Analysis of Gene Expression of Human Leukocyte Antigen B27 in Patients with Ankylosing Spondylitis and Correlation with Related Indicators

Chun Liu
   Tianjin First Central Hospital
Zhong-Yu Kang
   Tianjin First Central Hospital
Wei Liu
   Tianjin First Central Hospital
Yuan Zhao
   Tianjin First Central Hospital
Dai-Hong Li (ldh_hla@126.com)
   Tianjin First Central Hospital

Research Article

Keywords: ankylosing spondylitis, human leukocyte antigen-B27, polymerase chain reaction-sequence specific primers, genotyping

Posted Date: December 10th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-1066135/v1

License: ☝️ © This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License
Abstract

The aim of this study was to investigate the potential correlation between the human leukocyte antigen (HLA)-B*27 subtypes in patients with ankylosing spondylitis (AS) and the clinical features of AS. The prevalence of HLA-B*27 subtypes was investigated in 132 healthy donors recruited from our center and in 143 patients with AS. We investigated the differences in HLA-B27 subtype status for the patients and healthy donors by using a PCR-SSP (polymerase chain reaction with sequence-specific primer) method. The clinical outcomes of the patients, including lower back pain, uveitis, peripheral arthritis, stiffness, joints, erythrocyte sedimentation rate (ESR), Anti-streptolysin O (ASO) and C-reactive protein (CRP), were recorded. The male-to-female ratio was 2.5, and the mean age at diagnosis was 29.3 years. HLA-B*27 positivity was detected in 111 patients (77.6%) of patients with AS. The rate of HLA-B*27 positivity was significantly higher in the AS group than patients without AS. The subtypes observed in patients with AS were HLA-B*2704 (55.9%, 62/111), HLA-B*2705 (39.6%, 44/111), HLA-B*2702 (1.80%, 2/111), HLA-B*2707 (0.90%, 1/111), and HLA-B*2704/05(1.80%, 2/111). The main genotypes were HLA-B*2704 and HLA-B*2705. There were no significant differences in clinical manifestations. Multivariate analysis showed statistically significant differences in sex (P=0.01), disease duration (P=0.023), hematocrit (P=0.01), and CRP (P=0.01) between patients in the HLA-B*27(+) AS group and the HLA-B*27(-) AS group. In addition, HLA-B*15, HLA-B*40, HLA-B*13, and HLA-B*46 were the major alleles with associated HLA-B types in patients with HLA-B*27(+) AS. Therefore, we conclude that HLA-B*27 is highly correlated with AS and can be used as an early predictor of AS. In addition, sex, disease duration, ESR, and CRP were significantly different in the HLA-B*27(+) AS group and HLA-B*27(-) AS group. Finally, our study also found a correlation between HLA-B*15, HLA-B*40, HLA-B*13, and HLA-B*46 subtypes and the development of AS.

Introduction

Ankylosing spondylitis (AS) is a chronic progressive autoimmune disease of the spine and sacroiliac joints and is a common orthopedic disease; the incidence is 0.2–0.3% in China. The main clinical signs of the disease are sacroiliac arthritis and spondylitis, which lead to ankylosis and the eventual loss of spinal mobility. Peripheral arthritis, lower back pain, stiffness, and sacroiliac arthritis are also major symptoms in patients with AS. The disease can also be associated with extra-skeletal manifestations such as anterior uveitis and psoriasis. Therefore, early detection and timely treatment are particularly critical. Various hypotheses have been reported to explain the relationship between human leukocyte antigen B27 (HLA-B*27) and AS. The arthritic peptide hypothesis states that T-cell receptors can recognize complexes of foreign or unknown MHC autologous peptides and when bound together. The molecular mimicry hypothesis suggests a cross-reactivity between antigens of the associated bacteria and the HLA-B27 molecule, whereas a third hypothesis is based on the aberrant folding of HLA-B27, which induces the disorder of intracellular traffic of this molecule. However, none of these hypotheses has fully elucidated the pathogenicity mechanism of HLA-B27 in AS. The etiology and pathogenesis of AS are still not well understood, and it is generally believed that it they are closely related to genetics and infection.
Human leukocyte antigen B27 (HLA-B*27) is the gene that is reported to be most closely associated with AS, with over 155 subtypes identified to date\(^6\). However, the prevalence of HLA-B*27 varies across racial and ethnic populations, possibly as a result of the different genetic interactions and geographic origins\(^7\)–\(^10\). In general, HLA-B*2705 is the most common subtype in all populations. The subtype HLA-B*2702 is present in Asia, North Africa, Europe, and certain Mediterranean countries. It is reported to be the most common subtype in Caucasian and Arab populations\(^4\). HLA-B*2704 is the most common subtype in Chinese and Japanese populations\(^11\). It has been shown that patients with AS carrying the HLA-B*2704 gene are more likely to develop uveitis than patients with AS carrying the HLA-B*2705 gene. Recently, it has been found that some alleles at the B locus, other than HLA-B*27, are also closely associated with AS; for example, HLA-B*40 and HLA-B*51 increase the risk of developing AS, whereas HLA-B*07 has been reported to protect against AS.

