# HLA-B27 subtypes in the enthesitis-related arthritis category of juvenile idiopathic arthritis and ankylosing spondylitis in northern India

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## Abstract

### Objective

*Enthesitis-related arthritis (ERA) is the most common form of juvenile idiopathic arthritis (JIA) in the Asian and Indian populations. The presence of HLA-B27 has a strong association with JIA-ERA similar to that with adult ankylosing spondylitis (AS). The HLA-B27gene is highly polymorphic. Susceptibility to AS varies between different HLA-B27 subtypes; data on the relationship of susceptibility to JIA-ERA with HLA-B27 types are scant. In this study, we determined HLA-B27 subtypes in patients with JIA-ERA and AS to find out whether there is any difference in the HLA-B27 subtypes prevalent in these two diseases.*

### Methods

*Genomic DNA from 135 patients with JIA-ERA and 121 with AS was tested for the presence of HLA-B27. In patients testing positive, HLA-B27subtyping was done by sequencing a genomic region that contained second and third exons and the intervening intron of this gene; this method permitted identification of common HLA-B27 subtypes (HLA-B*27:01 to HLA-B*27:09).*

### Results

*One hundred and seven (79%) patients with JIA-ERA and 102 (84%) patients with AS tested positive for HLA-B27. In both groups, HLA-B*27:05 and HLA-B*27:04 were the common subtypes; some patients had HLA-B*27:07(7.4%) and HLA-B*27:18. Patients with JIA-ERA had a higher frequency of HLA-B*27:05 than those with AS (70% vs. 57%, p=0.047), and a lower frequency of HLA-B*27:04 (21% vs. 36%, p=0.018).*

### Conclusion

*HLA-B*27:05 and HLA-B*27:04 were the most common HLA-27 subtypes in both JIA-ERA and AS. However, HLA-B*27:05 was more frequent and HLA-B*27:04 was less frequent in JIA-ERA. It is possible that HLA-B*27:05 being the ancestral HLA-27 subtype leads to expression of disease early in life.*

**Key words**

juvenile idiopathic arthritis, ankylosing spondylitis, HLA antigens

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## Introduction

Juvenile idiopathic arthritis (JIA) is a heterogeneous disease that is classified into seven different categories, as per the criteria laid down by the International League of Associations for Rheumatism (1). Of these, enthesitis-related arthritis (ERA) is the most common type in India and in other parts of Asia (2). The ERA category of JIA is characterised by disease onset in boys older than 6 years of age, arthritis, enthesitis and family history of HLA-B27-related diseases. It resembles adult spondyloarthropathies in several characteristics, such as occurrence of enthesitis, inflammatory back pain and anterior uveitis, and presence of a strong family history. Nearly 30–40% of children with JIA-ERA develop inflammatory back pain and/or sacroilitis on follow up (3). Similar to AS (4) HLA-B27 also has a strong association with JIA-ERA, with a prevalence of 60–70% among Caucasian (5, 6) and 80% among Indian (2, 7) patients with this disease.

HLA-B27 subtypes association with AS has been studied in several populations around the world (8-14). Some subtypes, such as B*27:02, B*27:04, B*27:05, B*27:07 and B*27:08, confer particularly high susceptibility to AS, whereas HLA-B27 subtype B*27:06, which differs from B*27:04 in only two amino acids, is negatively or weakly associated with AS (8). Data on the relationship of various HLA-B27 subtypes with susceptibility to JIA-ERA are limited. In a study from Latvia, B*27:05 was found in 55% of 25 children with JIA-ERA (15). Another study in a southern Chinese population that compared patients with juvenile-onset and adult-onset AS found no significant difference in the distribution of B27 subtypes between these groups (11).

Data on HLA B27 subtypes in AS in the Indian population are sparse. These have shown prevalence rates of B*27:04 and B*27:05 of 14%–40% and 51%–77%, respectively (12-14). No data are available in JIA-ERA. We therefore decided to study the prevalence of HLA B27 subtypes in children with JIA-ERA and compared it with adult AS patients. Furthermore, we also assessed whether there was an association of specific HLA-B27 subtype with enthesitis, uveitis or family history in patients with JIA-ERA.

## Patients and methods

### *Patients*

Patients with onset before their 16th birthday and fulfilling the International League of Associations for Rheumatology (1) criteria for enthesitis-related arthritis were classified as JIA-ERA, whereas patients with onset after 18 years of age and who satisfied the modified New York criteria for AS were classified as AS (16). One ml of blood was collected from each patient in EDTA and stored at -40°C until DNA isolation. The study was approved by the ethics committee of our institution and written consent was obtained from each patient and/or one of the parents.

