# Increased frequency of HLA-B7 among B27-negative seronegative spondarthritis patients from Mumbai, western India

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**ABSTRACT**

Seronegative spondarthritis (SSA) is a group of inflammatory disorders that shares certain clinical features and has a strong association with the human leucocyte antigen (HLA)-B27 allele. Serologically, HLA-B27, HLA-B22, HLA-B7, HLA-B40 and HLA-B42 antigens belong to the HLA-B cross-reacting antigen group (CREG). In addition to B27, other B locus antigens are associated with B27-negative American black, Brazilian, French and Chinese SSA patients. Many B27-negative individuals in India have developed SSA with severe clinical and radiological findings. This stimulated the evaluation of the involvement of HLA-B7 CREG antigens among B27-negative SSA patients from western India. A total of 276 SSA patients who were B27-negative and fitted the modified New York criteria for AS and the European Spondyloarthropathy Study Group (ESSG) criteria for spondarthritis from western India were studied and compared with 637 normal, healthy individuals who were B27-negative and of the same ethnic background. A significantly increased phenotype frequency of HLA-B7 (PF=57.24% *vs.* 22.44%; $P<0.001$) and a significant decreased phenotype frequency of HLA-B40 (PF=18.11% *vs.* 31.86%; $P<0.001$) was observed when compared to the controls. These results suggest that HLA-B7 antigen may be associated with B27-negative SSA in patients from western India.

KEY WORDS: HLA-B7 antigen.
HLA-B22.
HLA-B27 antigen.
HLA-B40 antigen, human.
HLA-B42 antigen, human.
Spondylitis.

## Introduction

Seronegative spondarthritis (SSA) is a group of diseases that includes mainly ankylosing spondylitis (AS), Reiters syndrome/reactive arthritis (ReA), enteropathic spondylitis (Crohn's disease and ulcerative colitis), psoriatic arthropathy (PsA) and undifferentiated spondylitis (uSp).[1]

Ankylosing spondylitis is a chronic inflammatory disease that begins primarily in the sacroiliac joints and goes on to involve the spine and other large joints.[1] An association between human leucocyte antigen (HLA)-B27 and AS was first reported in 1973,[2,3] and an association with other members of the SSA group was confirmed later.[4–7] Furthermore, it is suggested that the other HLA-B locus alleles are also involved in B27-positive and -negative SSA patients around the world.

Human leucocyte antigens are highly cross-reactive in nature and share an amino acid sequence for most of their molecular structure. Antibodies bind to specific sites on these molecules and it would be expected that many different antigens would share a site (epitope) to which a specific antibody will bind. Thus cross-reactivity is the sharing of epitopes between antigens, and the term cross-reacting groups (CREGs) is often used in this respect.

Serological HLA typing has shown that there are many CREGs and they have been reported among the antigens of the HLA A, HLA B and HLA DR loci. The CREGs of HLA antigens can be strong or weak, depending on the antigen–antibody binding. The HLA-B27 CREG antigens, which include HLA-B27, -B7, -B22, -B40 and -B42, are very strong.

Khan *et al.* found an association between HLA-B7 CREG antigens and AS among American black patients.[8,9] Subsequently, these findings were confirmed in ReA patients with AS by Arnet.[10] Later, Benchetrit showed an association between HLA-B7 and -B22 and ReA in patients from Israel.[11] Similarly, HLA-B7 CREG antigens have been associated with Brazilian uSpA patients,[12] and an association between HLA-B7 and inflammatory arthritis has been reported in patients from France.[13]

The association of HLA-B40 with AS was reported by Robinson and colleagues who found that HLA-B60 was increased in B27-positive AS patients.[14] In another study, Cedoz reported that HLA-B40 is associated with prominent peripheral arthritis.[12] Subsequently, Brown and colleagues demonstrated that HLA-B60 is associated with AS both in B27-positive and B27-negative individuals.[15] Recently, in a study of a Taiwan Chinese population, it has been reported that HLA-B60 and HLA B-61 (splits of HLA-B40) are strongly associated with HLA-B27-negative AS patients.[16]

Previous reports show that the prevalence of B27 in a

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**Table 1.** Distribution of HLA-B7 CREG antigens among B27-negative SSA patients ($n$=276) and controls ($n$=637).

| HLA | Pos | PF% | Pos | PF% | OR | 95% CI | AF | Pr F | $\chi^2$Y | *P* value |
|---|---|---|---|---|---|---|---|---|---|---|
| B7 | 158 | 57.24 | 143 | 22.44 | 4.62 | 3.41-6.26 | 0.44 | | 103.94 | <0.0001* |
| B22 | 24 | 8.69 | 37 | 5.80 | 1.544 | 0.905-2.63 | | 3.12E-02 | 2.13 | 0.1085 |
| B40 | 50 | 18.11 | 203 | 31.86 | 0.473 | 0.337-0.670 | | 0.168 | 17.5 | <0.0001* |

PF: phenotype frequency; OR: odds ratio; CI: confidence interval; AF: aetiological fraction; PrF: preventive fraction; $\chi^2$Y: $\chi^2$ with Yates correction.
*Significant

western Indian population with SSA is 30–80%.[5–7] Recently, however, we observed that a higher number of B27-negative individuals develop SSA with typical clinical and radiological findings. Thus, we hypothesise that these patients must be carrying one of the B27 CREGs (B7, B22, B40 and B42). Therefore, the present study aims to discover whether or not other antigens are involved in HLA-B27-negative spondarthritis patients in western India.