Anti-streptolysin O (ASO) is an antibody produced by streptococcal infection and is associated with immune diseases, such as rheumatism and nephritis, and leukocytes, C-reactive protein (CRP), and sedimentation are all indicators of inflammation. In this study, the above indicators were tested to find associations with AS and to achieve early diagnosis and early treatment of AS.

In this study, we will investigate the correlation between HLA-B subtypes and the incidence and clinical symptoms of AS, as well as the differences in clinical symptoms between patients with AS carrying the HLA-B*2704 and those carrying the HLA-B*2705 gene, and to explore the correlation between AS and B locus alleles other than HLA-B*27. In addition, the relationships between AS and indicators such as ASO, leukocytes, CRP, and hematocrit were also explored through multifactorial and univariate analyses.

**Materials And Methods**

This study was approved by the ethics review board of the Tianjin First Central Hospital with Nankai Medical University. Written informed consent was provided by each enrolled patient. The study was performed in accordance with the Declaration of Helsinki and its recommendations.

**Selection of subjects**

This study was conducted in the division of orthopedics and immunology department at a government hospital. Between January 2020 and October 2020, 143 patients with AS were randomly selected were recruited for the purpose of HLA typing; another 132 patients without AS were selected over the same consultation period.

Medical charts were reviewed and the data of demography, lower back pain, uveitis, peripheral arthritis, and laboratory indicators, including erythrocyte sedimentation rate (ESR), anti-streptolysin O (ASO), and CRP, were collected. All data were obtained at diagnosis.

The inclusion criteria for patients with AS were: 1) history of pain in the lower back and lumbar region, morning stiffness for more than 3 months, symptom relief after activity, no improvement with rest; 2)
manifestation of restricted movement in the lateral flexion direction and anterior-posterior direction of the lumbar spine; 3) thoracic mobility lower than normal people of the same age and sex; 4) imaging showing bilateral grade II or higher sacroiliac arthritis or unilateral grade III or higher sacroiliac arthritis. Those who met the above imaging manifestations in 4 and any of the clinical manifestations of criteria 1–3 were enrolled.

Non-AS inclusion criteria: simple bone and joint disease, no abnormal liver or kidney function, abnormal hematopoietic function, no neurological disease, malignancy, and other immune system diseases.

**Detection of HLA-B*27 subtypes**

The HLA-B27 genotyping assay was performed by a PCR-SSP method. For DNA extraction (QIAGEN Nucleic acid extraction kit; HLA-B27 genotyping kit (Tianjin Xiu peng Biological), 200 µL of EDTA-anticoagulated blood was mixed thoroughly with 100 µL of template DNA in accordance with the operating instructions, at a concentration of 20–100 ng/µL, a purity (A260/A280 value) of 1.6–2.0, and stored at -20°C. For PCR amplification, the DNA template and working solution was mixed thoroughly and added to each well. The amplification was conducted in accordance with the operating instructions, and a 2% agarose gel was loaded with 5 µL of the amplification product and 2 µL of loading buffer. Sample electrophoresis was performed at 140 V for 10 min, and the gel was transferred to an imaging system to observe the results of electrophoresis.

CRP and ASO assays were performed by transmission turbidimetry with direct serum aspiration and sample addition, and analyzed by an automatic immunoassay analyzer. The ESR assay was performed using the sedimentation method with sodium citrate anticoagulant.