### *HLA typing*

DNA was extracted from frozen whole blood by rapid salting out method (17). Presence of HLA-B27 was detected using amplification-refractory-mutation-system polymerase chain reaction with HLA-B27 specific primers, which amplified a 149-base pair region (18). Each reaction used amplification of a conserved, 796-base pair, intronic region of HLA-DR gene using another set of primers as an internal control. Primers were synthesised by Sigma USA.

### *HLA B27 subtype analysis using genetic sequencing*

For the patients who tested positive for HLA-B27, a fragment of the HLA-B27 gene spanning the second and third exons and the intervening second intron was amplified using a touch-down PCR (19). The PCR reaction mixture contained 80mM Tris-HCl, pH 9.0, 2mM $MgCl_2$, 20mM $(NH_4)_2SO4$ (Fermentas), 200 mM of each dNTP (Invitrogen), 0.5 units TaqDNA polymerase (Bangalore Genei, Bangalore, India), 20 pM of each primer (forward: 5'-GCT ACG TGG ACG ACA CGC T-3' and reverse: 5'-GAG CCA CTC CAC GCA CTC-3'), 10% dimethyl sulfoxide and 5µl of genomic DNA at a concentration of 50 to 300 ng/µl. The PCR cycling conditions were: an initial denaturation at 96°C for 1 minute, 26 amplifi-

**Table I.** Clinical features of JIA-ERA patients.

| Characteristic | Value (n=107) |
|---|---|
| Sex (M:F), number | 98:9 |
| Median age, years ( range) | 17 (6-36) |
| Median age at disease onset, years (range) | 12 (4-16) |
| Median disease duration, years (range) | 5 (0.1-24) |
| Number with arthritis (%) | 103 (96%) |
| Number with enthesitis (%) | 63 (59%) |
| Number with uveitis (%) | 13 (12%) |
| Number with sacroiliitis (%) | 23 (21%) |
| Number with family history (%) | 15 (14%) |
| Number with inflammatory back pain (%) | 59 (55%) |

cation cycles consisting of denaturation at 96°C for 25 seconds, annealing (at 70°C for one cycle, 65°C for 21 cycles and 60°C for 4 cycles) for 45 seconds and extension at 72°C for 30 seconds, four additional amplification cycles of denaturation at 96°C for 25 seconds, annealing at 55°C for 1 min and extension at 72°C for 2 min and final extension at 72°C for 10 minutes. Formation of PCR product with expected length of 670 base pairs was confirmed using 2% agarose gel electrophoresis.

The PCR products were purified using QIAquick columns (Qiagen, Venlo, Limburg, Netherlands) to remove the unused primers and primer dimers. The purified PCR products were then amplified in both directions using the Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, CA, USA), and one of the above primers, the PCR steps included an initial denaturation at 96°C for 2 min, followed by 35 cycles of denaturation at 96°C for 10 seconds and annealing/extension at 50°C for 10 seconds, followed by final extension at 50°C for 4 min. The products of this sequencing reaction were cleaned up using the ethanol/EDTA/sodium acetate precipitation method to remove the unincorporated dideoxynucleotides as well as salts. The purified product was then denatured using hi-di formamide and run on the capillary electrophoresis system of a ABI 3130 Genetic Analyzer (Applied Biosystems). The electropherograms obtained with analyzed manually and using FinchTV (http://http://www.geospiza.com/Products/finchtv.shtml) and BioEdit(http://www.mbio.ncsu.edu/bioedit/bioedit.html) softwares. The sequences generated were compared with those available in IMGT/HLA Database, which is maintained by the international ImMunoGeneTics project (http://www.ebi.ac.uk/ipd/imgt/hla/align.html).

*Statistical analysis*

Frequencies of HLA-B27 subtypes in patients with AS and JIA-ERA were compared using chi-square test, *p*-values <0.05 were considered significant.

## Results

*HLA-B27 positivity and its clinical correlates*

HLA-B27 was positive in 107 of 135 (79%) patients with JIA-ERA, and in 102 of 121 (84%) patients with AS. The mean age of disease onset was 12 years for the patients with ERA and 23 years for those with AS. All but 4 patients with JIA-ERA had arthritis and most had involvement of lower limb joints. Four patients without arthritis presented with enthesitis and had other features to support diagnosis of JIA-ERA according to ILAR classification. Other symptoms included inflammatory back pain, sacroilitis, enthesitis and uveitis (Table I). The mean age of AS patients was 33 years and the mean duration of disease was 9.7 years. Twenty-one patients with AS had uveitis.