## Materials and methods

*Selection criteria for patients and controls*

A total of 276 HLA-B27-negative patients were selected according to the revised New York criteria for AS[17] and the European Spondyloarthropathy Study Group (ESSG) criteria for SSA between April 2004 and March 2007.[18] Patients include those who were suffering from arthritis and satisfied at least four of the following criteria: insidious onset, duration >3 months, radiological bilateral or unilateral sacroiliitis, limitation of motion of the lumbar spine and chest expansion, and association with morning stiffness that improves with exercise and is not relieved by rest. All patients were negative for rheumatoid factor.

Clinical evaluation was undertaken by rheumatologists at various hospitals around Mumbai, and cases were also re-evaluated by one of the authors (KG). The patients underwent various tests including X-ray (affected joints, sacroiliac joints, lumbar and cervical spine) full blood count with ESR and rheumatoid factor.

The study was approved by the local ethics committee. The control group (from the same socioeconomic and ethnic background) comprised 637 age- and gender-matched healthy individuals who were negative for B27.

*HLA typing*

Samples (5–10 mL) of heparinised blood (20 iu preservative-free heparin/mL) were freshly drawn from each individual after obtaining consent, and the lymphocytes were separated by Ficoll–Hypaque density gradient centrifugation.[19] The HLA-B27 typing was performed using the standard two-stage NIH microlymphocytotoxicity assay.[20] Two or three specific HLA antisera (Biotest, Germany; Pelfreez, USA) were used for each specificity ($r$≥0.8).

*Statistical Analysis*

Antigen frequencies, odds ratio (OR), 95% confidence interval (CI), $\chi^2$ with Yates correction ($\chi^2$Y), aetiological fraction (AF) and preventive fraction (PrF) were estimated using our database and software programs.[6]

**Table 2.** Clinical characteristics of HLA-B7-positive patients.

| Characteristics | | B7-positive (%) ($n$=158) | Controls (%) ($n$=637) |
|---|---|---|---|
| Age at onset | ≤15 | 20 (12.65) | 90 (14.12) |
| | 16-30 | 78 (49.36) | 135 (21.19) |
| | 31-45 | 48 (30.37) | 365 (57.29) |
| | >45 | 12 (7.59) | 47 (7.37) |
| Gender | M | 125 (79.11) | 329 (51.64) |
| | F | 33 (20.88) | 308 (48.35) |
| Family history of arthritis | | 34 (21.51) | 0 |
| Polyarticular arthritis | | 66 (41.77) | 0 |
| Pauciarticular | | 55 (34.81) | 0 |
| Monoarticular | | 25 (15.82) | 0 |
| Chest expansion <5 cm | | 56 (35.44) | 0 |
| Spinal flex <5 cm | | 88 (55.69) | 0 |
| Sacroiliac joint | | 106 (67.08) | 0 |
| Knee joint | | 84 (53.16) | 0 |
| Hip joint | | 83 (52.53) | 0 |

## Results

The distribution of HLA-B7 CREG antigens in B27-negative SSA patients and the B27-negative control group is shown in Table 1. Phenotype frequency of HLA-B7 was significantly increased ($P$<0.001), whereas HLA-B40 decreased in the B27-negative group ($P$<0.001).

An AF of 1 is considered highly significant, suggesting 100% infectivity, while the PrF indicates the opposite, with a lower value indicating greater protection. HLA-B7 showed 44% infectivity, while the value for HLA-B40 was 16.8%. HLA-B22 frequency was increased in the patient group, and it was statistically significant. HLA-B42 was not seen in either group.

The clinical characteristics of the B7-positive patients are shown in Table 2.

When Scober's test results were analysed, 55% of patients had restricted spine flexion and 35% had diminished chest expansion.

## Discussion

The association between HLA-B27 with AS and related arthropathies is well known, but it has been postulated that other class I and II genes may increase susceptibility to the

development of AS and related arthropathies.[14,21] In the present study, HLA-B7 was shown to be significantly increased among B27-negative SSA patients in western India. This confirms earlier reports of work in American, Brazilian, Jewish and French populations.[8–13] In addition, HLA-B60 and B61 (split antigens of HLA-B40) have been associated with HLA-B27-negative SSA patients in China,[16] but this connection was not observed in the present study.

HLA-B7 may be associated with B27-negative SSA either directly or because of linkage disequilibrium with an associated major histocompatibility complex (MHC) gene, which significantly differentiates in the structure and peptide presentation by HLA-B7 compared to B27. This suggests that different mechanisms are involved in the association of HLA-B7 antigen with AS and related arthropathies. The peptide binding motifs of HLA alleles show considerable differences, making it unlikely that the effect is mediated by the presentation of similar arthritogenic peptides

Population-specific distribution of HLA antigens has been shown in population genetics and in HLA disease association studies.[22] Anthropological studies have shown that the distribution of HLA antigens differs between ethnic groups, and new alleles may be discovered in the Indian population.[23]

Various Indian religious and caste groups differ in their origin, migration and settlement. Genetic analysis suggest that the inhabitants of the western part of the Eurasian steppes comprised Caucasoid people speaking Indo-European languages, and these were believed to be the earliest settlers of the Indian subcontinent. Migration has a linear effect on HLA antigen frequency and could be the cause of nationwide genetic gradients.

Clearly, extensive typing of the Indian population will be necessary to resolve the evolutionary implications of HLA-B7 allele subtypes and related haplotypes in relation to population demography and disease association/protection in the Indian context. However, the present study reveals a strong association between HLA-B7 and B27-negative SSA in patients from western India. □

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