

# Prevalence of HLA-B27 antigen in patients with juvenile idiopathic arthritis

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## Abstract

**Introduction:** Human leukocyte antigen B27 (HLA-B27) is considered as a risk factor for development of juvenile idiopathic arthritis (JIA).

The aim of this study was to analyse the prevalence of HLA-B27 antigen in JIA categories and its influence on disease onset and response to conventional therapy.

**Material and methods:** The retrospective analysis included 461 unselected children with JIA hospitalized in a single reference rheumatology centre between July 2007 and June 2012. The diagnosis was based on criteria by the International League of Association for Rheumatology. HLA-B27 was determined in 387 of all patients (84%) by hybridization of the amplified, labelled product to immobilize it on the microarray probe.

**Results:** HLA-B27 antigen was found in 104 of 383 affected children (27.2%), 48 of 206 girls (23.3%), and 56 of 177 boys (31.6%) – most frequently in patients with enthesitis-related arthritis (71%), psoriatic arthritis (50%) and unclassified cases (86.7%). The age of JIA onset was slightly (by 1 year) but significantly different in patients with and without HLA-B27 antigen [11 (8.5–14) vs. 10 (5–13.5) years.;  $p < 0.001$ ].

The use of disease-modifying antirheumatic drugs (DMARDs) and corticosteroids was more frequently clinically ineffective in HLA-B27 positive than negative patients (23.1% vs. 15.2%;  $p = 0.09$ ). Patients with polyarthritis, systemic, and psoriatic arthritis more frequently received biological therapy. HLA-B27 positive patients with enthesitis-related arthritis received biological therapy more frequently than HLA-B27 negative ones (20.4% vs. 0, respectively;  $p = 0.09$ ).

**Conclusions:** HLA-B27 antigen is a strong risk factor for the development of enthesitis-related arthritis, and to a lesser extent for psoriatic arthritis and extended course of oligoarthritis. The presence of this antigen does not affect the disease onset but seems to predict resistance to therapy with disease-modifying drugs and corticosteroids.

**Key words:** juvenile idiopathic arthritis, therapy, HLA-B27 antigen.

## Introduction

Juvenile idiopathic arthritis (JIA) is the most common group of chronic connective tissue diseases in the developmental period. The diagnosis of JIA is established when symptoms of arthritis persist for more than

6 weeks, primarily after the exclusion of all secondary forms of arthritis, in children aged up to 16 years [1–3]. The current classification system by the International League Against Rheumatism – ILAR (Edmonton 2001) distinguishes seven JIA categories, characterized by different clinical presentation and laboratory findings, as

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well as the outcome: systemic arthritis, oligoarthritis (persistent, extended), polyarthritis, rheumatoid factor positive [RF(+)], polyarthritis, rheumatoid factor negative [RF(-)], enthesitis-related arthritis (JIA-ERA), psoriatic arthritis (JIA-PA), and unclassified arthritis [2].

The etiology of this heterogeneous group of autoimmune diseases remains unknown. As with most autoimmune disorders, interactions between genetic predisposing factors, immune mechanisms, and environmental exposures are thought to contribute to JIA pathogenesis [3]. It is assumed that the genetic predisposition to JIA is determined by the major histocompatibility complex (MHC) loci. Among MHC loci the HLA-B27 allele has a special position.

The association with HLA-B27 antigen was described for the first time in adults with ankylosing spondylitis in 1973 [4]. In a similar way, high prevalence of this antigen was shown in, similar to spondyloarthritis, arthritis with enthesitis (JIA-ERA), which occurs in children with additional extra-axial joint involvement [5]. In children the occurrence of HLA-B27 antigen is also associated with other JIA categories, such as oligoarthritis and polyarthritis, especially among girls [6].

Furthermore, the prognostic effect of HLA-B27 antigen in various JIA categories has been suggested by some authors [7–10]. Recently we found that HLA-B27 antigen is associated with the occurrence of uveitis [11].