**Statistical analysis**

All statistical analyses were performed using Statistical Package for the Social Sciences (SPSS, Chicago, Illinois, USA; version 20.0). The results of the descriptive analysis were presented as the mean ± standard deviation if a normal distribution was present and, if not, as the median and interquartile range. For pairwise comparisons, an independent t-test was used for normally distributed numerical variables, and chi-square test or Fisher’s exact test was used, when appropriate, for categorical variables. Two-sided p values of less than 0.05 were considered statistically significant.

**Results**

**General clinical features of patients with AS and healthy controls in the Chinese population**

The mean age of the 143 patients was 38.5±14.5 years, the mean age at disease diagnosis was 29.3±10.2 years, with a mean disease duration of 9.3±8.0 years. The male-to-female ratio was 2.49, with 102 (77.6%) male patients. With respect to the frequency of symptoms, 56.6% had inflammatory back
pain, 71.3% had stiffness, 62.9% had sacroiliac joints, 7.0% had uveitis, and 18.9% patients had familial history. Of the 143 patients, HLA-B*27 positivity was detected in 111 patients (77.6%), include five allele subtypes. The distribution frequency was HLA-B*2704 (55.9%, 62/111), HLA-B*2705 (39.6%, 44/111), HLA-B*2702 (1.8%, 2/111), HLA-B*2707 (0.9%, 1/111), and HLA-B*2704/B*2705 homozygote (1.8%, 2/111). The results of the control group were essentially consistent with those of the AS group, but there was one HLA-B*27:15 and HLA-B*27:02 and HLA-B*27:07.

With regard to the laboratory indicators, ESR was 25.4±24.4 mm/h, CRP was 20.8±24.4 mg/L, and ASO was 53.0±56.0 IU/mL (Table 1).

The HLA-B27 subtypes in all HLA-B27(+) groups and the HLA-B27(-) groups

Of all subjects, 119 subjects were HLA-B*27 positive, of which 111 were patients with AS; 156 subjects were HLA-B*27 negative, of which 32 were patients with AS. From the 111 HLA-B*27-positive patients with AS, 81 patients (73.0% 81/111) were men, and the male-to-female ratio was 2.7. Of the 32 HLA-B*27(-) patients with AS, 21 subjects (65.6%, 21/32) were men.

The mean age of the patients with AS(+) was 37.7±13.3 years; the mean age at diagnosis was 28.9±10.1 years, and the disease duration was 8.8±7.9 years. The mean age of the patients with AS(-) was 41.5±18.2 years, the mean age at disease diagnosis was 30.7±10.7 years, and the disease duration was 10.8±8.5 years.

With respect to the frequency of symptoms, in the HLA-B*27(+) AS group, 9.0% (10/111) had uveitis, 58.6% (65/111) had inflammatory back pain, 31.5% (35/111) had peripheral arthritis, 73.9% (82/111) had sacroiliac arthritis, and 21.6% (24/111) had familial history. In the HLA-B*27(-) AS group, 6.3% (2/32) had uveitis, 50.0% (16/32) had inflammatory back pain, 28.1% (9/32) had peripheral arthritis, 71.9% (23/32) had sacroiliac arthritis, and 18.8% (6/32) had familial history. There was no significant difference between the two groups in the univariate analysis; however, the multivariate analysis showed that sex (OR=6.235, 95% CI=1.519–25.593, P=0.011) and disease duration (OR=0.918, 95% CI=0.853–0.988, P=0.023) showed significant differences.

Among the laboratory indicators, ESR in the HLA-B*27(+) group was 25.8±24.5 mm/h, CRP was 13.2±17.8 mg/L, and ASO was 56.8±58.6 IU/mL; in the HLA-B27(-) group, ESR was 23.8±24.2 mm/h, CRP was 14.7±15.6 mg/L, and ASO was 39.9±44.0 IU/mL. There were significant differences in CRP (P=0.001) and ASO (P=0.027) in the univariate analysis.

However, the multivariate analysis showed that ESR (OR=1.055, 95% CI=1.022–1.089, P=0.001) and CRP (OR=0.899, 95% CI=0.862–0.937, P=0.01) was significantly higher in the HLA-B*27(+) group than in the HLA-B*27(-) group (Table 2).

