Role of *HLA-DRB1* and *PTPN22* genes in susceptibility to juvenile idiopathic arthritis in Hungarian patients

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Abstract

Objectives

*Juvenile idiopathic arthritis (JIA) is a complex immune-mediated disease characterized by environmental influences along with several predisposing genes in the pathogenesis. The present study was undertaken to investigate the association of polymorphisms in two candidate genes for autoimmunity, human leukocyte antigen (HLA) DRB1 and protein tyrosine phosphatase N22 (*PTPN22*) with JIA in Hungarian patients.*

Methods

A case-control study including 150 Hungarian JIA patients and 200 sex and ethnically matched healthy controls was conducted. Genotyping for HLA-DRB1 *and* PTPN22 *C1858T single nucleotide polymorphism (SNP) (rs2476601) was carried out by group-specific PCR amplification and by real-time PCR allelic discrimination, respectively.*

Results

*In Hungarian patients JIA was associated with HLA-DRB1*01, DRB1*08, DRB1*13 (p=0.048, p=0.002, p=0.019, respectively) with marked differences between the disease subtypes classified according to the ILAR criteria. There was no association of the* PTPN22 *C1858T SNP with JIA (p=0.66). No correlation was found between the presence of this* PTPN22 *SNP and* HLA-DRB1 *alleles.*

Conclusions

Our results confirm 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.*

Key words

PTPN22, *HLA-DRB1*, polymorphism, juvenile idiopathic arthritis.

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Introduction

Juvenile idiopathic arthritis (JIA) represents a group of diseases that have chronic inflammation of one or more joints in children. JIA is one of the most common chronic illnesses in childhood and is a major cause of functional disability and eye disease leading to blindness. The prevalence varies worldwide among different ethnic and geographically distinct populations. Although both genetic and environmental factors are believed to play a role in the etiopathogenesis, the exact etiology and pathomechanism are still unclear. Hereditary basis of the immunopathogenesis is underscored by human leukocyte antigen (HLA) associations of the disease: HLA-A2 and specific HLA-DR, DQ, and DP genotypes were found to be associated with the type of onset and course subtype (1). Several studies suggest that polymorphisms in other candidate genes also influence susceptibility to JIA. A number of studies of single nucleotide polymorphisms (SNPs) implicate genes separate from those associated with the MHC in the pathogenesis of specific features of JIA. In systemic onset disease high levels of macrophage migration inhibitory factor (MIF) in serum and synovial fluid is associated with the G-C substitution at position -173 in the *MIF* gene (2). The C-T substitution at position -318 in the *CTLA4* gene is also found to be associated with JIA (3). Most recently, mutations in the *PTPN22* gene were identified as a risk factor of JIA in a UK (4), a Norwegian (5) and a Czech (6) JIA cohort.

The HLA region on chromosome 6p21 is a highly interesting chromosomal region for susceptibility to autoimmune disorders. The manifestation of JIA is influenced by both HLA class I and HLA class II genes, the associations between JIA and HLA polymorphisms are well recognized (7, 8). Results of genome-wide scan for JIA susceptibility loci showed the strongest evidence for linkage to JIA near the HLA-DRB1 locus, with a LOD score of 2.26 (9). Several studies identified certain HLA-DRB1 alleles associated with JIA. HLA-DRB1*01 and *04 alleles are reported to increase the risk of rheumatoid factor positive polyarticular JIA in many populations. HLA-DRB1*04 allele is associated with the oligoarticular subtype for which not DRB1*04 alleles are often reported to be protective (10). DRB1*01 is found to be associated with the extended oligoarticular subtype (11). The DRB1*08, DRB1*11 and DRB1*13 alleles are also associated with JIA (12-14).

The protein tyrosine phosphatase, non-receptor type 22 (*PTPN22)* gene on chromosome 1p13 encodes the protein lymphoid-specific phosphatase (Lyp). It is expressed in haemopoetic tissues, all subtypes of peripheral blood mononuclear cells (T cells, B cells, mononuclear, neutrophil, NK-cells). It is a negative regulator of T-cell activation. Recent studies found that *PTPN22* is the first gene since HLA-DR genes, which is considered as a true candidate gene in rheumatoid arthritis (RA). The presence of a C-T substitution (rs2476601) at position 1858 significantly increases susceptibility to RA. The missense SNP results in a change from tryptophan (W) to arginin (R) in the protein (R620W), and this amino-acid change leads to reduced ability to down-regulate T-cell activation. This risk allele presents in ~17% of Caucasian individuals from the general population, and ~28% of Causasians with RA (15). Individuals homozygous for the T allele have more severely reduced effect on T-cell regulation. The mutant T allele is found to be associated with T-cell mediated diseases as RA, type I diabetes (T1D) (16, 17), Graves' disease (18) and Hashimoto thyroiditis (19) among others. Some recent studies identified the C1858T SNP of the *PTPN22* gene as a risk factor of JIA as well, in a UK (4), Norwegian (5) and a Czech (6) JIA cohort. A recent Finnish study supported evidence for the association with RA, but it showed only minimal effect of the polymorphism on susceptibility to JIA (20). While similar HLA associations have been found in JIA patients of different ethnicity, there are only a few and contradicting studies available regarding the role of *PTPN22* in the genetic susceptibility to JIA. In the present study we, for the first time, performed a case-control association study to determine the *HLA-DRB1* and *PTPN22* polymorphism associations in Hungarian JIA patients.

