Molecular Biology Reports (2021) 48:7819–7829
https://doi.org/10.1007/s11033-021-06801-3

ORIGINAL ARTICLE

A proposed HLA-B*27 screening method for ankylosing spondylitis detection based on tag-single nucleotide polymorphisms: a preliminary study

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Received: 26 July 2021 / Accepted: 1 October 2021 / Published online: 13 October 2021
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Abstract

Background Ankylosing spondylitis (AS) is a type of inflammatory arthritis that affects primarily the spine. There is a strong association of the HLA-B*27 allele with AS pathogenesis, but recent studies have demonstrated the participation of *ERAP1* gene in the genetic susceptibility. The aim of this study was to determine whether HLA-B tag-single nucleotide polymorphisms (SNPs) and *ERAP1*-related genetic variations associated with AS have equal or similarly performance in patients´ screening compared to HLA-B*27 standard genotyping in Mexican population.

Methods and Results Genomic DNA from patients with AS and population-based controls from Mexico City was analyzed for five single nucleotide polymorphisms (SNPs): rs4349859, rs13202464, rs116488202, tagging HLA-B*27; and rs30187 and rs27044 in *ERAP1* gene. TaqMan genotype assay method was used for SNPs genotyping. We found a significant association between AS and the heterozygote genotypes and minor alleles of the HLA-B*27 tag-SNPs, as well as for their haplotypes. With respect to *ERAP1* polymorphisms, no significant associations were observed ($p > 0.05$). The sensitivity and specificity analysis showed values of 0.96 and 1.00 for the rs4349859 SNP, and 0.96 and 0.94 for the rs116488202 SNP, respectively, in detecting HLA-B*27 compared to the B27 test as the gold standard.

Conclusions HLA-B*27 tag-SNPs are associated with AS susceptibility; furthermore, the rs4349859 SNP by its own have an outstanding performance in detecting HLA-B*27 and therefore can be proposed as screening marker in the identification of HLA-B*27 in our population.

Keywords Ankylosing spondylitis · HLA-B*27 · Single nucleotide polymorphisms

Introduction

Spondyloarthropathies (SpA) are interrelated diseases that share common epidemiological, pathogenic and clinical characteristics; they include ankylosing spondylitis (AS), psoriatic arthritis (PsA), arthritis associated with inflammatory bowel disease, and reactive arthritis [1]. SpA are characterized by chronic inflammation, affecting mainly the spine and sacroiliac joints. Furthermore, joint and back pain, enthesitis, intestinal inflammation and acute anterior uveitis are common [2, 3]. At the vertebral level, the inflammatory process erodes the fibrocartilage, the hyaline cartilage, and the bone, causing chondral ossification, and fibrous ankylosis (fusion) of the joints involved, which leads to the characteristic "bamboo spine". Additionally, bony protrusions, called syndesmophytes are also gradually generated [4].

AS prevalence as reported in the United States and Europe ranges from 0.4 to 1.3%; in China, it is reported to be 0.29%, and in Mexico, 0.09%. It affects men more than women, with a ratio of 5:1, and the first symptoms usually show between 15 and 40 years of age [5–8]. AS has heritability patterns of up to 97%, but those patterns take on greater relevance when accompanied by clinical manifestations, such as radiographic severity, age under 45 at the

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onset of symptoms, persistent pain for over three months, and elevated acute-phase proteins in serum [9, 10].

AS pathogenesis involves interactions of environmental and genetic factors, where the latter are well represented by their strong association with the HLA-B*27 allele [11–14], as it has been observed that between 80 and 90% of AS patients are positive for HLA-B*27 [10], compared to the low prevalence in the open population, which is approximately 1.3% [15]. Nevertheless, being a carrier of HLA-B*27 is not a conditioning factor to develop this disease since there are AS patients that are negative for B27. Similarly, there are other non-HLA molecules that take part in antigen processing, such as the endoplasmic reticulum aminopeptidases 1 and 2 (ERAP-1 and ERAP-2), which affect significantly the susceptibility of AS [16]. Even though single-nucleotide polymorphisms (SNPs) near HLA-B*27 locus and within *ERAP1* genes associated with AS development have been identified [17–19], gene–gene or SNP-SNP interactions, better known as epistasis, may explain more accurately the genetic origin or severity of AS [20].

The standard technique for HLA-B*27 typing is by PCR-SSP, where several primers are used in a single sample. Despite this technique is highly used it can be optimized by using AS associated SNPs, mainly those that tag HLA-B*27 locus.

Based on the foregoing, the objective of this study was to analyze the association between HLA-B*27 tag-SNPs and *ERAP1*-related polymorphisms in AS patients, and determine their performance in HLA-B*27 screening compared to the standard genotyping method in Mexican population.