Disease characteristics of patients with AS carrying HLA-B*27:04 and HLA-B*27:05
In this study, there were 62 patients with AS with HLA-B*27:04 and 44 patients with HLA-B*27:05. The mean age of the patients with AS with HLA-B*27:04 was 37.7±14.3 years, the mean age at diagnosis was 29.2±11.4 years, and the disease duration was 8.5±8.9 years. The male-to-female ratio was 2.65; 45 patients (72.6%) were men. With regard to HLA-B*27:05, the mean age of the 44 patients was 38.5±11.9 years, the mean age at diagnosis was 29.0±8.4 years, and the disease duration was 9.6±6.5 years. The male-to-female ratio was 3.4; 34 patients (77.3%) were men.

With regard to the occurrence of symptoms for B27:04, 71.0% had inflammatory back pain, 77.4% had sacroiliac arthritis, 9.7% had uveitis, 27.4% had peripheral arthritis and 24.2% patients had familial history. Of the B27:05 patients, 59.1% had inflammatory back pain, 72.7% had sacroiliac arthritis, 6.8% had uveitis, 34.1% had peripheral arthritis, and 22.7% patients had familial history. No factors were statistically significant (Table 3).

**Allele With The Associated Hla-b Types In B27(+)**

As the main isoforms of HLA-B*27 are B*27:04 and B*27:05, only the linked genes of HLA-B*27:04 and HLA-B*27:05 were tested. The major genotypes were HLA-B*15 (19.8%, 21/106), HLA-B*40 (22.6%, 24/106), HLA-B*13 (17.0%, 18/106), HLA-B*46 (11.3%, 12/106), and HLA-B*58 (8.5%, 9/106). The HLA-B*15 subtypes included 4 cases of 1501/2704, 5 cases of 1501/2705, 3 cases of 1502/2705, 2 cases of 1511/2704, 3 cases of 1511/2705, 4 cases of 1518/2704; HLA-B*40 included 4 cases of 4001/2704, 5 cases of 4001/2705, 7 cases of 4002/2704, 3 cases of 4002/2705, 5 cases of 4006/2704; HLA-B*13 included 4 cases of 1301/2704, 9 cases of 1303/2704, 5 cases of 1303/2705; HLA-B*46 included 4 cases of 4601/2704, 9 cases of 4601/2705, 5 cases of 4603/2705; HLA-B*46 included 4 cases of 4601/2704, 8 cases of 4601/2705 2705; and HLA-B*58 included 5 cases of 5801/2704 5 cases and 4 cases of 5801/2705 (Table 4).

**Discussion**

Ankylosing spondylitis (AS) is a chronic progressive immune disorder with the involvement of the medial joints as the main manifestation; it occurs mostly in young and middle-aged men. The disease has a slow course with no obvious symptoms in the early stages, and when patients develop clinical manifestations or histopathology, the disease has often reached an intermediate to advanced stage, with the presence of irreversible damage owing to the formation of connective tissue and spinal ankylosis, with varying degrees of dysfunction, which can lead to disability and affecting the quality of life of patients[12,13]. Therefore, early the detection and early treatment of AS are particularly critical. In recent years, although AS has been well studied, the pathogenesis and principles of the disease are still less clear, although it is generally believed that it is closely related to genetics and infection. In this study, 71.3% (102/143) of the patients were men and 28.7% (41/143) were women, with a male-to-female ratio of 2.49, which was consistent with previous reports in the literature[14]. Some studies have shown that there is a stronger association with family genetic history for patients with a lower age of onset[15,16]. An
association of early disease onset with a high familial history of HLA-B27-positive patients have also been reported\[17\]. This pattern was not found in this study, but in terms of patient age and age at first diagnosis, the AS-positive group was younger than the AS-negative group, but there was no statistically significant difference between them. In terms of family history, the genetic incidence was 21.6% (24/111) in the AS-positive group compared with 18.8% (6/32) in the AS0-negative group, which was not a statistically significant difference (p=0.725). However, the disease duration was 8.8 ± 7.9 years in the AS-positive group and 10.8 ± 8.5 years in the AS-negative group, which confirmed to be a statistically significant difference through the multifactorial analysis (P=0.023).