*HLA B27 subtypes*

HLA-B*27:05 and HLA-B*27:04 were the most common subtypes observed in both the patient groups (Table II). Some patients had HLA-B*27:02, HLA-B*27:07 and HLA-B*27:18. The frequency of HLA-B*27:05 was higher in patients with JIA-ERA (70%) than in those with AS (56%, $p$=0.047). By contrast, HLA-B*27:04 was found less frequently in patients with JIA-ERA than in AS (21% *vs*. 36%, $p$=0.018) (Table II). Among patients with JIA-ERA, no significant difference was found between patients with HLA-B*27:05 and those with HLA-B*27:04 in the frequency of any clinical feature, except for family history of JIA-ERA (Table III).

**Table II.** Comparison of HLAB27 allele distribution in patients with AS and JIA-ERA.

| HLA-B*27allele | Number positive for the particular allele among 102 patients with AS who were B*27 positive | Number positive for the particular allele among 107 patients with JIA-ERA who were B*27 positive | *p-value* |
|---|---|---|---|
| B*27:05 | 58 (56.8%) | 75 (70%)* | 0.047 |
| B*27:04 | 37 (36.2%) | 23 (21.4%)* | 0.018 |
| B*27:07 | 5 (4.9%) | 8 (7.4%) | NS |
| B*27:02 | 2 (1.9%) | 0 (0%) | NS |
| B*27:18 | 0 (0%) | 1 (0.93%) | NS |

Data are shown as number (%).

**Table III.** Relationship of clinical features of patients with JIA-ERA with the HLA-B27 subtype.

| Clinical feature | HLA-B*27:05 (n=75) | HLA-B*27:04 (n=23) | Others* (n=9) | Total (n=107) |
|---|---|---|---|---|
| Sex (M:F) | 69:6 | 20:3 | 9:0 | 98:9 |
| Enthesitis | 44 (58.6%) | 15 (65.2%) | 3 (33.3%) | 63 (59%) |
| Uveitis | 8 (10.6%) | 2 (8.6%) | 2 (22.2%) | 13 (12.14%) |
| Sacroiliitis | 17 (22.6%) | 5 (21.7%) | 1 (11.1%) | 23 (21.4%) |
| Family history | 7 (9.3%) | 7 (30.4%)* | 1 (11.1%) | 15 (14.0%) |
| History of inflammatory back pain | 44 (58.6%) | 11 (47.8%) | 3 (33.3%) | 59 (55.1%) |

*$p$<0.05 for comparison of B*27:05 *vs*. B*27:04.

## Discussion

We found HLA-B27 in 79% of our patients with JIA-ERA and 84% of those with AS. Among patients with JIA-ERA

as well as those with AS who had HLA-B27, the most common subtypes were HLA-B*27:05 and HLA-B*27:04. The prevalence of HLA-B2*27:05 was higher and that of HLA-B2*27:04 was lower in patients with JIA-ERA than in those with AS.

The prevalence rate of HLA-B27 of 79% among our patients with JIA-ERA is somewhat higher than the 60%-70% prevalence reported in series from UK (5). HLA-B27 is known to be a marker of disease severity in patients with AS. It is possible that its presence is also related to more severe disease in JIA-ERA. The higher prevalence rate of HLA-B27 in our patients with JIA-ERA may thus be related to the selective inclusion of patients with severe and persistent disease in our study which enrolled patients from a tertiary care referral hospital.

Both HLA-B*27:05 and HLA-B*27:04 subtypes were observed frequently among our patients with JIA-ERA as well as AS. HLA B*27:05 is the predominant HLA-B27 subtype in Caucasians and Koreans (20-23). Whereas HLA B*2704 as the predominant HLA-B27 subtype in Chinese and Thai patients with AS (24, 25; Table IV). Previous data from India also show nearly equal prevalence of HLA-B*27:05 and HLA-B*27:04 in AS (12, 26). The finding of both HLA-B*27:05 and HLA-B*27:04 among patients with both AS and JIA-ERA may be related to the fact that the northern Indian population has a genetic admixture, due to multiple invasions in last 5–6 centuries and because of trade links with China, Southeast Asia and Europe.

B*27:07 was found in five of our patients with AS. This subtype has been infrequent in previous studies from Asia (22, 27) and India (12, 26). We also found B*27:02 subtype in two of our patients with AS. This subtype is the predominant subtype in Tunisinan patients (27) and has been the second commonest subtype in some Asian countries (22). Similar to our finding, B*27:02 was rarely found in Europe (23) and in previous Indian studies (12, 26).

