instability and impaired DNA repair. It is unclear how this genomic instability contributes to diseases pathogenesis.
Objectives: The aim of this study was to analyze the sensitivity and repair efficiency of mononuclear cells isolated from RA patients to DNA damaging agents.
Methods: The study group consisted of 22 patients with RA (age years - 60,77±13,00 women-17, men - 5) hospitalized in the Department of Rheumatology between 2017 and 2018 and 10 healthy controls without autoimmune and oncological diseases in clinical history (age 44,09±16,56; women-5, men-6). The peripheral blood mononuclear cells (PBMC) from all subjects were isolated. Using comet assay the degree of intracellular DNA damage as a result of exposure to standard damage factors: tert-butyl hydroperoxide (TBH), bleomycin, methyl methanesulfonate (MMS) and UV radiation was assessed.
Results: RA patients show a statistically significant higher level of endogenous damage in PBMC DNA than controls (mean RA- 8,64% vs 4,68% in control; p<0,001). The extent of the DNA damage induced by TBH, MMS as well as UV was significantly higher in PBMC derived from RA patients than in healthy counterparts (p<0,001). The DNA of RA patients treated with TBH was repaired less effectively than in control (p<0,0001). Significantly higher percentage of DNA damage in RA DNA (p<0,0001) under the influence of bleomycin and clearly marked repair processes were observed. Among the healthy controls lower percentage of DNA was damaged, and although the repair process was slower but the final percent of DNA damage was lower than in RA cases (p<0,0001).
Conclusion: DNA of people with rheumatoid arthritis is significantly more susceptible to damage in baseline and induced. The kinetics of DNA repair from RA patients after the introduction (TBH and bleomycin) was statistically less effective as compared to healthy control. Understanding the ethology of this phenomenon in RA may provide insight into disease pathogenesis and explain the increased susceptibility of patients to malignancies.
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AB0003 GENETICS OF PAIN IN WOMEN WITH FIBROMYALGIA: THE PROMISING ROLE OF REDUCING SEDENTARY BEHAVIOUR

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Background: Fibromyalgia is characterised by chronic pain and a heterogeneous presentation of other symptoms (e.g., fatigue and depression) [1]. It is widely accepted that pain is promoted by both genetic susceptibility and environmental factors such as people's behaviours [2]. In addition to genotype individual associations and gene-gene interactions, when considering complex phenotypes such as pain, gene-environmental interactions are likely present and can help to better understand the disease (i.e. by unravelling underlying mechanisms [3]).
Objectives: To test the individual association of 64 polymorphisms (34 candidate-genes) and the gene-gene, gene-physical activity, and gene-sedentary behaviour interactions with pain and pain-related cognitions in fibromyalgia.
Methods: In 274 women with fibromyalgia, saliva samples were collected for extracting DNA. We measured physical activity and sedentary behaviour by accelerometers for a week, pain with algometry and questionnaires, and pain cognitions with questionnaires. Age, body fat, and analgesics and antidepressants consumption were included as covariates. Significance was set at P-values lower than the Bonferroni's correction or P- and false discovery rate values lower than 0.05.
Results: The rs6311 and rs6313 polymorphisms were individually related to algometer scores. The interaction of rs4818 and rs1799971 polymorphisms was related to pain catastrophizing. Five gene-behaviour interactions were significant: the interactions of sedentary behaviour with rs1383914, rs6860, rs4680, rs165599, and rs12994338 polymorphisms were associated with the bodily pain subscale of the SF-36.
Conclusion: The HTR2A gene (individually), COMT and OPRM1 gene-gene interaction, and the interactions of sedentary behaviour with ADRA1A, CHMP1A, COMT, and SCN9A genes were associated with pain-related outcomes in fibromyalgia females. Besides indicating the relevance of genetic background for pain and pain-catastrophizing, the observed genotype-behaviour interactions suggest that the effects of sedentary behaviour on pain may depend on the genotype of women with fibromyalgia. Future clinical experimental research should examine whether reducing sedentary behaviour is particularly beneficial for reducing pain in women with specific genotypes.