HLA-B*27 is an allele at the B locus of the MHC-1 class of molecules on the short arm of human chromosome 6 and consists of an alpha chain with a molecular weight of $4.4 \times 10^4$ Da and a beta chain with a molecular weight of $1.2 \times 10^4$ Da \[18\]. The alpha chain includes $\alpha_1$, $\alpha_2$, and $\alpha_3$ chains, and the beta chain is bound by $\beta_2$ macroglobulin; together, they form the HLA-B*27 molecule. In 1973, British scientists first reported that AS was closely associated with the HLA-B*27 gene, with significant familial aggregation. Although research into AS has progressed in recent years, HLA-B*27 remains the most strongly associated gene with AS identified to date. One study showed that about approximately 90% of patients in AS carry the HLA-B*27 antigen, whereas only 4–9% of the healthy population are positive for HLA-B*27 [19]. In this study, the positive percentage of B27 in the AS group was 77.6%, which is low and might relate to the inclusion criteria; some early-stage patients positive for HLA-B*27 but without symptoms on imaging were not included, whereas a total of 111 of 133 patients positive for HLA-B*27 were diagnosed with AS (83.5%), indicating that HLA-B*27 was very useful for the diagnosis of AS, especially in early diagnosis.

The current methods for detecting HLA-B*27 are micro lymphocytotoxic assay, flow cytometry, and PCR-SSP gene assay. The micro lymphocytotoxic assay is a classic method for the detection of HLA-B*27, but it is influenced by cell purity, complement difference, monoclonal antibody potency, and operator experience, which can lead to false negatives. In recent years, the PCR-SSP method has been applied to the detection of HLA-B*27. This is because under specific reaction conditions, the first base at the 3’-end of the primer is complementary to the allele-specific base, and the specific primer amplifies only the allele that matches it under strict experimental operations and in molecular biology laboratories, to avoid the highly polymeric HLA-B*27 gene, with only individual site differences between the nucleotide sequences of each isoform. Overall, 160 HLA-B*27 isoforms have been identified, named HLA-B*27:01 to 27:161 in order (with no HLA-B*27.22), encoded by 213 alleles, respectively [20], of which HLA-B*27:05 is the current prototype of all subtypes, and HLA-B*27:04/05 is the dominant subtype in Chinese patients. In the present study, of the 111 patients with HLA-B*27-positive AS, there were 62 cases of HLA-B*27:04, 44 cases of HLA-B*27:05, 2 cases of HLA-B*27:02, 1 case of HLA-B*27:07, and 2 cases of HLA-B*27:04/05, which also verified this result. However, among the 22 HLA-B*27-positive patients in the non-AS group, there were 13 cases of HLA-B*27:04 (P=0.818), 8 cases of HLA-B*27:05 (P=0.816), and 1 case of HLA-B*27:15. The results of this study showed that HLA-B*27:04 and HLA-B*27:05 were predominant in both the AS and non-AS groups, and the subtype composition in both groups was not significantly different.
The difference in the polymorphisms of HLA-B*27 isoforms in the development of HLA-B*27-related diseases leading to the associated symptoms shows their different involvement in the development of the disease [21, 22]. Uveitis is considered to be the most common extra-articular manifestation of AS, with a distribution of 5.3–33.2% in different races [14], and some studies showed that HLA-B*2705-positive patients were more closely associated with uveitis than HLAB*2704 [23, 24]. Uveitis accounted for 9.0% (10/111) of patients in this study, but the positivity rate was not statistically significant for either (p=0.732). Peripheral arthritis was found in 35 cases in this study, accounting for 31.5%, which was consistent with previous studies [25]. There were 82 cases (73.9%) of sacroiliac arthritis and 65 cases (58.6%) of lower back pain. These clinical manifestations were not statistically significantly different from the various subtypes of B27, which was consistent with some studies [26]. There were two cases of pure B27:04/05 in this study, which were not counted owing to the small number of cases, but some scholars found no significant difference in clinical presentation and imaging progression between pure and heterozygous HLA-B27 [27, 28].

