Is Ankylosing Spondylitis a single disease? A clinical and genetic perspective

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Research article

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Abstract

Background:

The aim of the present study was to evaluate **whether** ankylosing spondylitis (AS) and non-radiographic axial spondyloartritis (nr-axSpA) are subsets of a single disease from a genetic perspective.

Methods:

We analyzed from a clinical and genetic perspective 62 patients from the University Hospital of Basurto (Bilbao, Spain) diagnosed with axial spondyloarthritis. Forty three SNPs previously associated with spondyloarthritis (SpA) were selected. The DNA samples were genotyped through SNP Type Assay, using the BioMark HD platform of Fluidigm.

Results:

Regarding clinical characteristic we found statistically significant differences between the patients with AS and nr-axSpA in the age at diagnosis, the disease duration, the presence of syndesmophytes and the BASMI index. In relation to genetic markers, we only found statistically significant differences in *HLA-B27* allele frequency between the two groups. Regarding the frequencies of the SNPs, no statistically significant differences were found between the two groups. Despite the high genetic heterogeneity observed among the patients, it is worth highlighting that some of the most important risk SNPs associated with AS, located in genes (*ERAP1*, *ERAP2*, *IL-23R*, *GPR25*) and intergenic region (2p15), appeared at high frequencies in all the patients.

Conclusion:

We have observed that AS and nr-axSpA have a common genetic background associated with the pathogenic development of these diseases; therefore, we suggest that the two entities constitute two different expressions of the same disease. Among the genetic factors, the present study shows the importance of genes involved in the pathogenesis of AS, such as *HLA-B27*, *ERAP1*, *ERAP2*, *IL-23R*, *GPR25* and intergenic region 2p15, whose role may influence the onset, development and severity of the disease. However, the pathogenesis of SpA is very complex, indicating the involvement of environmental factors (smoking and obesity), in the triggering of the disease, so that patients with different genotypes would have the same pathogenic phenotype.

Background

Spondyloarthritis (SpA) are a group of rheumatic, inflammatory diseases that share a series of characteristics, such as affected axial skeleton [spine and sacroiliac joints (SIJs)], and peripheral and extra-articular manifestations [1]. SpA traditionally include ankylosing spondylitis (AS), psoriatic arthritis (PsA), reactive arthritis, undifferentiated spondyloarthritis, juvenile spondyloarthritis and inflammatory bowel disease-associated spondyloarthritis (IBD) (Crohn´s disease (CD) and ulcerative colitis) [2].

The classification criteria for the diagnosis of SpA developed by the Assessment of Spondyloarthritis International Society (ASAS) **(Table 1)**, were created and published in 2009 [3,4], due to the diagnostic limitations of the previous criteria [modified New York criteria [5] and European Spondyloarthropathy Study Group criteria [6]]. Based on the ASAS criteria, SpA can be classified as axial (axSpA) or peripheral [3,4]. Within axSpA, there are patients with radiographic changes in SIJs (AS) and other patients without radiographic manifestations (nr-axSpA).

The ASAS classification criteria (image branch) use magnetic resonance imaging (MRI) to diagnose nr-axSpA by detecting the active inflammation (bone marrow edema). However, in the case of AS, conventional x-ray imaging shows advanced and irreversible changes of the sacroiliitis [7], although several years are necessary for these changes to take place, which makes the diagnosis delay years after the beginning of the symptoms [7], thus decreasing the quality of life of the patient.

Sacroiliitis on Imaging + ≥1 SpA Feature OR *HLA-B27* + ≥2 Other SpA Features	
SpA Features	**Sacroiliitis on Imaging**
Inflammatory back pain Arthritis Enthesitis (heel) Uveitis Dactylitis Psoriasis Crohn´s disease/ulcerative colitis Good response to NSAIDs Family history for SpA *HLA-B27*(+) Elevated CRP	Active (acute) inflammation on MRI highly suggestive of sacroiliitis associated with SpA OR Definitive radiographic sacroiliitis according to modified New York criteria

Table 1. The ASAS classification criteria for axial spondyloarthritis in patients with back pain 3 months or more and age at onset younger than 45 years [3,4]. ASAS, Assessment of Spondyloarthritis International Society; SpA, spondyloarthritis; NSAIDs, non-steroidal anti-inflammatory drug; *HLA-B27*, human leukocyte antigen-B27; CRP, C-reactive protein, MRI, magnetic resonance imaging.

There is current debate on whether nr-axSpA is a different form of AS [8,9], an early form of AS [10,11], or whether both are two expressions of the same disease [12,13]. Some patients with nr-axSpA will develop AS after years of disease [14-17], which occurs in 10% of patients with over 2 years of follow-up, and in over 20% of patients after 2 years with high levels of C-reactive protein (CRP) or an active inflammation detected by MRI [15]. However, other patients with nr-axSpA will suffer the disease for decades, and probably for life, without any evidence of radiographic damage [18], whereas remission will take place in others, either spontaneously or through the administration of drugs.