Materials and methods

Patients and controls

DNA samples were obtained from 150 Hungarian JIA patients (40 males and 110 females) with a mean age of 5.49±4.19 years (range: 1-15 years). All JIA patients were treated and followed-up at the National Institute of Rheumatology and Physiotherapy in Budapest. Diagnosis and classification were made according to the ILAR criteria (21). To determine rheumatoid factor (RF) status, we performed a particle agglutination test, and a positive result was defined as a titer of ≥60. Anti-nuclear antibody (ANA) positivity was defined as a titer of ≥200. As a control group, we included 200 sex and ethnically matched healthy controls (83 males and 117 females), mean age: 38.11±10.71, ranging from 19 to 56 years. Control subjects were all recruited from the National Medical Center, Institute of Haematology and Immunology, Budapest. Informed written consent was obtained from each subject, and the local institutional review board's approval was secured at both recruitment sites.

Genotyping

DNA was isolated from peripheral blood samples with the Genomic DNA Purification Tray II (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions using 6100 Nucleic Acids PreStation instrument (Applied Biosystems). DNA samples were stored at 4°C until use.

The *HLA-DRB1* genotyping was performed by polymerase chain reaction (PCR) using sequence-specific oligonucleotides of the Inno-Lipa HLA-DRB1 Plus Kit (Innogenetics, Gent, Belgium). The *PTPN22* SNP analysis was performed with real-time PCR allelic discrimination TaqMan assays (Applied Biosystems). Real-time PCR analysis was carried out in a total volume of 10 µl with 10 ng of genomic DNA, 1 pmol gene-specific Forward and Reverse primer in 1 x TaqMan 2x Universal PCR Master Mix No AmpErase UNG (Applied Biosystems). Specifically, forward TaqMan primers for the PTPN22 alleles were as follows: *FAM*-TCAGGTGTCCGTACAGG for the wild allele, and *VIC*-TCAGGTGTCCATACAGG for the variant allele. Real-time PCR was performed using an ABI 7300 Real-Time PCR System (Applied Biosystems) according to the manufacturer's instructions.

Statistical analysis

Allele and genotype frequencies were tested for the Hardy-Weinberg equilibrium in case and control groups. Allele and genotype frequencies analysis was performed using HelixTree® Genetics Analysis Software (Golden Helix Inc, Bozeman, MT, USA). Associations between the investigated polymorphisms and JIA as well as between the presence of the PTPN22 C1858T SNP and HLA-DRB1 alleles were analysed using the chi-square test or the Fisher-exact test if appropriate. A p-value <0.05 was considered significant. The magnitude of association was expressed as the odds ratio with a 95% confidence interval. Stratification analysis was carried out to investigate the polymorphisms associations in the separate ILAR subgroups, and to investigate the effect of gender, RF and ANA status. All statistical calculations and power estimations were performed using the Statistica Software version 7.1 (StatSoft, Tulsa, OK, USA).

Results

HLA-DRB1 alleles

The HLA DRB1*01, DRB1*08, DRB1*13 alleles were significantly more frequently represented in our Hungarian JIA cohort whereas the HLA DRB1* 03 allele was less frequent (Table I).

Stratifying our data by gender we managed to confirm the positive associations of the HLA-DRB1*01, HLA-DRB1*08 and the HLA-DRB1*13 among females. The HLA-DRB1*03 was underrepresented in this subgroup. In males, only the HLA-DRB1*08 association was significant (data not shown).

