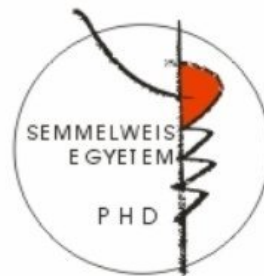


Role of non-MHC genes in the pathogenesis of immune-mediated rheumatic diseases

Doctoral Thesis

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Introduction

Several genetic factors play role in the pathogenesis of immun-mediated rheumatic diseases (rheumatoid arthritis (RA), juvenile idiopathic arthritis (JIA), ankylosing spondylitis (AS)). In all three diseases well-known MHC associations have already been described. Recent studies show evidence of non-MHC genes as susceptibility factors of the immune-mediated diseases as well. We investigated the significance of the following non-MHC genes in the pathogenesis of immune-mediated rheumatic diseases.

Toll-like receptor genes

Toll-like receptors are principal recognition structures of the innate immune system. Their activation results in the production of proinflammatory cytokines, chemokines, metalloproteases, type I interferons. Besides, their activation on antigen presenting cells induce the expression of co-stimulatory molecules therefore they play a role in the regulation of the adaptive immune system as well. These two important functions of the Toll-like receptors presume that changes in their structure or their function leads to pathological immune responses and the development of immune-mediated diseases.

Functional studies confirm the role of the TLR signaling pathway in the pathogenesis of arthritis. It is still unclear whether the TLR pathway contributes to acute or chronic inflammation, but recent experiments underlay the basic function of the TLR/IL-1 receptor family in the development of joint inflammation.

Mannose binding lectin

The three pathways of complement activation are the classical, the alternative and the lectin pathway. Mannose binding lectin is the main molecule of the lectin pathway. It is an acute phase protein, synthesized in the liver and takes part in the innate immune response. Low MBL serum level results in a defect of opsonisation and this leads to development of chronic, recurrent infections in childhood. MBL serum concentration is determined by certain mutations in the MBL2 gene. To date 6 SNPs have been described in the MBL2 gene: three in the promoter region and three in exon I. Five of these polymorphisms affect the MBL serum concentration. MBL2 haplotypes that

determine serum MBL level are defined according to the different variants of these SNPs.

Protein Tyrosin Phosphatase, Nonreceptor 22 (PTPN22)

PTPN22 encodes an intracellular lymphoid tyrosine phosphatase (LYP). Recently a C-T SNP has been described in the PTPN22 gene in the +1858-as position that results in a tryptophan-arginin change in the matured peptid. The variant allele of this polymorphism was first described in Type 1 diabetes (T1D). In 2004 the association of the variant 1858T allele with rheumatoid arthritis was reported by Anne Begovich. According to their results, the variant T allele is present in 17% of white healthy individuals while it is observed in 28% of white subjects with rheumatoid arthritis. The mutant peptid that contains tryptophan is not able to inhibit T cell activation. Since the discovery of the significance of the HLA genes, the PTPN22 C-T SNP, considered as a new candidate gene of RA, is the first genetic variation that shows strong association with several autoimmune diseases and it is supposed to have a great significance in the development of autoimmunity.

ARTS1 (Aminopeptidase regulator of TNFR1 shedding)

ARTS1 is an endoplasmic reticulum associated aminopeptidase (otherwise ERAP1). It has two known functions: during MHC class I. antigen presentation it is involved in trimming peptides to optimal length; and it cleaves cell surface receptors for proinflammatory cytokines. The Wellcome Trust Case Control Consortium (WTCCC) AS whole genome association study performed on 1000 AS patients and 1500 control subjects revealed two SNPs on chromosome 5 that showed significant association with AS (rs27044 and rs30187) both SNPs located in the ARTS1 gene. The WTCCC study reported three other SNPs (rs17482078, rs10050860, rs2287987) in the ARTS1 gene in association with AS with a modest significance level. Results of the WTCCC study indicate that the ARTS1 gene might have an importance in the pathogenesis of AS.

Interleukin 23 receptor (IL23R)

The other, recently identified AS possible candidate gene is the IL23R gene. It encodes a receptor for the IL23 together with the $\beta 1$ subunit of the IL12R. IL23 is a proinflammatory cytokine, member of the IL12 cytokine family. It has a main role in the transformation of the naive CD4⁺ T cells into Th17 cells. Polymorphisms in the IL23R were reported first in a whole genome association study performed on patients with inflammatory bowel disease (IBD). Later these polymorphisms were reported in psoriasis as well. In the Wellcome Trust Case Control Consortium (WTCCC) AS whole genome association study 8 SNPs in the IL23R gene showed significant association with the disease.

Objectives

The Asp299Gly and the Thr399Ile polymorphisms in the TLR4 gene show association with several autoimmune diseases. We investigated the presence of these SNPs in Hungarian AS and reactive arthritis (ReA) patients.

A GT microsatellite polymorphism has been described in the intron II of the TLR2 gene. The length of the GT repeats is associated with inflammatory and immune-mediated diseases according to recent data. We determined the length of the GT microsatellite polymorphism in the intron II of the TLR2 gene in Hungarian RA, AS and JIA patients. To assess the functional significance of this polymorphism we evaluated TLR2 gene expression as well.