Patients with AS and nr-axSpA share some clinical characteristics, such as peripheral manifestations (arthritis, enthesitis, dactylitis) and extra-articular manifestations [uveitis, IBD, psoriasis (Ps)] [10-12], with uveitis being slightly more prevalent in AS [19]. Different researchers believe that intestinal dysbiosis plays a fundamental role in the genesis of SpA [20]; in fact, SpA have been associated with IBD, since approximately 5-10% of patients with SpA develop IBD, and approximately 70% may have a subclinical intestinal disease [21]. Furthermore, common genes and loci have been identified for these two diseases [22], as well as the influence of specific alterations in the composition of the gut microbiota with several immune-mediated disorders [23]. Moreover, 10% of patients with AS suffer from Ps, whereas in the case of Ps, 7-42% of patients develop SpA-type axial affectation [24].

Other common characteristics among patients with AS and nr-axSpA are related to the clinical activity, damage, functional deterioration, quality of life [10,12,15,25], similar response to anti-TNF treatment and similar average age in the appearance of symptoms, with small differences in the duration of the disease.

Regarding the differences between AS and nr-axSpA, we can highlight the presence of structural changes in the spine and SIJs, which limit the mobility of patients with AS, whose functionality is influenced by inflammation and the formation of new bone material [26]. Furthermore, several authors have reported a greater number of inflammatory lesions and high levels of CRP in patients with AS [27]. This could be due to the fact that patients with axSpA and high levels of CRP or positive MRI progress more rapidly to radiographic sacroiliitis and, therefore, there may be a larger percentage of patients with radiographic progression if there are signs of inflammation (CRP and MRI) [28]. It has been described that smoking increases the pace and severity of the progression to the radiographic form of SpA [29]. With respect to gender, there is a larger proportion of women among the population of patients with nr-axSpA and a larger percentage of men among patients with AS [10,16,30]. In studies with Asian cohorts, the prevalence of nr-axSpA was twice as high in women with respect to men [31,32], whereas in European and North American cohorts the prevalence of nr-axSpA was similar in both gender [8,11,12]. Therefore, basal radiographic damage, high levels of CRP, smoking and being male are factors that could predict the future radiographic damage in patients with early SpA [33].

The etiopathogenesis of SpA results from the complex interaction between genetic and environmental factors [34]. Although some studies report that genetic factors have a great influence on the susceptibility to develop AS (up to 80-90% in studies with relatives and twins [34]), this type of disease is very complex from the genetic perspective. It has been suggested that allele *HLA-B27* contributes to 20-25% of the genetic inheritance of this disease [35], and the single nucleotide polymorphisms (SNPs) found in genome-wide association studies (GWASs) seem to contribute to 3-7% [36]. These data suggest that there may still be many unknown genetic variants that are part of the background of the disease.

Among the SpA, AS has the strongest association with allele *HLA-B27* [37,38], since this allele has been found in almost 90% of patients with AS [38]. However, in the general population, only 5% of *HLA-B27*(+) individuals will eventually develop this disease, with the peculiarity that homozygous for this allele have a

greater risk of suffering from AS compared to heterozygous [39], although not all subtypes of *HLA-B27* have a predisposition for the disease [40]. Despite the reported association between *HLA-B27* and susceptibility to AS, it is currently unknown how *HLA-B27* contributes to the development of AS [41]. Different studies have stated that *HLA-B27* does not play a direct role in the formation of new bone and cartilage [42,43], and that it is indirectly involved in the inflammatory process [43]. It has been reported that *HLA-B27*(+) patients have a younger disease onset age and a shorter delay in the diagnosis than *HLA-B27*(-) patients [30]. Furthermore, it has been suggested that other alleles of the *HLA-B* system increase or decrease the susceptibility to develop SpA, such as alleles *HLA-B07*, *HLA-B13*, *HLA-B40*, *HLA-B47*, *HLA-B51* or *HLA-B57* among others [44,45]. In recent years, thanks to GWAS, alleles of different genes, both inside and outside of the Major Histocompatibility Complex (MHC), have been associated with the susceptibility to develop AS [34,36,46-49].

Some studies indicate that the prevalence of *HLA-B27* is similar in patients with nr-axSpA and in those with AS [8,10,11,27,50-52]. However, others indicate that patients with AS have a greater frequency of *HLA-B27* [34,36,46-49].

Considering the hypothesis that AS and nr-axSpA are subsets of a single disease with common clinical characteristics and a similar disease burden, the genetic background could be expected to be similar for the two entities. The aim of the present study was to evaluate this hypothesis, through the analysis of a set of risk SNPs localised in genes involved in the pathogenesis of AS, Ps, PsA and IBD in a sample of patients with axSpA.

Methods

Patients

DNA was obtained from blood samples of 62 patients of the University Hospital of Basurto (Bilbao, Basque Country, Spain). These samples were supplied through the Basque Biobank for Research (Biobanko, Bioef), along with the informed consent of the patients, following the protocols of the Drug Research Ethics Committee of Euskadi (CEImE, PI2017141).