Materials and methods

Study population

One hundred and five well-characterized men and women of Mexican origin older than 18 years old and unrelated were included in this pilot study. Forty of them were axial AS outpatients of the Rheumatology Service of the Instituto Nacional de Rehabilitación “Luis Guillermo Ibarra Ibarra” (INR-LGII). AS diagnosis was based on the New York Modified Criteria [21, 22]. Patients with other autoimmune diseases were excluded.

The control group consisted of 65 healthy individuals, men and women over 18 years of age from the same geographic region as the patients, with no family history of AS or symptoms suggestive of the disease. The selected individuals were blood bank donors, people who accompanied patients, or staff of the Human Resources and Human Communications Departments of the INR-LGII.

DNA extraction and genotyping

A sample of peripheral blood of all participants was obtained by venipuncture in tube with EDTA-K2. From the total blood, genomic DNA was isolated using a commercial kit (QIAmp 250 DNA Blood Kit, Qiagen, Hilden, Germany) following the manufacturer’s instructions. The obtained DNA was adjusted to 50 ng/μl with molecular grade water through spectrophotometry using a Nanodrop 2000 (Thermo Scientific) and maintained at -80 °C until it was analyzed.

Three SNPs tagging HLA-B*27 (rs4349859, rs13202464, and rs116488202), and two SNPs from *ERAP1* gene (rs30187 and rs27044) were selected according to: (a) the information of each SNP was obtained from the public databases of HapMap (http://www.hapmap.ncbi.nlm.nih.gov/) and the National Center for Biotechnology Information dbSNP (http://www.ncbi.nlm.nih.gov/snp); (b) the selected SNPs had previously been evaluated in AS patients from other populations [18, 19]; and (c) the minor allele frequency (MAF) in the Mexican population had to be > 1% according to phase 3 of the 1000 Genomes Project (Supplementary Table 1). SNPs genotyping was carried out with the allelic discrimination method using TaqMan probes (Applied Biosystems, Foster City, CA, USA). Amplification was performed with 5 μl of TaqMan Universal PCR Master Mix (Applied BioSystems, Warrington, UK), 0.25 μl of TaqMan probe, 0.25 μl of water, and 4.5 μl of genomic DNA. All samples were genotyped using the StepOne Plus Real-Time PCR System (Applied Biosystems), following the manufacturer’s protocol. The allelic discrimination analysis was conducted with the StepOne software v2.3 (Applied BioSystems).

HLA-B*27 typing

HLA-B*27 typing was performed with the HLA-FluoGene B27 commercial kit (Inno-Train, Diagnostik GmbH, Germany). PCR was performed in a Veriti thermal cycler (Applied BioSystems) following the manufacturer’s instructions, and the fluorescence lecture was done with the FluoVista equipment (Inno-Train, Germany) using Fluogene software v1.5.5.

Statistical analysis

The obtained data were expressed as median (interquartile range, IQR) and mean ± standard deviation (SD). The *p*-values were calculated with the Mann–Whitney U test, Student’s *t* test (for continuous variables), or Fisher’s exact test (for categorical variables), when appropriate. Hardy–Weinberg equilibrium (HWE) was calculated using

the χ^2 test for the control group. The genetic and allelic frequency distribution in both study groups was compared with the χ^2 test. The association of the SNPs with AS risk was estimated with logistic regression models adjusted by age and gender. Bonferroni's test was used to correct multiple test errors, and considering the five selected SNPs, an adjusted p value ≤ 0.01 (α/number of loci) was deemed statistically significant.

Linkage disequilibrium (LD) was evaluated between SNPs present in the same chromosome with Haploview software v4.2 [23]. Those SNPs with high LD were taken into account when constructing haplotypes. Additionally, the SNP-SNP epistatic interaction analysis for high and low risk genotypes identification with the five selected SNPs, was determined with the multifactor dimensionality reduction (MDR) method [24, 25], using the MDR software v3.0.2 (freely available at http://sourceforge.net/projects/mdr).

Considering the B27 test as the gold standard, the specificity and sensitivity values for the HLA-B*27 tag-SNPs alleles were calculated with receiver operating characteristic (ROC) curves. The positive predictive value (PPV) and negative predictive value (NPV) were calculated using Bayes' theorem to determine their diagnostic value. The area under the curve (AUC) of each ROC curve was estimated and compared with the gold standard by χ^2 test adjusted by Bonferroni multiple comparisons approach. All statistical analyses were performed with the STATA statistical package v14.0 (StataCorp, Texas, USA), with a significance level of $\alpha = 0.05$. This study meets all criteria contained in the Helsinki Declaration, and obtained the approval of the INR-LGII Ethics and Research Committee (Reg. INR-51/19). All participants signed an informed consent letter.