The limited knowledge concerning the presence of antigen HLA-B27 in patients with JIA categories in the Polish population prompted us to perform this retrospective analysis. In addition we analysed the influence of the antigen on the disease onset and response to conventional therapy.

## Material and methods

This retrospective analysis included all patients [ $N = 461$ ] diagnosed with JIA, hospitalized between 1 July 2007 and 30 June 2012 in the Department of Older Children with subunits of Neurology, Rheumatology and Rehabilitation, St Ludwik Hospital in Cracow. Seven categories of JIA were distinguished according to the current classification system by ILAR revised in 2001 [2].

Among 461 patients diagnosed with JIA, there were 387 patients with assessed HLA-B27 antigen. In general, patients with clinical presentation of the systemic JIA category and RF(+) were not tested. Additionally, in a few samples no amplification was obtained (DNA degradation during the sample transport).

Laboratory measurements included in the analysis were performed in certified laboratories. HLA-B27 was determined in peripheral lymphocytes by genotyping (EUROArray, Euroimmun Polska, Wrocław, Poland). The

test is based on amplification of exon 2 and 3 of the HLA-B\*27 gene and detection of the labelled product by hybridization to immobilize it on the microarray probe. The analyses were performed by Euroimmun Laboratory in Wrocław.

The data were retrieved from the registry database maintained by the institution (approved by the Bioethical Committee).

## Data analysis

The prevalence of HLA-B27 antigen in different JIA categories was analyzed in relation to prevalence of the antigen in 240 healthy unrelated adults inhabiting the area of Southern Poland ( $N = 23$ ; 9.6%) [12].

Additionally, we analyzed the association between the prevalence of HLA-B27 and the onset of JIA and response to conventional therapy with disease-modifying drugs and corticosteroids.

## Statistical analysis

Statistical analyses were performed using the STATISTICA 10.0 PL for Windows software package (StatSoft Polska, Kraków, Poland) and MedCalc 12.3.0.0. (Mariakerke, Belgium). Values are presented as medians with interquartile ranges. For comparison of groups, the  $\chi^2$  test (qualitative variables) and Kruskal-Wallis ANOVA (quantitative variables) were used. In all statistical tests the ' $p$ ' values below 0.05 were considered statistically significant.

## Results

Age of onset of JIA sign and symptoms ranged from 15 months to 16 years (mean 11.5 years). The mean period of follow-up at the time of analysis was more than 4 years.

HLA-B27 was determined in 383 of 461 JIA patients (83.2%), 206 girls, and 177 boys. In 104 patients (48 girls and 56 boys) HLA-B27 antigen (27.2%) was found – Table I. There were more HLA-B27 positive boys (31.6%) than girls (23.2%), but the difference was not statistically significant ( $p = 0.09$ ).

HLA-B27 antigen was most frequently found in patients with enthesitis-related arthritis (71%), psoriatic arthritis (50%), unclassified cases (86.7%), and a small subset of oligoarthritis with extended course (42.9) – Table I. The prevalence of HLA-B27 antigen in patients with enthesitis-related arthritis was 23.1 times greater than in the general population. Even greater prevalence was found in the unclassified JIA category – OR = 61.3 (95% CI: 13.0–289). Increased prevalence of the antigen was found in psoriatic arthritis – OR = 9.43 (2.21–40), and oligoarthritis with extended course – OR = 7.08 (1.49–33).