Environmental factors are also important influences in the development of AS; among which the influence of infection cannot be ignored [29]. Some studies have proposed a close relationship between streptococcal infection and the development of arthritis. Anti-streptolysin O (anti-streptolysin O ASO) is an antibody produced by streptococcal infection, a positive ASO indicates that the body has streptococcal infection, and the antibody potency reaches a 3–6-week peak [30]. Streptococcal infection stimulates the body’s immune response, induces the production of multiple antibodies, and activates T and B lymphocytes, promoting the release of various inflammatory factors, mediating the aggravation of damage to joints, and therefore participating in the development of AS. CRP is an acute temporal reactive protein, a non-specific indicator of inflammation, and hematocrit (ESR) is also an indicator of inflammation. In the present study, a univariate analysis showed statistically significant differences in CRP (P=0.001) and ASO (P=0.027), but no statistically significant ESR (P=0.188) in both groups, whereas a multifactorial analysis showed statistically significant ESR (P=0.01) and CRP (P=0.01), but no statistically significant ASO (P=0.16), indicating that these inflammatory indicators need to be analyzed together to aid the diagnosis of AS.

Recent studies on the B locus allele of HLA-B*27 in patients with AS have emerged; the association of HLA-B*40 with AS has now been convincingly demonstrated now in East Asian studies [31, 32]. Other studies have found that 18.2% of patients with AS carry HLA-B*227/B40 and that HLA-B*240 increases the susceptibility of HLA-B*27 to AS [33, 34]. In this study, only 105 patients positive for HLA-B*2704 and HLA-B*2705 were tested. In total, 24 cases of HLA-B*40 (22.9% 24/105) were detected, including nine cases of HLA-B*4001, ten cases of HLA-B*4002, and five cases of HLA-B*4006. The frequency was generally consistent with previous studies. A total of 21 cases of HLA-B*15 (20.0%, 21/105) was detected, including 9 cases of HLA-B*1501, 9 cases of HLA-B*1502 and 3 cases of HLA-B*1511. Some studies have shown that B15 positivity increases the risk of peripheral joint involvement [33], and HLA-B*07 and HLA-B*51 alleles have been shown to have a protective influence on the risk of developing AS in several Mediterranean populations [35, 36]. The number of B7 and B51 cases in this study was low, with
only one HLA-B*7 and four HLA-B*51 cases. Other B loci found in higher numbers in this study were HLA-B*13 (17.1% 18/105), HLA-B*46 (11.4% 12/105), and HLA-B*58 (8.6% 9/105). In terms of diagnostic value, further research is needed regarding the significance of heterozygosity for HLA-B loci owing to the small number of patients.

Our study has some limitations. First, as it is a retrospective study, some clinical manifestations may have been missed owing to the specific patients; secondly, the number of cases in both groups was small and there was some bias in the results; and finally, owing to the reagents, only B27-positive patients were tested for the B locus allele, whereas the control group was only B27-negative and no B locus testing was performed, preventing some corroboration of the results.

In conclusion, our study showed that in addition to the HLA-B*27 and HLA-B*27 subtypes, the allelic typing of HLA-B*27, and patients family genetic background was closely associated with AS. This paper may be somewhat different from the real-word situation owing to specimen size and patient enrollment. Moreover, some results are not sufficiently clear and require further study.

Declarations

Funding: This work was supported by no specific funding. The funding sources had no role in the study design, data collection, analysis or interpretation, or the writing of this manuscript.

Conflict of interest: The authors declare that they have no competing interests.

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent: Informed consent was obtained from all individual participants included in the study.

References

1. Feldtkeller E, Khan MA, van der Heijde D, van der Linden S, Braun J (2003) Age at disease onset and diagnosis delay in HLA-B27 negative vs. positive patients with ankylosing spondylitis. Rheumatol Int 23(2):61–66
2. Brown MA (2008) Breakthroughs in genetic studies of ankylosing spondylitis. Rheumatology (Oxford) 47(2):132–137
3. Ebringer A (1983) The cross-tolerance hypothesis, HLA-B27 and ankylosing spondylitis. Br J Rheumatol 22(4 Suppl 2):53–66
4. Khan MA, Mathieu A, Sorrentino R, Akkoc N (2007) The pathogenetic role of HLA-B27 and its subtypes. Autoimmun Rev 6(3):183–189
5. Mear JP, Schreiber KL, Munz C, Zhu X, Stevanovic S, Rammensee HG et al (1999) Misfolding of HLA-B27 as a result of its B pocket suggests a novel mechanism for its role in susceptibility to spondyloarthopathies. J Immunol 163(12):6665–6670