Table 1 Demographic data of participants and clinical characteristic of AS patients

	AS patiens N = 40	Controls N = 65	p
Age (years)			
Median (IQR)	46 (16.5)	53 (8)	**< 0.01***
Gender (%)			
Male	23 (57.5)	5 (7.69)	**< 0.01****
Female	17 (42.5)	60 (92.3)	
HLA-B*27 (%)			
Negative	4 (10.00)	64 (98.4)	**< 0.01*****
Positive	36 (90.00)	1 (1.54)	
Age at diagnosis (years)			
Mean ± SD	36.4 ± 14.11	NA	
Current medicaments use (%)			
No	5 (12.5)	NA	
Yes	35 (87.5)	NA	
Treatment response (%)			
Not favorable	7 (20.0)	NA	
Favorable	28 (80.0)	NA	
Median (IQR)	7.5 (14)	NA	
CRP mg/dL			
Median (IQR)	2.45 (4.88)	NA	
Leukocytes 10^3/mm^3			
Median (IQR)	7.55 (3.35)	NA	
BASFI	4.9	NA	
BASDAI	3.73	NA	

Data are expressed as mean ± SD

AS ankylosing spondylitis, *IQR* Interquartile range, *ESR* erythrocyte sedimentation rate, *CRP* C-reactive protein, *BASFI* Bath Ankylosing Spondylitis Functional Index, *BASDAI* Bath Ankylosing Spondylitis Disease Activity Index, *NA* Not applicable

*p-values were estimated using Mann–Whitney U test, $\alpha = 0.05$; **p-values were estimated using X^2 test; ***p-values were estimated using Fisher's exact test, $\alpha = 0.05$; Significant p-values are in bold

Results

Characteristics of the study population

In total, 40 AS patients and 65 controls were analyzed. The clinical, demographic, and biochemical characteristics of the two study groups are summarized in Table 1. The age median of cases and controls was 46 years (IQR = 16.5) and 53 years (IQR = 8), respectively ($p < 0.01$); 57.5% of patients were male, and 42.5% were female. With regards to the analyzed B27, 90.0% of patients were positive and 10.0% negative, whereas 98.4% of controls were negative and only 1.54% were positive. Average age of the patients at the time of diagnosis was 36.4 ± 14.11 years, and 77.5% of them had AS symptoms at < 45 years old.

Genetic and allelic frequencies of SNPs studied in AS patients and controls

Table 2 shows the distribution of genotypes and alleles of SNPs in the study groups. The control group was consistent with HWE. Only the heterozygote genotypes and minor alleles of the HLA-B*27 tag-polymorphisms showed a significant association with AS risk (rs13202464, A/G genotype: OR 15.5, 95% CI 4.54–52.7, $p = 1.2 \times 10^{-5}$, G allele: OR 5.87, 95% CI 2.42–14.2, $p = 9.3 \times 10^{-5}$; rs4349859, G/A genotype: OR 572, 95% CI 24.6–13,345, $p = 7.7 \times 10^{-5}$, A allele: OR 167 95% CI 16.2–1717, $p = 1.7 \times 10^{-5}$; and rs116488202, C/T genotype: OR 36.0, 95% CI 11.3–114.8, $p = 1.4 \times 10^{-9}$, T allele: OR 15.0, 95% CI 5.51–40.8, $p = 1.2 \times 10^{-7}$). Likewise, the dominant model of these three polymorphisms showed a significant association with

Table 2 Genetic and allelic frequencies of SNPs studied in AS patients and controls