**Table I.** Structure of juvenile idiopathic arthritis (JIA) categories and HLA-B27 distribution in the study group (N = 461)

JIA categories	All JIA (N = 461)	JIA analysed for HLA-B27 (N = 383)	HLA-B27 positive (N = 104)	Odds ratio* (95% CI)
	N (%)	N (% of analysed)	N (% of positive)	
Systemic arthritis	33 (7.2)	14 (42.4)	0	0.31 (0.02–5.52)
Oligoarthritis	239 (51.9)	202 (84.5)	31 (15.3)	1.71 (0.96–3.04)
Persistent course	230 (50.0)	195 (84.8)	28 (14.3)	1.58 (0.88–2.85)
Extended course	9 (1.9)	7 (77.8)	3 (42.9)	7.08* (1.49–33)
Polyarthritis, RF(+)	8 (1.7)	7 (87.5)	1 (14.3)	1.57 (0.18–13.6)
Polyarthritis, RF(-)	96 (20.8)	75 (78.1)	11 (14.7)	1.62 (0.75–3.50)
Enthesitis-related arthritis	62 (13.4)	62 (100)	44 (71.0)	23.1*** (11.5–46)
Psoriatic arthritis	8 (1.7)	8 (100)	4 (50.0)	9.43** (2.21–40)
Unclassified	15 (3.3)	15 (100)	13 (86.7)	61.3 (13.0–289)

\*In relation to the occurrence of HLA-B27 antigen in 23 out of 240 (9.6%) healthy unrelated adults inhabiting the area of Southern Poland [12].

RF – rheumatoid factor, \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

No significant gender differences in the prevalence of HLA-B27 antigen in JIA categories were found (Table II). However, the analysis was underpowered for many categories due to the small number of cases within the subgroups.

The age of JIA onset was slightly (by 1 year) but significantly different in patients with and without HLA-B27 antigen [11 (8.5–14) vs. 10 (5–13.5) years;  $p < 0.001$ ]. However, in each JIA category we failed to find any difference at the age of the onset related to the presence of HLA-B27 antigen (Table II).

The therapy of HLA-B27 positive patients differed significantly from that of HLA-B27 negative individuals (Table III), as the frequency of sulfasalazine use in the HLA-B27 positive group exceeded more than four times that observed in the HLA-B27 negative group. Additionally, the HLA-B27 positive patients with oligoarthritis presented elevated frequency of glucocorticoid treatment. Moreover, in the group of HLA-B27 negative patients with polyarticular seronegative JIA, more than 90% of the individuals were treated with glucocorticoids.

The use of disease-modifying antirheumatic drugs (DMARDs) and glucocorticoids was more frequently clinically ineffective in HLA-B27 positive than negative patients (24–23.1% vs. 42–15.2%, respectively;  $p = 0.09$ ), especially in patients with persistent course oligoarthritis (7–22.6% vs. 11–6.4%, respectively;  $p < 0.001$ ). In these patients biological agents (adalimumab, etanercept, tocilizumab, golimumab) were used. Biological therapy was most frequently required among patients with polyarthritis [57.0% of RF(+) and 37.3% RF(-)], systemic arthritis (28.6%), and psoriatic arthritis (25%) (Table III).

In addition, there were 9 patients with enthesitis-related arthritis (14.5%) refractory to disease-modifying drugs and corticosteroids, all HLA-B27 positive (20.4% vs. 0 in HLA-B27 positive vs. negative;  $p = 0.09$ ).

## Discussion

The results of our study demonstrate the increased prevalence of HLA-B27 antigen in the cohort of JIA patients. It should be stressed that the prevalence of the antigen in Polish JIA patients has not been reported yet.

31.6% of boys and 23.2% of girls were HLA-B27 positive. The prevalence in the general population is markedly lower. We adopted the data in healthy unrelated adults, estimating the frequency of HLA-B27 at 9.6% [12] as the reference value. The reported prevalence of HLA-B27 antigen in the European populations reaches even 16.6% [5, 13–15]. Our results demonstrate the increased prevalence of HLA-B27 antigen in the cohort of JIA patients, which is in line with data presented by other authors [5, 10].

The prevalence of HLA-B27 in our cohort (27.2%) is higher than that reported by Murray et al. (14%) in U.S. JIA patients [16], by Thomson et al. (16.9%) for a UK cohort [5], and Nordic countries reported by Berntson et al. (21%) [10], but in a similar range as in Estonia (28.6%) [17]. Additionally, the prevalence is lower than that reported in JIA cohorts from Ukraine (43%) [18]. The discrepancies are mostly related to varied distribution of HLA-B27 antigen in ethnic cohorts.