6. Robinson J, Halliwell JA, Marsh SG (2014) IMGT/HLA and the Immuno Polymorphism Database. Methods Mol Biol 1184:109–121

7. Reveille JD, Maganti RM (2009) Subtypes of HLA-B27: history and implications in the pathogenesis of ankylosing spondylitis. Adv Exp Med Biol 649:159–176

8. Hou TY, Chen HC, Chen CH, Chang DM, Liu FC, Lai JH (2007) Usefulness of human leucocyte antigen-B27 subtypes in predicting ankylosing spondylitis: Taiwan experience. Intern Med J 37(11):749–752

9. Cipriani A, Rivera S, Hassanhi M, Marquez G, Hernandez R, Villalobos C et al (2003) HLA-B27 subtypes determination in patients with ankylosing spondylitis from Zulia, Venezuela. Hum Immunol 64(7):745–749

10. Park SH, Kim J, Kim SG, Kim SK, Chung WT, Choe JY (2009) Human leucocyte antigen-B27 subtypes in Korean patients with ankylosing spondylitis: higher B*2705 in the patient group. Int J Rheum Dis 12(1):34–38

11. Reveille JD (2006) Major histocompatibility genes and ankylosing spondylitis. Best Pract Res Clin Rheumatol 20(3):601–609

12. Deodhar A, Mittal M, Reilly P, Bao Y, Manthena S, Anderson J et al (2016) Ankylosing spondylitis diagnosis in US patients with back pain: identifying providers involved and factors associated with rheumatology referral delay. Clin Rheumatol 35(7):1769–1776

13. van der Heijde D, Braun J, Deodhar A, Baraliakos X, Landewe R, Richards HB et al (2019) Modified stoke ankylosing spondylitis spinal score as an outcome measure to assess the impact of treatment on structural progression in ankylosing spondylitis. Rheumatology (Oxford) 58(3):388–400

14. Stolwijk C, van Tubergen A, Castillo-Ortiz JD, Boonen A (2015) Prevalence of extra-articular manifestations in patients with ankylosing spondylitis: a systematic review and meta-analysis. Ann Rheum Dis 74(1):65–73

15. Chung HY, Machado P, van der Heijde D, D'Agostino MA, Dougados M (2011) HLA-B27 positive patients differ from HLA-B27 negative patients in clinical presentation and imaging: results from the DESIR cohort of patients with recent onset axial spondyloarthritis. Ann Rheum Dis 70(11):1930–1936

16. Rudwaleit M, Haibel H, Baraliakos X, Listing J, Marker-Hermann E, Zeidler H et al (2009) The early disease stage in axial spondylarthritis: results from the German Spondyloarthritis Inception Cohort. Arthritis Rheum 60(3):717–727

17. Arevalo M, Gratacos Masmitja J, Moreno M, Calvet J, Orellana C, Ruiz D et al (2018) Influence of HLA-B27 on the Ankylosing Spondylitis phenotype: results from the REGISPONSER database. Arthritis Res Ther 20(1):221
18. Ridley A, Hatano H, Wong-Baeza I, Shaw J, Matthews KK, Al-Mossawi H et al (2016) Activation-Induced Killer Cell Immunoglobulin-like Receptor 3DL2 Binding to HLA-B27 Licenses Pathogenic T Cell Differentiation in Spondyloarthritis. Arthritis Rheumatol 68(4):901–914

19. Faham M, Carlton V, Moorhead M, Zheng J, Klinger M, Pepin F et al (2017) Discovery of T Cell Receptor beta Motifs Specific to HLA-B27-Positive Ankylosing Spondylitis by Deep Repertoire Sequence Analysis. Arthritis Rheumatol 69(4):774–784

20. Khan MA (2017) An Update on the Genetic Polymorphism of HLA-B*27 With 213 Alleles Encompassing 160 Subtypes (and Still Counting). Curr Rheumatol Rep 19(2):9