Gene (SNP rs ID)	Controls (%) N = 65	AS patients (%) N = 40	Adjusted OR[b]	(CI 95%)	*p*
HLA-B*27 (rs13202464)					
A/A	53 (81.5)	7 (15.7)	1.00		
A/G	11 (16.9)	32 (80.0)	15.5	(4.54–52.7)	**1.2×10^{-5}**
G/G	1 (1.54)	1 (2.50)	6.57	(0.31–138.4)	0.23
Inheritance models					
A/G + G/G	12 (18.5)	33 (82.5)	14.5	(4.38–48.2)	**1.2×10^{-5}**
G/G[a]	1 (1.54)	1 (2.50)	1.88	(0.10–36.1)	0.67
Alleles					
A	117 (90.0)	46 (57.5)	1.00		
G	13 (10.0)	34 (42.5)	5.87	(2.42–14.2)	**9.3×10^{-5}**
HWE	0.62	< 0.01			
HLA-B*27 (rs4349859)					
G/G	64 (98.5)	10 (25.0)	1.00		
A/G	1 (1.54)	26 (65.0)	572	(24.6–13,345)	**7.7×10^{-5}**
A/A	0 (0.00)	4 (10.0)	–		–
Inheritance models					
A/G + A/A	1 (1.54)	30 (75.0)	601	(24.5–13,656)	**5.9×10^{-5}**
A/A[a]	0 (0.00)	4 (20.0)	–		–
Alleles					
A	129 (99.2)	46 (57.5)	1.00		
G	1 (0.77)	34 (42.5)	167	(16.2–1717)	**1.7×10^{-5}**
HWE	0.95	0.03			
HLA-B*27 (rs116488202)					
C/C	60 (92.3)	10 (25.0)	1.00		
C/T	5 (7.69)	30 (75.0)	36.0	(11.3–114.8)	**1.4×10^{-9}**
T/T	0 (0.00)	0 (0.00)	–		–
Inheritance models					
C/T + T/T	5 (7.69)	30 (75.0)	32.2	(7.51–137.8)	**2.9×10^{-6}**
T/T[a]	0 (0.00)	0 (0.00)	–		–
Alleles					
C	125 (96.1)	50 (62.5)	1.00		
T	5 (3.85)	30 (37.5)	15.0	(5.51–40.8)	**1.2×10^{-7}**
HWE	0.74	< 0.01			
ERAP1 (rs30187)					
C/C	24 (36.9)	14 (35.0)	1.00		
C/T	31 (47.7)	20 (50.0)	1.65	(0.51–5.28)	0.40
T/T	10 (15.3)	6 (15.0)	2.66	(0.63–11.2)	0.18
Inheritance models					
C/T + T/T	41 (63.1)	26 (65.0)	1.90	(0.64–5.65)	0.25
T/T[a]	10 (15.4)	6 (15.0)	2.00	(0.57–7.05)	0.28
Alleles					
C	79 (60.8)	48 (60.0)	1.00		
T	51 (39.2)	32 (40.0)	1.70	(0.81–3.54)	0.16
HWE	0.99	0.79			
ERAP1 (rs27044)					
C/C	28 (43.1)	16 (40.0)	1.00		
C/G	28 (43.1)	18 (45.0)	1.29	(0.42–4.02)	0.44
G/G	9 (13.8)	6 (15.0)	2.67	(0.65–11.0)	0.17
Inheritance models					
C/G + G/G	37 (56.9)	24 (60.0)	1.60	(0.56–4.55)	0.38

Table 2 (continued)

Gene (SNP rs ID)	Controls (%) N = 65	AS patients (%) N = 40	Adjusted OR[b]	(CI 95%)	*p*
G/G[a]	9 (13.85)	6 (15.0)	2.34	(0.65–8.50)	0.19
Alleles					
C	84 (64.6)	50 (62.5)	1.00		
G	46 (35.6)	30 (37.5)	1.66	(0.79–3.45)	0.18
HWE	0.64	0.80			

If $p < 0.05$, not consistent with HWE in controls; Significant *p*-values are in bold

AS Ankylosing spondylitis, *OR[b]* Odds ratio adjusted by age and gender, *CI* confidence interval, *HWE* Hardy–Weinberg equilibrium

[a]Recessive inheritance model, the reference group is made up of carriers of the homozygous genotype for the most frequent allele and of the heterozygous genotype

AS (OR 14.5, $p = 1.2 \times 10^{-5}$; OR 601, $p = 5.9 \times 10^{-5}$; and OR32.2, $p = 2.9 \times 10^{-6}$, respectively). Regarding the polymorphisms of *ERAP1* gene, none of them showed a significant association with AS risk ($p > 0.05$).

Haplotype and gene–gene interactions: MDR

We detected a high LD between the three polymorphisms located in chromosome 6 near HLA-B gene, being the highest D' between rs4349859, and rs116488202. The two *ERAP1* gene polymorphism also showed high LD with D' of 1 (Supplementary Fig. 1). The carriers of the haplotypes AAC or GAT for rs13202464, rs4349859, and rs116488202, respectively, had an increased risk for AS than the carriers of the AGC or AGT haplotypes (Supplementary Table 2). Since we did not detect significant association between both *ERAP1* gene polymorphisms and AS we did not carry out the analysis by haplotypes.

The comprehensive MDR analysis illustrates that the best SNP-SNP interaction model to predict AS development comes from the interaction between rs4349859, rs13202464, and rs30187, which was the most significant and had the highest test accuracy (Table 3).