Regardless of interethnic differences, the highest prevalence is reported in enthesitis-related arthritis, as

**Table II.** Age at onset and gender of patients with different categories of juvenile idiopathic arthritis (JIA) with respect to the occurrence of HLA-B27 antigen ( $N = 383$ ). Age is presented as median value with interquartile range in parentheses.

JIA categories	Age at onset [years]			Gender	
	All	B27(+)	B27(-)	HLA-B27(+) girls	HLA-B27(+) boys
Systemic arthritis ( $N = 14$ )	10.5 (4–13)	–	10.5 (4–13)	0/6 (0%)	0/8 (0%)
Oligoarthritis ( $N = 202$ )	10 (5–13)	10 (8–12)	9 (4.5–13)	19/114 (16.7%)	12/88 (13.6%)
Persistent course ( $N = 195$ )	10 (5–13)	10 (8–13)	9 (4.5–13)	17/110 (17.7%)	11/85 (12.9%)
Extended course ( $N = 7$ )	9 (4–11)	9 (4–11)	6.5 (3.5–10)	2/4 (50%)	1/3 (33.3%)
Polyarthritis, RF(+) ( $N = 7$ )	8 (7.5–15)	11 (11–11)	7.5 (7.5–15)	1/5 (20%)	0/2 (0%)
Polyarthritis, RF(-) ( $N = 75$ )	8.5 (5–14)	9 (5–14)	8 (4–14)	6/52 (11.5%)	5/23 (21.7%)
Enthesitis-related arthritis ( $N = 62$ )	12 (10–15)	12.5 (10.5–15)	10.5 (4–13)	13/18 (72.2%)	31/44 (70.4%)
Psoriatic arthritis ( $N = 8$ )	13 (11–16)	11 (8–15)	14.5 (13–16)	1/4 (25%)	1/4 (25%)
Unclassified ( $N = 15$ )	11 (7–13)	11 (7–13)	13 (13–13)	6/7 (85.7%)	7/8 (87.5%)

in our study (71%). Similar values (72% and 76%) were reported in Nordic and UK cohorts [5, 10]. We have calculated that HLA-B27 antigen increases the risk of enthesitis-related arthritis by 23.1 (11.5–46) times.

Of interest, we have found a high prevalence of HLA-B27 antigen in psoriatic arthritis (50%), oligoarthritis with extended course (42.8%) and unclassified cases (86.7%). However, the sizes of these groups were relatively small, so we could not exclude an accidental finding. It is worth stressing that also Berndson et al. [10] reported a higher prevalence of HLA-B27 in patients with extended course oligoarthritis than with persistent course (20% vs. 15%). Therefore, we may suggest that HLA-B27 antigen may predispose to a more severe course of oligoarthritis.

There is limited evidence suggesting that HLA-B27 antigen is associated with more severe clinical course of JIA. Savolainen et al. [7] described accumulation of HLA-B27 positive cases among the most severe JIA cases, lower remission rate, earlier development of amyloidosis and joint destruction necessitating arthroplasty. In addition, Berndson et al. [10] found that HLA-B27 positive patients during 8-year follow-up less frequently had clinical remission. Furthermore, Hsu et al. [19] found that HLA-B27, high CRP level, and thrombocytosis at diagnosis are the major factors related to the failure of first remission. In line with these findings, we observed

that HLA-B27 patients with oligoarthritis and RF(-) polyarthritis more frequently required glucocorticoids, and the second group also sulfasalazine administration. Additionally, DMARDs and glucocorticoids are more frequently ineffective in HLA-B27 positive patients (23.1% vs. 15.2%, respectively), necessitating modern biologic therapy, especially patients with oligoarthritis (22.6% vs. 6.4%;  $p < 0.001$ ) and enthesitis-related arthritis (20.4% vs. 0;  $p = 0.09$ ). Unfortunately, the low number of patients precluded statistical significance of this observation.