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AB0004 DETECTION OF THE SNP-SNP INTERACTIONS IN THE JUVENILE ARTHRITIS SUSCEPTIBILITY USING MDR ANALYSIS

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Background: Juvenile idiopathic arthritis (JIA) is the most common chronic arthropathy of childhood which is considered to be a complex disease. The genes encoding HLA complex account for only 14% of the JIA risk. Hence, it is suggested that substantial role in genetic predisposition to JIA belongs to the non-HLA gene variations.
Objectives: The aim of the study was to estimate interactions between SNPs of the genes implicated in immune and inflammatory responses: *STAT4* (rs7574865), *CTLA4* (rs5742909), *MIF* (rs755622), *TRAF1/C5* (rs3761847), *RUNX3* (rs11249215) and their effect on the JIA susceptibility.
Methods: 119 patients diagnosed with JIA (mean age 8.48 ± 5.06), and 197 hospital controls with no signs of autoimmune or inflammatory diseases (mean age 14.19 ± 2.56) were included into the study. DNA extraction from peripheral blood samples was performed with phenol-chloroform method. SNPs were genotyped using the PCR-RFLP assay. Multifactor dimensionality reduction (MDR) analysis was performed using MDR 3.0.2 software package with the following configuration: attribute count range – from 1 to 5; cross-validation count – 10; track top models – 1000; search method configuration – exhaustive; ambiguous cell analysis – Fisher's exact test; ambiguous cell assignment – unclassified. The best model was selected on the basis of maximum crossvalidation consistency and testing balance accuracy values.
Results: Model-free nonparametric statistical approach of MDR analysis revealed the best model for JIA susceptibility prediction with cross-validation consistency of 9/10 and testing balanced accuracy of 0.5768. The model includes SNPs of *STAT4*, *TRAF1/C5* and *RUNX3* genes and is characterized by 0.6727 sensitivity; 0.7083 specificity, OR = 4.9921; 95%CI [2.4–10.20], p < 0.0001. Gene-gene interaction analysis discovered three genotype combinations for higher JIA risk. The most statistically significant was: GA (*RUNX3* rs11249215), GT (*STAT4* rs7574865) and GG (*TRAF1/C5* rs3761847), OR = 2.92, combined entropy – 4.83%. Separate data analysis for males and females didn't show any statistically significant model of SNP interactions associated with JIA. However, *MIF* rs755622 with entropy of 2.92% was more informative in females, while *STAT4* rs7574865 with entropy value of 1.12% – in males.
Conclusion: MDR analysis of the JIA case-control data set identified a statistically significant high-order interaction of three polymorphisms: *STAT4* (rs7574865), *TRAF1/C5* (rs3761847), *RUNX3* (rs11249215). This combination may contribute to JIA genetic susceptibility in the Belarusian population.
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AB0005 FAVORABLE RESPONSE TO RITUXIMAB IN A PATIENT WITH HYPOCOMPLEMENTEMIC URTICARIAL VASCULITIS ASSOCIATED WITH A HOMOZYGOUS FRAMESHIFT AGBL3 VARIANT