21. Akassou A, Bakri Y (2018) Does HLA-B27 Status Influence Ankylosing Spondylitis Phenotype? Clin Med Insights Arthritis Musculoskelet Disord; 11:1179544117751627

22. Chen B, Li J, He C, Li D, Tong W, Zou Y et al (2017) Role of HLA-B27 in the pathogenesis of ankylosing spondylitis (Review). Mol Med Rep 15(4):1943–1951

23. Qi J, Li Q, Lin Z, Liao Z, Wei Q, Cao S et al (2013) Higher risk of uveitis and dactylitis and older age of onset among ankylosing spondylitis patients with HLA-B*2705 than patients with HLA-B*2704 in the Chinese population. Tissue Antigens 82(6):380–386

24. Konno Y, Numaga J, Tsuchiya N, Ogawa A, Islam SM, Mochizuki M et al (1999) HLA-B27 subtypes and HLA class II alleles in Japanese patients with anterior uveitis. Invest Ophthamlol Vis Sci 40(8):1838–1844

25. Firat SN, Yazici A, Yilmazer B, Cosan F, Savli H, Cefe A (2017) Low frequency of HLA-B27 in ankylosing spondylitis and its relationship with clinical findings in patients from Turkey. Eur J Rheumatol 4(4):268–271

26. Wu Z, Lin Z, Wei Q, Gu J (2009) Clinical features of ankylosing spondylitis may correlate with HLA-B27 polymorphism. Rheumatol Int 29(4):389–392

27. Kim TJ, Na KS, Lee HJ, Lee B, Kim TH (2009) HLA-B27 homozygosity has no influence on clinical manifestations and functional disability in ankylosing spondylitis. Clin Exp Rheumatol 27(4):574–579

28. Kim TJ, Sung IH, Lee S, Joo KB, Choi JH, Park DJ et al (2013) HLA-B27 homozygosity has no influence on radiographic damage in ankylosing spondylitis: Observation Study of Korean spondyloArthropathy Registry (OSKAR) data. Joint Bone Spine 80(5):488–491

29. Gonullu E, Bilge NSY, Cansu DU, Bekmez M, Musmual A, Akcar N et al (2017) Risk factors for urolithiasis in patients with ankylosing spondylitis: a prospective case-control study. Urolithiasis 45(4):353–357

30. Sahin MS, Yalcin MU, Kocyigit D (2016) Prevalence of rheumatic heart disease in patients with recurrent tonsillitis and elevated anti-streptolysin O titers. Int J Pediatr Otorhinolaryngol 89:133–135

31. Wei JC, Tsai WC, Lin HS, Tsai CY, Chou CT (2004) HLA-B60 and B61 are strongly associated with ankylosing spondylitis in HLA-B27-negative Taiwan Chinese patients. Rheumatology (Oxford) 43(7):839–842
32. Yi L, Wang J, Guo X, Espitia MG, Chen E, Assassi S et al (2013) Profiling of hla-B alleles for association studies with ankylosing spondylitis in the chinese population. Open Rheumatol J 7:51–54

33. van Gaalen FA, Verduijn W, Roelen DL, Bohringer S, Huizinga TW, van der Heijde DM et al (2013) Epistasis between two HLA antigens defines a subset of individuals at a very high risk for ankylosing spondylitis. Ann Rheum Dis 72(6):974–978

34. Cortes A, Pulit SL, Leo PJ, Pointon JJ, Robinson PC, Weisman MH et al (2015) Major histocompatibility complex associations of ankylosing spondylitis are complex and involve further epistasis with ERAP1. Nat Commun 6:7146

35. Robinson WP, van der Linden SM, Khan MA, Rentsch HU, Cats A, Russell A et al (1989) HLA-Bw60 increases susceptibility to ankylosing spondylitis in HLA-B27+ patients. Arthritis Rheum 32(9):1135–1141

36. Hajjej A, Hmida S, Kaabi H, Dridi A, Jridi A, El Gaa l ed A, et al (2006) HLA genes in Southern Tunisians (Ghannouch area) and their relationship with other Mediterraneans. Eur J Med Genet; 49(1):43-56

Tables

Please leave html that Tables 1-4 are not available with this version.