Serum concentration of the MBL is determined by polymorphisms in the MBL2 gene. We determined prevalence of the MBL haplotypes and the MASP2 SNP in Hungarian JIA patients.

Autoimmune diseases show strong association with two genetic loci: the MHC genes on chromosome 6 and the PTPN22 gene on chromosome 1. We determined the prevalence of HLA-DRB1 and PTPN22 genotypes in Hungarian JIA patients

The association of HLA B27 and AS is well known, but the role of the HLA-B27 in other seronegative arthropaties is not clearly defined. We investigated the presence of HLA-B27 in Hungarian patients with psoriatic arthritis (PsA). There are 25 known subtypes of the HLA-B27 gene to date, the 2702 and the 2705 show strongest association with AS. We determined the prevalence of the HLA-B27 subtypes in B27 positive healthy Hungarians, AS and PsA patients. We examined the possible effect of HLA-B27 subtypes on clinical parameters.

Recent genome wide association studies revealed several new non-MHC genes with presumable role in the pathogenesis of AS. The most interesting findings are the IL23R gene and the ARTS1 gene. We examined 9 SNPs of the IL23R (rs11805303, rs10889677, rs1004819, rs2201841, rs11209032, 11209026, rs104889629, rs7517847 és rs7530511) and 5 SNPs of the ARTS1 (rs27044, rs17482078, rs10050860, rs30187 és rs2287987) in AS patients and healthy Hungarians. We determined the ILR23R and ARTS1 haplotypes as well and we evaluated the possible connection between these two genes and HLA-B27 in conferring susceptibility to AS.

Methods

Patients were recruited from the National Institute of Rheumatology and Physiotherapy, Budapest and the Department of Rheumatology, Institute of Medicine, University of Debrecen. Genomic DNA was isolated from peripheral blood. Genotyping was performed with several different methods: restriction fragment length polymorphism, allelic discrimination, sequence specific PCR, automatic DNA sequencing. For functional evaluations we used real-time PCR and ELISA methods.

Results

No significant differences in allele or genotype frequencies were observed between controls and either the AS patients or the ReA patients. Clinical characteristics of these groups were unrelated to the presence of any of these polymorphisms.

Genotypes with longer GT repeats were more frequent among RA and AS patients than as compared into controls. Higher TLR2 mRNA expression levels were observed in RA and AS patients as compared to than in controls.. Longer GT repeats were associated with elevated TLR2 mRNA expression levels in both disease case groups and as well as in the control groups.

Variant allele frequencies of both codon 52 and 57 polymorphisms in the MBL2 gene were significantly overrepresented in JIA. The frequency of low MBL genotypes (XA/XA, YA/YO, XA/YO, and YO/YO) in JIA was higher than that in healthy controls. Serum MBL concentrations were found to be significantly lower in JIA patients versus control subjects. The 2 promoter polymorphisms and codon 54 SNP of the MBL2 gene were not associated with JIA. No association was found between the MASP2 SNP and JIA.

In Hungarian patients JIA was associated with HLA-DRB1*01, DRB1*08, DRB1*13 with marked differences between the disease subtypes classified according to the ILAR criteria. There was no association of the PTPN22 C1858T SNP with JIA. No correlation was found between the presence of this PTPN22 SNP and HLA-DRB1 alleles.

In Hungarian AS patients we observed two HLA-B27 subtypes: the B*2705 and the B*2702. However, only the B*2705 subtype was significantly associated with AS when we compared the data with HLA-B27 subtypes of Hungarian healthy individuals. Thus only presence of the B*2705 subtype is considered as a susceptibility factor of AS in the Hungarian population. Clinical parameters showed no association with the presence of any of the HLA-B27 subtypes.

We observed a significant increase in the minor allele frequency of rs27044 in the AS group compared to controls. The minor allele frequencies of rs10050860 and rs2287987 showed a significant decrease in AS patients compared to controls. No significant difference was found in allele frequencies of rs30187 and rs17482078 between the AS and the control group.

Genotype distributions showed significant differences between AS and control groups for rs27044, rs10050860 and rs2287987. For the rs10050860 and rs2287987 variants the prevalence of homozygosity for minor alleles (TT, CC, respectively) showed a more than 3-fold increase in the control group compared to AS patients. For rs17482078 and rs30187 no significant difference was observed in the distribution of genotypes between the AS patients and controls subjects after statistical correction. The haplotypes GCCTT and GCCCT were associated with risk of AS in Hungarian patients, while the CCCTC haplotype were associated with protection against AS.

The presence of the mutant T allele of the IL23R rs11805303 was significantly increased in patients with AS compared to the healthy controls. Carrying the minor allele confers a 1.6-fold risk for the development of AS. Similarly to the rs11805303 allele, carrying the rs1004819 A allele confers a more than 2-fold risk for ankylosing spondylitis ($p=0.003$), moreover, the allele frequencies showed also significant difference between the AS patients and controls. For the rs10889677 variant the prevalence of the AA genotype and the rs2201841 CC both showed a more than 2-fold increase in the AS group compared to the controls. The haplotypes ATCACAG and ATCACAA were associated with risk of AS in Hungarian patients, while the GGCATCG and AGCACAA haplotypes were associated with protection against AS respectively.