As a result of these interactions, it was possible to identify genotypes of low and high risk associated with AS development. The genotype combination that was the most representative and with the highest risk for AS was A/G + G/A + C/T (SNP1, SNP2, SN4, respectively). Additionally, the low-risk genotypes are visualized, of which the most representative combinations are A/A + A/A + C/C; A/A + A/A + C/T; and A/G + A/A + T/T, respectively (Supplementary Fig. 2). The participants were categorized by the genotypes proposed by the MDR as follow: high risk for the carriers of A/G + G/A + C/T; low risk for the carriers of A/A + A/A + C/C, A/A + A/A + C/T, or A/G + A/A + T/T genotypes; and neutral risk for any other genotype combination. The neutral risk category of the risk genotypes proposed by MDR showed a significant association with AS compared to the low risk genotypes carriers. Additionally, none of the controls subjects carried the risk genotype (Supplementary Table 2).

Performance of associated polymorphisms for B27 detection

When comparing the performance of rs13202464, rs4349859, and rs116488202 polymorphisms with the test of B27 as gold standard, the sensitivity and specificity of each polymorphism were 0.96 and 0.80 (AUC = 0.88, $p < 0.05$), 0.96 and 1.0 (AUC = 0.98, $p > 0.05$), and 0.96

Table 3 Results of MDR analysis

Number of risk factors	Interaction models	Testing accuracy	CVC	*p*
1	SNP2	0.8673	10/10	0.0113
2	SNP1, SNP2	0.8596	10/10	0.0139
3	SNP1, SNP2, SNP4	0.8692[a]	10/10	< 0.001
4	SNP1, SNP2, SNP3, SNP4	0.8615	10/10	0.0172
5	SNP1, SNP2, SNP3, SNP4, SNP5	0.8462	10/10	0.0242

The model with the maximum testing accuracy and maximum CVC was considered as the best model

CVC cross-validation consistency, *SNP1* HLA-B*27 rs4349859, *SNP2* HLA-B*27 rs13202464, *SNP3* HLA-B*27 rs116488202, *SNP4 ERAP1* rs30187, *SNP5 ERAP1* rs27044

[a]The best interaction model in MDR analysis

and 0.94 (AUC = 0.96, $p > 0.05$), respectively (Table 4 and Fig. 1). The PPV and NPV, which measure the effectiveness of a diagnostic test, for each polymorphism are showed in Table 4. All three HLA-B tag-SNPs had excellent NPV for B27 detection, nevertheless only the rs4349859 polymorphism had high PPV.

When performing the diagnostic test for the joint effect of the evaluated SNPs we found that the tag-polymorphisms haplotypes had a low sensitivity but high specificity for detecting HLA-B*27 (54.69% and 100%, respectively). As for the MDR proposed risk genotypes the sensitivity and specificity, taking into account neutral or high-risk genotypes as cut off value were 97.06% and 80.82% respectively. However, the predicted values were higher in the tag-SNPs haplotypes (PPV = 100%, NPV = 99.96%) than the MDR proposed risk genotypes (PPV = 0.45%, NPV = 99.99%) (Table 5).

Interestingly, when comparing the HLA-B*27 screening performance obtained from the SNPs individually and the joint effects evaluated by haplotypes and MDR proposed genotypes, we did not observe a better HLA-B*27 screening performance in the AUC from the ROC curve analysis than the effect of rs4349859 on its own (Fig. 2).

Table 4 Sensitivity and specificity analysis by ROC curve using B27 as gold standard

SNP	Genotypes n (%)			Sensitivity (%)	Specificity (%)	AUC**	PPV (%)	NPV (%)
rs13202464	A/A	A/G	G/G	0.96	0.80	0.88	0.0045	1.00
B27+	1 (3.13)	31 (96.8)	0 (0.0)					
B27-	59 (80.8)	12 (16.4)	2 (2.74)					
rs4349859	G/G	A/G	A/A	0.96	1.00	0.98*	1.00	1.00
B27+	1 (3.13)	27 (84.3)	4 (12.5)					
B27-	73 (100)	0 (0.0)	0 (0.0)					
rs116488202	C/C	C/T	T/T	0.96	0.94	0.96*	0.015	1.00
B27+	1 (3.13)	31 (96.9)	0 (0.0)					
B27-	69 (94.5)	4 (5.48)	0 (0.0)					

AUC area under the ROC curve, *PPV* positive predictive value, *NPV* negative predictive value

*$p > 0.05$, AUC** 0.90–1.00 = excellent, 0.80–0.90 = good, 0.70–0.80 = fair, 0.60–0.70 = poor, 0.50–0.60 = fail