Our analysis failed to find significant differences between HLA-B27 positive and negative patients regarding categories of age of JIA onset. In all but psoriatic arthritis and unclassified categories the median age of HLA-B27 positive patients was slightly (non-significantly) higher. Our observation supports the previous finding that this antigen does not accelerate development of JIA [10].

Our study has some limitations, e.g. the lack of analysis of other HLA loci that were reported as increasing susceptibility to JIA in affected families [20], and the lack of HLA-B27 assessment in each study subject.

In conclusion, HLA-B27 antigen is a strong risk factor for the development of enthesitis-related arthritis, and to a lesser extent for psoriatic arthritis and extended course of oligoarthritis. Presence of this antigen does not affect the disease onset but seems to predict resis-

**Table III.** Current therapy in patients with different categories of juvenile idiopathic arthritis (JIA) with respect to the occurrence of HLA-B27 antigen (N = 383)

JIA categories	Methotrexate (MTX) [N/N in the group, (%)]		Other# DMARDs [N <sub>s</sub> /N <sub>o</sub> /N in the group, (%)]		Corticosteroids [N/N in the group, (%)]		Biological agents [N/N in the group, (%)]	
	All	B27(+)	All	B27(+)	All	B27(+)	All	B27(+)
Systemic arthritis (N = 14)	14/14 (100)	- (0)	0/10/14 (0/71.4)	- (0)	14/14 (100)	- (0)	4/14 (28.6)	- (0)
Oligoarthritis (N = 202)	90/202 (44.6)	12/31 (38.7)	116/34/202 (57.4/16.8)	21/3/31 (67.7/9.7)	14/202 (6.9)	8**/31 (25.8)	18/202 (8.9)	7**/31 (22.6)
Persistent course (N = 195)	83/195 (42.6)	9/28 (32.1)	113/31/195 (57.9/15.9)	20/2/28 (71.4/7.1)	10/195 (5.1)	6***/28 (21.4)	13/195 (6.7)	4/28 (14.3)
Extended course (N = 7)	7/7 (100)	3/3 (100)	3/3/7 (42.9/42.9)	1/1/3 (33.3/33.3)	4/7 (57.1)	2/3 (66.7)	5/7 (71.4)	3/3 (100)
Polyarthritis, RF(+) (N = 7)	7/7 (100)	1/1 (100)	0/7/7 (0/100)	0/1/1 (0/100)	5/7 (71.4)	1/1 (100)	4/7 (57.1)	1/1 (100)
Polyarthritis, RF(-) (N = 75)	72/75 (96)	10/11 (90.9)	14/34/75 (18.7/45.3)	6**/4/11 (54.5/36.4)	15/75 (20)	10***/11 (90.9)	28/75 (37.3)	5/11 (45.4)
Enthesitis-related arthritis (N = 62)	27/62 (43.5)	22/44 (50)	53/5/62 (85.5/8.1)	38/3/44 (86.4/6.8)	5/62 (8.1)	4/44 (9.1)	9/62 (14.5)	9*/44 (20.4)
Psoriatic arthritis (N = 8)	2/8 (25)	1/4 (25)	8/4/8 (100/50)	4/2/4 (100/50)	3/8 (37.5)	2/4 (50)	2/8 (25)	1/4 (25)
Unclassified (N = 15)	9/15 (60)	8/13 (61.5)	11/8/15 (73.3/53.3)	9/7/13 (69.2/53.8)	6/15 (40)	5/13 (38.5)	1/15 (6.7)	1/13 (7.7)

\*p = 0.09; \*\*p &lt; 0.01; p &lt; 0.001 vs. corresponding B27(-) group.

# Other DMARDs include: sulfasalazine (SSZ), hydroxychloroquine (HCQ), cyclosporine A (CsA).

DMARDs – disease-modifying anti-rheumatic drugs

tance to therapy with disease-modifying drugs and corticosteroids.

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