Stratifying the JIA patients by the presence or absence of autoantibodies (RF or ANA), we found that HLA-DRB1*01 (OR=2.72 CI=1.32-5.61 p=0.005) was significantly more prevalent in RF positive JIA patients, whereas HLA-DRB1*14 (OR=0, p=0.01) was absent in this subgroup. HLA-DRB1*08 (OR=4.87 CI=0.89-12.54 p<0.001) and HLA-DRB1*13 (OR=1.83 CI=1.18-2.81 p=0.006) were associated with RF negative JIA. HLA-DRB1*03 (OR=0.48 CI=0.27-0.86 p=0.012) and HLA-DRB1*07 (OR=0.42 CI=0.20-0.85 p=0.014) were underrepresented in the RF negative JIA patients.

HLA-DRB1*08 was found to be strongly associated with JIA regardless of the presence or absence of ANA (OR=5.97 CI=2.02-17.64 p<0.001, OR=3.72 CI=1.30-10.61 p=0.03; respectively). HLA-DRB1*07 (OR=0.32 CI=0.095-1.05 p=0.022) and HLA-DRB1*14 (OR=0.22 CI=0.052-0.94 p=0.011) were significantly less frequent in the ANA positive subgroup. In the ANA negative JIA patients HLA-DRB1*03 (OR=0.44 CI=0.24-0.83 p=0.01) was underrepresented.

A subgroup analysis stratified by the ILAR criteria revealed a strong association between HLA-DRB1*13 and the persistent oligoarticular subgroup. DRB1*08, an allele that showed strong association with JIA in general, was also more prevalent in the persistent oligoarticular subgroup and in the RF-negative polyarticular type. Presence of the DRB1*16 allele was characteristic of extended oligoarticular JIA. The DRB1*01 allele was significantly associated with the RF-positive polyarticular group as well as with the enthesitis-related subgroup. Interestingly, significantly increased frequency of the DRB1*15 allele was observed in the psoriatic arthritis subgroup. The allelic frequencies in other subgroups for other alleles did not differ significantly from the frequencies in the control group. Over and underrepresented HLA-DRB1 alleles are shown in Table I.

Thirty-seven different HLA-DRB1 genotypes in the JIA group, and 44 different HLA-DRB1 genotypes in the control group were found. DRB1*13-DRB1*13, DRB1*11-DRB1*13 DRB1*08-DRB1*13 proved to be susceptibility genotypes whereas DRB1*03-DRB1*07 was protective (Table II).

The only significant genotypic association was found in the persistent oligoarticular subgroup. Patients carrying DRB1*08-DRB1*13 genotype

Table I. HLA-DRB1 allele frequencies in controls and JIA patients.

DRB1 allele	Control (n=200)	JIA patients (n=150)		Persistent oligoarthritis (n=45)		Extended oligoarthritis (n=15)		RF-negative polyarthritis (n=38)		RF-positive polyarthritis (n=26)	
	n (%)	n (%)	p; OR (95%CI)	n (%)	*p*; OR (95%CI)	n (%)	*p*; OR (95%CI)	n (%)	*p*; OR (95%CI)	n (%)	*p*; OR (95%CI)
01	38 (9.50)	43 (14.33)	0.048; 1.59 (1.00-2.54)	10 (11.11)	n.s.	4 (13.33)	n.s.	11 (14.47)	n.s.	10 (19.23)	0.032; 2.27 (1.05-4.88)
03	53 (13.25)	21 (7.0)	0.008; 0.49 (0.29-0.48)	5 (5.56)	0.041; 0.38 (0.14-0.99)	0 (0)	0.017 0	5 (6.58)	n.s.	4 (7.69)	n.s.
04	41 (10.25)	25 (8.33)	n.s.	2 (2.22)	0.006; 0.19 (0.05-0.84)	2 (6.67)	n.s.	7 (9.21)	n.s.	10 (19.23)	n.s.
07	37 (9.25)	16 (5.33)	n.s.	1 (1.11)	0.003; 0.11 (0.01-0.81)	0 (0)	n.s.	4 (5.26)	n.s.	6 (11.54)	n.s.
08	6 (1.50)	17 (5.67)	0.002; 3.94 (1.54-10.13)	7 (7.78)	0.001; 5.54 (1.81-16.9)	2 (6.67)	n.s.	4 (5.26)	0.047; 3.65 (1.0-13.25)	2 (3.84)	n.s.
09	2 (0.50)	3 (1.0)	n.s.	1 (1.11)	n.s.	1 (3.33)	n.s.	1 (1.32)	n.s.	0 (0)	n.s.
10	6 (1.50)	3 (1.0)	n.s.	3 (3.33)	n.s.	0 (0)	n.s.	0 (0)	n.s.	0 (0)	n.s.
11	66 (16.50)	58 (19.33)	n.s.	16 (17.78)	n.s.	8 (26.67)	n.s.	16 (21.05)	n.s.	9 (17.31)	n.s.
12	10 (2.50)	6 (2.0)	n.s.	4 (4.44)	n.s.	2 (6.67)	n.s.	0 (0)	n.s.	0 (0)	n.s.
13	49 (12.25)	56 (18.67)	0.019; 1.64 (1.08-2.49)	27 (30.0)	0.001; 3.07 (1.78-5.27)	6 (20.0)	n.s.	13 (17.10)	n.s.	5 (9.62)	n.s.
14	35 (8.75)	15 (5.0)	n.s.	2 (2.22)	0.016; 0.23 (0.056-1.0)	0 (0)	n.s.	9 (11.84)	n.s.	0 (0)	0.012 0
15	31 (7.75)	16 (5.33)	n.s.	5 (5.56)	n.s.	0 (0)	n.s.	4 (5.26)	n.s.	1 (1.92)	n.s.
16	26 (6.25)	21 (7.0)	n.s.	7 (7.78)	n.s.	5 (5)	0.039; 2.85 (1.01-8.07)	2 (2.63)	n.s.	5 (9.62)	n.s.