Conclusions

1. Toll-like receptor 4 Asp299Gly and Thr399Ile polymorphisms do not contribute to disease susceptibility in either AS or ReA. Functional abnormalities of the TLR4 signalling pathway suggested in spondylarthropathies seem not to be genetically determined by these two common polymorphisms.
2. Genotypes containing longer GT repeats in intron II2 of the TLR2 gene may confer susceptibility to inflammatory rheumatic diseases in Hungarians. Elevated TLR2 mRNA levels in both RA and AS confirm that TLR2 is involved in the development of systemic inflammation. Correlation between the TLR2 genotype and TLR2 mRNA levels indicates that TLR2 expression could be altered according to genotype.
3. Our results suggest that genetically determined low MBL levels may predispose children to JIA in a Hungarian population. These data warrant further research to investigate the role of the lectin-dependent complement system in the pathogenesis of JIA.
4. We confirmed that certain HLA-DRB1 alleles reported previously as susceptibility factors are strongly associated with JIA in a Hungarian population. However, C1858T polymorphism of PTPN22, another candidate gene of autoimmunity seems to be independent of JIA in Hungarian patients. Our data taken together with various findings in different populations suggest that associations related to PTPN22 seem to be more ethnicity-specific in contrast to the general and less population-dependent role of HLA-DRB1 in JIA.
5. We assessed HLA-B27 subtype frequencies in Hungarian patients with AS, and we compared these frequencies with those of healthy controls. We observed two HLA-B27 subtypes: the B*2705 and the B*2702 in Hungarian AS patients. These subtypes were the most frequent subtypes among healthy subjects as well, and only the B*2705 was associated significantly with AS in Hungarians.

6. We studied the rs27044, rs17482078, rs10050860, rs30187, and rs2287987 SNPs recently identified by the WTCCC as risk conferring variants for AS. In our Hungarian population sample rs27044, rs10050860 and rs2287987 variants were significantly associated with the disease. In agreement with recent studies, we confirmed the associations of certain ARTS1 polymorphisms with AS in a Hungarian population study. These results suggest that this association is population-independent, and strongly contributes to susceptibility to AS.

7. Similarly to previous studies on US and UK populations, we confirmed the susceptibility or protective associations of IL23R polymorphisms (rs10889677, rs1004819, rs2201841, rs11209032, rs7530511, rs11209026, rs10489629, rs7517847 és rs11805303) with ankylosing spondylitis in a Hungarian population.

Publications

The dissertation is based on the following publications:

1. **Pazár B**, Gergely P Jr, Nagy ZB, Gombos T, Pozsonyi E, Rajczy K, Balogh Z, Sevcic K, Orbán I, Szodoray P, Poór G. Role of HLA-DRB1 and PTPN22 genes in susceptibility to juvenile idiopathic arthritis in Hungarian patients. *Clin Exp Rheumatol.* 2008;26:1146-52 **IF: 2.364**
2. Sáfrány E, **Pazár B**, Csöngéi V, Járomi L, Polgár N, Sipeky Cs, Horváth IF, Zeher M, Poór G and Melegh B. Variants of the IL23R gene are associated with ankylosing spondylitis but not with Sjögren syndrome in Hungarian population samples. *Scand J Immunol.* 2009;70:68-74. **IF: 2.186**
3. Gergely P Jr, **Pazár B**, Nagy ZB, Gombos T, Rajczy K, Balogh Z, Orbán I, Sevcic K, Poór G. Structural polymorphisms in the mannose-binding lectin gene are associated with juvenile idiopathic arthritis. *J Rheumatol.* 2009;36:843-7 **IF: 3.282**
4. Gergely P Jr, Blazsek A, Weiszhar Z, **Pazár B**, Poór G. Lack of genetic association of the Toll-like receptor 4 (TLR4) Asp299Gly and Thr399Ile polymorphisms with spondylarthropathies in a Hungarian population. *Rheumatology (Oxford)* 2006;45:1194-6 **IF: 4.052**
5. **Pazár B**, Nagy Zs, Gergely Jr. P, Poór Gy. Toll-like receptorok szerepe a gyulladásos reumatológiai betegségek patogenezisében. *Magyar Reumatológia* 2007; 48: 219-230

Other publications:

1. **Pazár B**. Spondylodiscitis. *Hungarian Rheumatology.* 2006; 47: 235-245
2. **Pazár B**. Mozgásszervi szűrővizsgálatok jelentősége, módszerei. *Hungarian Rheumatology.* 2004;45:29-53
3. Horváth Zs, Márialigeti Zs, Király M, **Pazár B**. A vallás és a reumás betegségek kapcsolata. *Hungarian Rheumatology.* 2003;44;129-192.