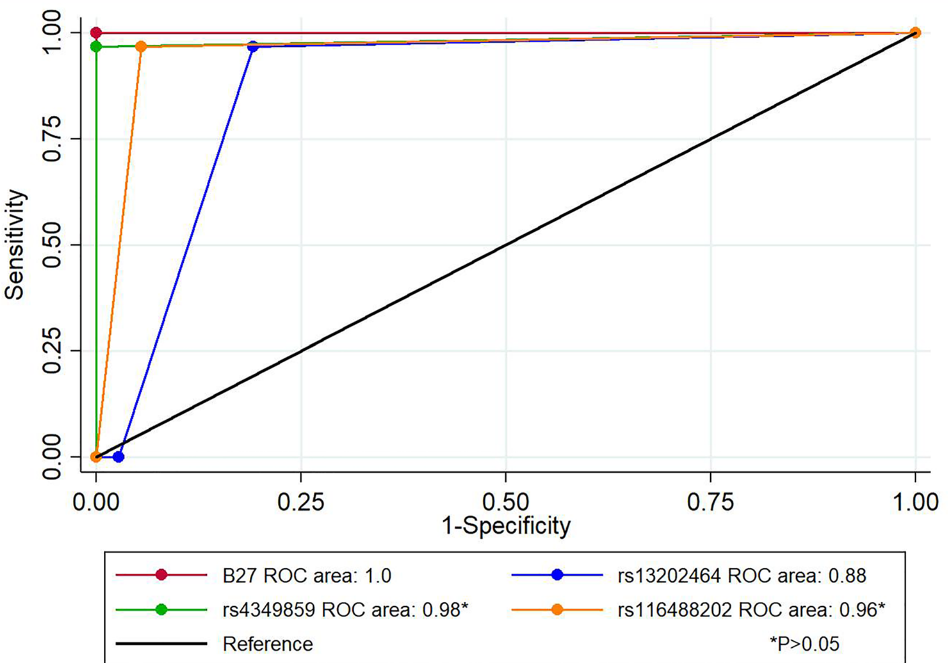


Fig. 1 ROC curve. The sensitivity and specificity of the evaluated polymorphisms were calculated considering to B27 as reference. *p value obtained from χ^2 test adjusted by Bonferroni multiple comparison method

Table 5 Sensitivity and specificity analysis by ROC curve using B27 as gold standard for the joint effect of studied-SNPs

SNPs Joint Effect	B27-	B27+	Sensitivity (%)	Specificity (%)	AUC**	PPV (%)	NPV (%)
AGC + AGT	130 (100.0)	29 (45.31)	54.6	100	0.77	100	99.96
AAC + GAT	0 (0.00)	35 (54.69)					
Low risk	59 (80.8)	1 (2.94)	95.2	80.8	0.96	0.45	99.99
Neutral risk	14 (19.1)	20 (58.8)					
High risk	0 (0.00)	13 (38.2)					

Haplotype analysis was carried out by alleles, so the numbers of B27 + and - represents the number of chromosomes

AUC area under the ROC curve, *PPV* positive predictive value, *NPV* negative predictive value

AUC** 0.90–1.00 = excellent, 0.80–0.90 = good, 0.70–0.80 = fair, 0.60–0.70 = poor, 0.50–0.60 = fail

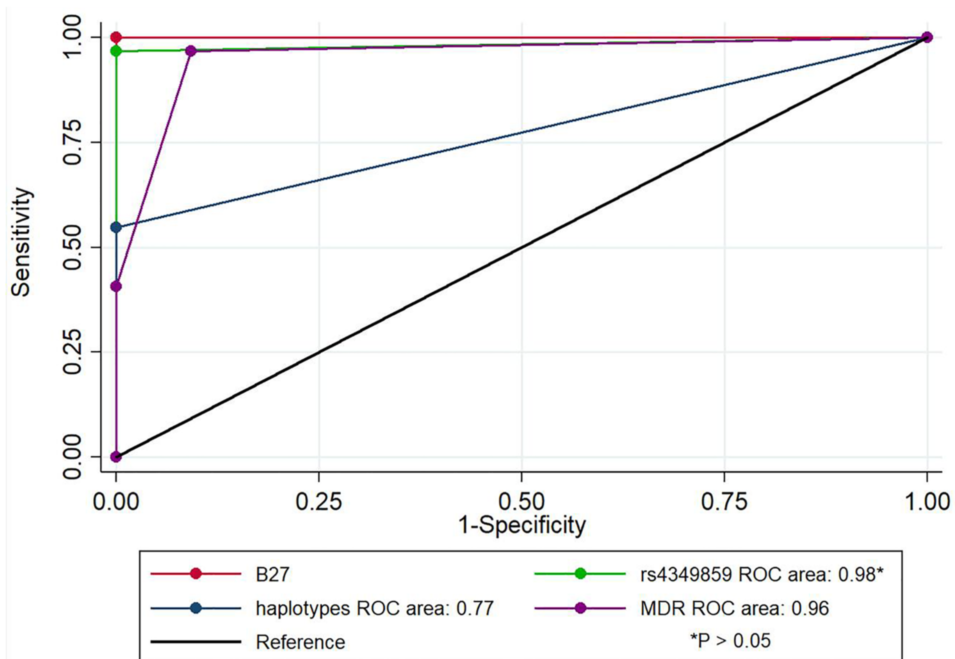


Fig. 2 ROC curve for rs4349859, haplotypes and MDR proposed risk genotypes compared to the gold-standard method for B27 genotyping. **p* value obtained from χ^2 test adjusted by Bonferroni multiple comparison method