In JIA-ERA, we found B*27:05 to be the most common HLA-B27 subtype. A small study of 25 Latvian patients with ERA found B*27:05 in 55% of patients with ERA (15). A study from southern China found HLA-B*27:04 in majority of children with juvenile AS (11). No other data is available on HLA B27 subtyping in JIA. We found HLA-B*27:18 in one and HLA-B*27:07 in eight ERA patients. HLA-B*27:07 has not been reported in JIA but has an association with adult AS (12, 22, 26).

In our study, the prevalence of HLA* B27:05 was higher in patients with JIA-ERA than in those with AS and that of HLAB*27:04 was lower. In studies from Southern China and Mexican Mestizos no difference in distribution of HLA-B27 subtypes was found between juvenile and adult onset AS (11, 29) This may suggest that in Indian population the ancestral HLA B27 *i.e.* HLAB*27:05 may contribute to expression of disease in younger age. The correlation with presence of family history in JIA-ERA further suggests that the genetic contribution of HLA B27 may be more in juvenile disease as compared to adult disease. Lack of association with extra-articular manifestation with different HLA B27 subtypes has also been reported in studies on AS patients from Korea (20, 21).

The strength of our study are a homogenous group of ERA category of JIA rather than all categories of JIA together as was done in a study from Latvia and a larger sample size. The limitations of our study are lack of subtype data on HLA B27 healthy controls.

**Table IV.** Comparison of HLA B27 allele distribution among different patient populations.

| First author | Country | Disease | n. | HLA-B27 subtype (%) | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | B*27:04 | B*27:05 | B*27:02 | B*27:07 | Other |
| Present study | India | ERA | 107 | 21.4 | 70.0 | 0 | 7.2 | B*2718 (0.9) |
| | | AS | 102 | 36.2 | 56.8 | 1.9 | 4.9 | 0 |
| Johnsen, 2014 | Norway | AS | 124 | 0 | 98.0 | 2.0 | 0 | 0 |
| Diyarbakir, 2012 | Turkey | AS | 43 | 2.3 | 39.5 | 48.8 | 0 | B*27:12 (4.7), B*27:17 (2.3), B*27:49 (2.3) |
| Acar, 2012 | Turkey | AS | 51 | 6.5 | 65.2 | 26.1 | 2.2 | 0 |
| Chavan, 2011 | India | AS | 81 | 28.4 | 67.9 | 1.2 | 2.4 | 0 |
| Mou, 2010 | China | Adult AS | 319 | 86.2 | 11.9 | 0.9 | 0 | B*27:15(1.0) |
| | | Juvenile AS | 134 | 89.5 | 8.2 | 0 | 0 | B*27:15 (2.2) |
| Lopez de Castro, 2010 | China | AS | 583 | 85 | 12.5 | 0.5 | 0 | B*27:10 (0.2), B*15 (1.0) |
| Stanevich, 2010 | Latvia | ERA | 24 | 0 | 54.2 | 4.2 | 0 | B*27:03 (29.2), B*27:10 (4.2), B*27:17 (4.2), B*27:25 (4.2) |
| Liu X, 2010 | China | AS | 172 | 69.2 | 24.0 | 0.6 | 0 | B*27:03 (5.8), B*27:13 (0.6) |
| Liu Y, 2010 | China | AS | 130 | 80.7 | 18.5 | 0 | 0 | B*27:10 (0.7) |
| Park, 2009 | Korea | AS | 143 | 7.7 | 90.9 | 0 | 0 | B*27:10 (1.4) |
| Wu, 2009 | China | AS | 93 | 81.7 | 12.9 | 0 | 0 | B*27:15 (5.4) |
| Lee, 2008 | Korea | AS | 266 | 8.3 | 91.7 | 0 | 0 | 0 |
| Ben Radhia, 2008 | Tunisia | AS | 121 | 0.8 | 47.1 | 47.1 | 3.0 | B*27:09 (0.8), B*27:14 (0.8) |
| Ma, 2006 | China | AS | 111 | 88.3 | 10.8 | 0 | 0 | B*27:24 (0.9) |
| Fraile, 1998 | Spain | AS | 52 | 0 | 92.3 | 7.7 | 0 | 0 |
| Lopez-Larrea, 1996 | India | AS | 45 | 41.0 | 51.0 | 2.2 | 6.0 | 0 |
| | Thailand | AS | 45 | 91.1 | 4.4 | 0 | 4.4 | 0 |

n: number of subjects studied.

In conclusion, nearly 80% children with JIA-ERA are HLA-B27 positive and among them HLA-B*27:05 is the most common subtype followed by HLA-B*27:04. In comparison with adult AS, JIA-ERA patients had a higher prevalence of HLA-B*27:05.

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