OR (95% CI) listed where *p* was significant.

had increased risk for developing this type of JIA (OR=3.86, CI=1.47-10.12, *p*=0.008).

PTPN22 *C1858T single nucleotide polymorphism*

The allele and genotype frequencies for the *PTPN22* SNP were in Hardy-Weinberg equilibrium. Allele frequencies in our Hungarian cohort (89.6 %) did not differ significantly from the previously reported frequency in the European population (85.8%). We found no evidence of association between the *PTPN22* SNP and JIA in our Hungarian JIA cohort. The allele and genotype frequencies for the disease case group and the control group did not differ significantly (Table III).

Allele and genotype frequencies stratified by gender, RF and ANA status did not show significant difference between case and control groups (data not shown). When stratifying our data by the seven ILAR subgroups we found similar allele and genotype frequencies. The presence of the variant T allele or the CT genotype was not significant in any of the seven ILAR subgroups (Table III).

Examining the association for the first time in JIA, we found that the presence of *PTPN22* C1858T SNP and *HLA-DRB1* alleles was independent from each other in Hungarian patients. The distribution of the *HLA-DRB1* alleles between subject homozygous for the CC wild type and subjects heterozygous for the CT variant type did not differ significantly (data not shown).

Table II. Association of *HLA-DRB1* genotypes with JIA.

DRB1 genotype	*p*	OR (95%CI)
DRB1*13-DRB1*13	<0.001	infinite
DRB1*11-DRB1*13	0.046	2.31 (0.26-20.54)
DRB1*08-DRB1*13	0.018	2.75 (1.16-6.55)
DRB1*03-DRB1*07	0.024	0.34 (0.13-0.90)

Discussion

This is the first study to determine the frequency of the *PTPN22* C+1858T

Table III. Allele and genotype frequencies of the PTPN22 R620W SNP in JIA patients and control subjects.

	Controls (n=200)		JIA (n=150)		Persistent oligoarthritis (n=45)		Extended oligoarthritis (n=15)		RF-negative polyarthritis (n=38)		RF-positive polyarthritis (n=26)	
	n	%	n	%	n	%	n	%	n	%	n	%
Allele												
C	369	92.25	274	91.33	81	90.0	29	96.67	69	90.79	46	88.46
T	31	7.75	26	8.67	9	10.0	1	3.33	7	9.21	6	11.54
OR			1.13		1.32		0.41		1.21		1.55	
(95% CI)			(0.66-1.95)		(0.61-2.89)		(0.05-3.12)		(0.51-2.85)		(0.62-3.92)	
p			0.66		0.48		0.24		0.67		0.35	
Genotype												
CC	169	84.50	124	82.67	36	80.0	14	93.33	31	81.58	20	76.92
CT	31	15.50	26	17.33	9	20.0	1	6.67	7	18.42	6	23.08
TT	0	0	0	0	0	0	0	0	0	0	0	0
OR			1.14		1.36		0.39		1.23		1.64	
(95% CI)			(0.65-2.02)		(0.59-3.11)		(0.05-3.07)		(0.49-3.04)		(0.61-4.39)	
p			0.65		0.46		0.23		0.65		0.33	

variant allele in a Hungarian population, and to investigate the role of the *HLA-DRB1* and *PTPN22* polymorphisms in genetic susceptibility to JIA in Hungary. We found a significant association between the HLA-DRB1*01, DRB1*08 and DRB1*13 alleles and JIA in Hungarian children which is in accordance with previous findings regarding the *HLA DRB1* associations of JIA of different ethnicity (14). Furthermore, stratification analysis revealed DRB1*08 allele as a risk factor for the oligoarticular and polyarticular JIA also confirming other observations (3, 14). Moreover, our results correspond to former data as well regarding the significant association of the HLA-DRB1*01 allele with the RF-positive polyarticular group and a higher DRB1*13 allele frequency in the persistent oligoarticular subgroup (12-14). Our data from stratification analyis are to be taken cautiosuly due to the relatively low number of patients in the subgroups, although the coincidence with previous observations is reassuring.