Discussion

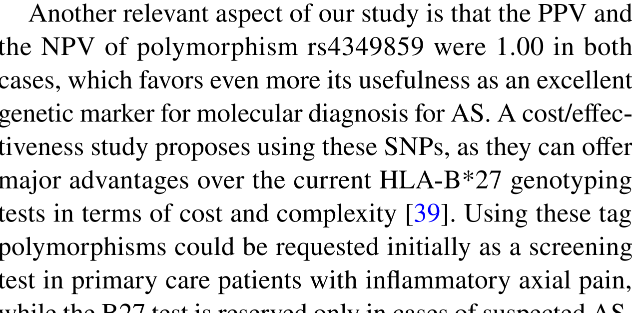
In the early '70 s, the association of B27 antigen with AS patients was identified for the first time [26, 27]. Even though several factors associated with AS development have been described, the pathogenic role of HLA-B*27 allele remains as the most representative in AS genetic susceptibility. Later on, derived from the GWAS Wellcome Trust Case Control Consortium (WTCCC) [19] and IGAS [28], it was possible to identify new non-HLA loci involved in AS development, with *ERAP1* gene standing out as the most important, followed by HLA-B*27.

In the present pilot study, we evaluated both the association between three polymorphisms of HLA-B*27 allele and two of *ERAP1* gene in the Mexican population, as well as their usefulness as potential genetic markers for AS. The results revealed a strong risk association of the rs13202464, rs4349859, and rs116488202 polymorphisms in AS patients. In contrast, rs30187 and rs27044 polymorphisms of the *ERAP1* gene did not reach a significant association, which coincides with that reported by Su et al., in Chinese population [29]. The association of *ERPA1* polymorphisms with AS is controversial. Interestingly, it has been described that *ERAP1* is only associated in B27 positive patients [16, 30], but the studies conducted have been limited mainly to patients with European origins [19, 31, 32]. Nonetheless, Hemmatzadeh et al., evaluated three polymorphisms of *ERAP1* in the Iranian population, and they observed that only the rs2703 showed a significant association in B27 positive patients [33]. This suggests that, besides other variants of *ERAP1*, genetic factors in the population structure might play a key role in AS susceptibility. Unfortunately, the statistical power for the association magnitude of *ERAP1* polymorphisms with AS was insufficient for us to make a conclusion. We cannot

deny that these SNPs could be associated with AS development in Mexicans.

Both B27 and ERAP-1 have a central role in antigen presentation. B27 is regulated by the HLA system, which is located in the short arm of chromosome 6 and its biological function is to encode membrane glycoproteins that act as mediators in antigenic peptide presentation to T lymphocytes to modulate immune response [34]. To date, 343 subtypes of allele HLA-B*27 (Release 3.42.0, October 2020, https://www.ebi.ac.uk/ipd/imgt/hla/allele.html) have been detected at the DNA sequence level, but many of those variants have no effect in protein synthesis. There are approximately 167 subtypes of HLA-B*27 whose products are different from one another in one or more amino acids, and only a few have a pathogenic role in AS [35, 36]. Of these suballeles, HLA-B*2701, HLA-B*2702, HLA-B*2704, HLA-B*2705, and HLA-B*2707 have been associated with AS; as yet there are few data on whether the other suballeles are associated with altered disease susceptibility [37]. Meanwhile, ERAP-1 is encoded by the *ERAP1* gene (also known as *ARTS1*), located in the long arm of chromosome 5, and it is a zinc-dependent metalloproteinase with two critical roles in the immune system. Firstly, it acts as a 'molecular rule' within the endoplasmic reticulum, cutting 9–17 amino acid long peptides that have been partially processed by the proteosome until they are 9 amino acid long, which is the optimal length for binding and presenting in class I HLA molecules. Secondly, in vitro studies reveal that ERAP-1 excises several cytokine receptors on the cell surface, including IL-6R, IL-1R2, and TNFR, and the excision of these receptors reduces their capability to transmit chemical signals to the cell, promoting the inflammatory process of AS [16, 17].