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Background: Last year we described a homozygous AGBL3 variant in a patient with autoinflammatory features and hypocomplementemic urticarial vasculitis. Whole exome sequencing revealed a deleterious homozygous c.769C>T mutation in AGBL3 (ATP/GTP binding protein-like 3) gene, which results in early termination of the protein (p.Gln257Ter) and deletion of the functional carboxypeptidase domain. This protein belongs to metallocarboxypeptidases that mediate both deglutamylation and deaspartylation of target proteins, and AGBL3 is suggested to catalyze the deglutamylation of polyglutamate side chains, especially in proteins such as tubulins. This variant was not found before in all reported databases including 1000 Genomes Project data.
Objectives: To define the clinical phenotype and treatment responses of a patient with newly defined monogenic hypocomplementemic urticarial vasculitis associated with a homozygous AGBL3 variant.
Methods: We collected all clinical, serologic, and pathological data regarding the clinical findings of the index case as well as recorded all treatment responses throughout the follow-up period during the last 8 years.
Results: The index case was 23-year-old male patient of Assyrian origin, who had consanguineous parents. He was evaluated in our clinic because of recurrent attacks of fever, urticarial rash on the extremities and trunk, conjunctival injections and arthralgia, without a trigger or more frequently following an infection. His 2 to 3 days lasting attacks started when he was 13 and recurred more frequently during warm weather conditions or following hot baths. He had highly elevated CRP and ESR during attacks, but his acute phase response did not return to normal values in between the flares. Low C3 and C4 values were also observed during asymptomatic periods. His ANA test became positive during the course of his disease with an increase in titers during the last years. The biopsy of skin lesions revealed findings compatible with urticarial vasculitis. He responded only partially to corticosteroids, canakinumab and anakinra treatments. His treatment was switched to rituximab last year, and a favorable response was observed following the first two infusions. He developed less frequent and milder attacks only after infections, and acute phase response was reduced to near normal values in between attacks.
Conclusion: The AGBL3 metallocarboxypeptidase gene was recently idenfied as a novel autoinflammatory gene associated with hypocomplementemic urticarial vasculitis phenotype, and it was different from the previously defined variants including DNASE1L3 mutations. The clinical features of the index case included both autoinflammatory and autoimmune findings including autoantibodies; and his inflammatory attacks did respond to rituximab treatment but not IL-1 blockade. Long term follow-up and search for other patients associated with AGBL3 variants among idiopathic hypocomplementemic urticarial vasculitis are required for better clarification of the AGBL3-associated clinical phenotype.
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AB0006 ASSOCIATION OF HLA-DPB1*16:01 ALLELE WITH PR3-ANCA POSITIVE GRANULOMATOSIS WITH POLYANGIITIS IN TURKISH PATIENTS

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Background: ANCA associated vasculitides (AAV) comprise an important subset of small vessel vasculitides with a multifactorial pathogenesis, which is considered to be associated with the interaction of genetic and environmental factors. Certain HLA Class 2 alleles have been reported among the genetic risk factors for AAV from different geographic regions such as Europe and Asia (1,2).
Objectives: In this study, we aimed to analyse the distribution of HLA Class 2 genotypes among a series of Turkish AAV patients in comparison with the healthy Turkish subjects and the previously reported data of AAV cohorts from other countries.
Methods: Ninety-eight patients (aged between 18–75 years) diagnosed with AAV according to 2012 Chapel Hill Consensus Criteria were enrolled into the study. Records of age-and birthplace-matched 196 healthy kidney donors were used as the control group. Patients were classified according to the clinical subgroups (GPA, e-GPA, MPA) and ANCA serotypes (MPO-AAV, PR3-AAV). HLA Class II genotyping were carried out by using NGS-Omixon Holotype HLA Kit at the EFI accredited HLA Typing Lab of Department of Medical Biology. Allele distributions were compared by Chi-square test, and P value lower than 0.05 was accepted as significant.
Results: AAV patients were classified as PR3-AAV (54%) and MPO-AAV (31.6%) according to autoantibodies and GPA (71.4%), MPA(20.4%), e-GPA (8.2%) according to clinical subgroups. HLA-DPB1*04:01 (8.5% vs. 35.9% p<0.01, OR= 0.16 %95 CI 0.08–0.34) and HLA-DPB1*02:01 (%7.5 vs. %22.4 p<0.05, OR=0.28 %95 CI 0.13–0.6) alleles were significantly lower in the PR3-AAV group. HLA-DPB1*16:01 allele was significantly higher in the PR3-AAV group (%8.5 vs. %0.26 p<0.01, OR=36.2 %95 CI 4.5–289.7). In clinical subgroups, HLA-DPB1*04:01 (%7.9 vs. %35.9 p<0.01, OR=0.15 %95 CI 0.08–0.29) and HLA-DPB1*02:01 (%7.9 vs. %22.4 p<0.01 OR= 0.29 %95 CI 0.15–0.57) alleles were significantly lower in the GPA-AAV group. HLA-DPB1*16:01 allele