Previous studies found the DRB1*11 allele significantly associated with different subtypes (12, 14). In our study, we found increased frequency of this allele in the persistent oligoarticular and the RF negative polyarticular subtype. However, it did not attain statistical significance. DRB1*14 was underrepresented in RF positive and ANA positive JIA patients suggesting that it might be a protective allele against autoantibody production. It is of note that DRB1*14 also proved to be a protective factor in Korean patients with idiopathic inflammatory myopathies having no myositis-specific autoantibodies (22) and in patients with multiple sclerosis, another autoimmune disorder in a large cohort of individuals mostly of Northern European origin (23).

PTPN22 is the first gene since the HLA class II genes that is showed to be a true candidate gene with RA in different populations. It plays a role in the TCR signalling pathway, and normally inhibits autoantibody induced T-cell activation. Knockout mice deficient for the *PTPN22* homologue PEP showed less capacity to down regulate T-cell activation (24), and these findings lead to the hypothesis, that *PTPN22* is a gene playing important role in many different autoimmune disorders. Recent findings showed association of the *PTPN22* R620W SNP with T1D (16, 17), Graves' disease (18), SLE (25), Hashimoto's thyroiditis (19), Addison's disease (18), and generalized vitiligo (26). The lack of this association has also been reported in several cases, as multiple sclerosis (27), psoriasis (28), psoriatic arthritis (4), Sjögren's syndrome (29), celiac disease (30), Crohn's disease (31), systemic sclerosis (32) and ankylosing spondylitis (33). Associated diseases are mostly autoantibody related, and lack of correlation was showed in disorders which do not show autoantibody production. That correlates with the function of the PTPN22 in down-regulation of the T-cell activation caused by autoantibody binding.

We failed to provide evidence for the association of the *PTPN22* R620W SNP with JIA in a Hungarian population; and thus, we were unable to confirm former studies that showed association between the *PTPN22* R620W SNP and JIA in different populations (4-6, 34). Our results are in accordance with the recent data by Seldin *et al.*, which showed no allelic association with JIA and only a weak evidence for genotypic effect in a Finnish population (20). Our negative findings regarding the PTPN22, however, cannot exclude an association of this SNP with JIA due to the relatively low sample size. For a 80% statistical power, more than 8500 patients should be included, which cannot be achieved without loosing the ethnic homogeneity of the population.

By stratification analysis our results are in accordance with former data that in the systemic onset subgroup there is no allelic or genotypic effect of this SNP (4, 34). We found increased T allele frequency in the persistent oligoarthritis, the RF-positive polyarthritis and the RF- negative polyarthritis subgroups. These data do not confirm former results that reported the strongest association in the RF-negative polyarthritis subgroup (4, 5, 34). Considering these findings together with the data of Seldin *et al.* (20), and Ikari *et al.* (35), which showed no association of the *PTPN22* polymorphism with RA in a Japanese population, we suggest,

that the *PTPN22* association with specific autoimmune diseases is present only in specific ethnic groups.

The possible association of *HLA-DRB1* polymorphisms and *PTPN22* C1858T SNP has not been investigated in juvenile idiopathic arthritis yet. In our study, we confirmed lack of association of these two polymorphisms in JIA, that has already been reported in RA (15, 36) and we support further evidence that *PTPN22* acts independently from *HLA-DRB1*.

JIA is a very heterogeneous disease and specific subgroups show several features resembling RA, which raises the possibility that these two candidate genes for RA, *HLA-DRB1* and *PTPN22* have an important role in the pathogenesis of JIA. It is difficult to provide roboustly powered studies on ethnically homogeneous JIA populations in smaller countries; therefore, sample size included in these JIA association studies usually does not exceed 200 (37, 38). In this study examing the genetic background of Hungarian patients with JIA for the first time, we confirmed the previously reported *HLA-DRB1* associations of JIA. On the other hand, we failed to provide evidence for the association of the *PTPN22* C1858T SNP with JIA in Hungarian patients. Taken this together with earlier positive and negative findings in different populations, our data 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.

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