While the B27 test is considered the gold standard for the molecular diagnosis of AS, a search for new biomarkers with sensitivity and specificity values similar to B27 has recently begun in order to have less expensive options. Our results show that polymorphisms rs4349859 and rs116488202 have high sensitivity and specificity for HLA-B*27, whereas polymorphism rs13202464 has high sensitivity but low specificity, which matches the report by Akar et al., in a Turkish population. In that study, it was reported that polymorphism rs4349859 has a great capacity to tag HLA-B*27 in patients from England and Australians of European origin [18]. It is worth mentioning that it has been reported that the rs4349859 polymorphism has been found to tag the major Caucasian AS-associated suballeles HLAB*2702, HLA-B*2705 and HLA-B*2708 with 98% sensitivity and 99% specificity. However, this SNP does not tag the African AS-associated suballele HLA-B*2703, nor the Asian suballeles HLA-B*2704, HLA B*2706, and HLA-B*2707. More recently another tagging SNP, rs116488202, has been identified that may accurately tag both Caucasian and Asian HLA-B27 suballeles [38].



Another relevant aspect of our study is that the PPV and the NPV of polymorphism rs4349859 were 1.00 in both cases, which favors even more its usefulness as an excellent genetic marker for molecular diagnosis for AS. A cost/effectiveness study proposes using these SNPs, as they can offer major advantages over the current HLA-B*27 genotyping tests in terms of cost and complexity [39]. Using these tag polymorphisms could be requested initially as a screening test in primary care patients with inflammatory axial pain, while the B27 test is reserved only in cases of suspected AS.

The interaction between HLA-B*27 and *ERAP1* in positive B27 individuals suggests that the presence of polymorphisms within *ERAP1* gene reduces the presentation of multiple antigens mediated by HLA-B*27, affecting AS pathogenesis [40, 41]. Using the MDR method we identified a strong epistatic interaction between HLA-B*27 and *ERAP1* SNPs, which points to a multi-loci effect, i.e., both genes need each other to have an effect on AS development. Since the polymorphisms of HLA-B*27 allele and *ERAP1* gene are located in different chromosomes, their interaction is epistatic, i.e., the expression of one of them can cancel or "conceal" the expression of the other. *ERAP1* is thought to interact directly with HLA-B*27, in contrast with other AS susceptibility loci (e.g., IL23R, IL12B), regardless whether B27 is positive or negative [16, 42]. Finally, the epistasis analysis allowed us to detect high and low risk genotypes, which may constitute an excellent tool to identify individuals with a genetic susceptibility to develop AS. Nevertheless, the screening performance of the interaction model did not surpass the performance observed for the HLA-B tag-SNP rs4349859 on its own. These results support the notion that using this only SNP as screening test could be enough in a first AS screening procedure.

Notwithstanding the foregoing results, our study has certain limitations. Firstly, the size of the analyzed population is very small and samples included in the study came from only one affiliated hospital, so it would be advisable to replicate our findings in a bigger sample and multi-centre studies are needed in future studies, particularly for the association of *ERAP1* polymorphisms with AS. Secondly, other populations need to be evaluated to corroborate our results and, if possible, extrapolate these findings to others populations with different genetic background. Thirdly, the selected genes have polymorphisms that were not taken into account, and other genes involved in genetic susceptibility were not considered either or their impact on AS is still unknown.

Conclusions

In this preliminary study the rs4349859 and rs116488202 polymorphisms (particularly the former) tag HLA-B*27 with high specificity and sensitivity in the Mexican population,

which suggests they may be used as potential genetic markers in screening tests, including cases of suspected AS. Further studies are needed to validate our findings.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11033-021-06801-3.

Acknowledgements This work was supported by federal resources from the INR-LGII. The authors we thank to Adrián García Soto and Dulce Reyes from Diagnomol laboratory for their facilities in typing HLA-B*27. We thank all the individuals who participated in this study.

Author contribution JFT, YZC and GAMN conceived the study, sample processing, interpretation of results, writing first draft of manuscript and final manuscript approval. JFT and GAMN performed the statistical analysis. NATO, KMF, REM, FMD, SMJB, CLP, MVF, RSS and AHB recruited subjects, applied the questionnaire, and reviewed the manuscript draft. All authors reviewed and approved the final version of the manuscript.

Funding This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

Consent to participate All the authors listed have approved the manuscript that is enclosed.

Consent for publication The manuscript is approved by all authors for publication.

Ethical approval This study was approved by the Ethics Committee of the INR-LGII (No. 19/51). All procedures performed in this study involving human participants were in accordance with the ethical standards of the INRLGII-Institutional Research and Ethical Committee (CONBIOETICA-09-CEI-031-20171207) and with the Helsinki Declaration (1964). Informed consent was obtained from all individual participants included in the study.

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