This study included patients over 18 years of age who suffered from inflammatory back pain and met the New York modified criteria for AS or the ASAS classification criteria for axSpA. Moreover, clinical, demographic and laboratory data were also included (CRP, ESR, *HLA-B27*). The functional state, the activity of the disease and the spinal and pelvic mobility were evaluated through Bath Ankylosing Spondylitis Functional Index (BASFI), Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) and Bath Ankylosing Spondylitis Metrology Index (BASMI), respectively.

Pelvic plain radiography was evaluated by three rheumatologists and the existence of sacroiliitis, defined according to the New York modified criteria [5], was decided by consensus with the positive opinion of at least 2 of the 3 rheumatologists. The patients fulfilling criteria with radiographic sacroiliitis were

diagnosed with AS, whereas those without radiographic criteria, were diagnosed with nr-AxSpA by MRI, according to ASAS image branch criteria [3,4].

Selection of SNPs associated with rheumatic diseases

For the selection of SNPs, a search was carried out in different bibliographic databases: 1) PubMed (*http://www.ncbi.nlm.nih.gov/pubmed/*), 2) *dbSNP* (*http://www.ncbi.nlm.nih.gov/snp/*) of *NCBI* (*National Center for Biotechnology Information*, USA), and 3) databases that gather the results obtained in different GWAS analyses (*GWAS Catalog*, *GWAS Central*, *Ensembl Genome Browser*), which identify those allele variations of SNPs associated with the susceptibility to developing a disease.

In this study, we selected those SNPs that had been previously associated with the diagnosis, prognosis and treatment of AS, Ps, PsA and IBD **(Supplementary table)**. These SNPs are located in genes that are involved in different routes and mechanisms that could partially explain the pathogenesis of the disease.

For the selection of the SNPs, the following criteria were applied: (1) GWAS described in Caucasian populations, and (2) the p_{value} of the association between the SNP and the disease had to be lower than $5*10^{-6}$. Moreover, the SNPs that were in linkage disequilibrium ($r^2 > 0.8$) were excluded from the study.

Genotyping

The DNA samples were genotyped through SNP Type Assay, using the BioMark HD platform of Fluidigm (96.96 Dynamic Array^{TM} IFC, Fluidigm, South San Francisco, CA, USA). The genotyping was carried out at the Sequencing and Genotyping Department of the SGIker General Genomic Services of the University of the Basque Country (UPV/EHU, Bizkaia, Spain). All the SNPs that did not obtain a call rate greater than 0.90 were discarded from the analysis. The genotyping of *HLA-B27* was conducted using tagSNP rs11688202 in the mentioned platform. The sensitivity and specificity of this SNP is >98% for the typing of *HLA-B27* [49]. The rest of the alleles of the *HLA-B* gene were analysed through sequencing, following the methodology proposed by Laza et al., 2016 [56].

Statistical analysis

The continuous variables, expressed as mean and standard deviation, were compared using the Student's T-test, and the results were shown as mean and standard deviation. Regarding the categorical variables, a statistically significant $p_{value}<0.05$ was considered for the clinical and laboratory variables, as well as for the allele and genotype frequencies, which were compared using the Fisher's exact test (applying the Bonferroni correction for multiple comparisons). These analyses were carried out using SPSS Statistics software v25 [57]. The Hardy-Weinberg equilibrium test for the frequencies of the SNPs was conducted using the HardyWeinberg package of RStudio [58,59], considering that there was no equilibrium in those with $p_{value}<0.05$ (applying the Bonferroni correction for multiple comparisons). To carry out the Principal Component Analysis (PCA) and Heatmap, we used the FactoMineR [60] and Pheatmap [61] packages of RStudio [59], respectively.

Results

In the present study, 62 patients diagnosed with axSpA were analysed, all of whom had a history of inflammatory back pain. Of these, 49 patients (79%) met the radiographic criteria being diagnosed with AS, and 13 (21%) were diagnosed with nr-axSpA using MRI. Table 2 shows the demographic and clinical characteristics and laboratory parameters of the patients of both groups. The patients of the AS group showed a higher inclusion age and a significantly longer duration of the disease with respect to those of the nr-axSpA group (p_{value}<0.05), although the symptoms onset age was lower, the diagnostic delay was shorter and the diagnosis age was significantly lower (p_{value}<0.05). In the group of patients with AS there was a predominant proportion of males (77.55% males *vs* 22.45% females), whereas in the nr-axSpA group the proportions of males and females were similar (53.85% *vs* 46.15%). The prevalence of smokers (including ex-smokers) was larger in the AS group compared to the nr-axSpA group **(Table 2)**. The presence of syndesmophytes in the spine was statistically higher in the AS group (p_{value}<0.05), confirmed by the BASMI index (p_{value}<0.05), which measures the mobility of the spine and SIJs **(Table 2)**. In the present study, no statistically significant differences were found for peripheral and extra-articular manifestations, although dactylitis, peripheral arthritis and uveitis were mostly diagnosed in the AS group **(Table 2)**.

Regarding the laboratory variables analysed in this study, the levels of CRP were higher among the patients with AS. It is worth highlighting the significantly higher prevalence of allele *HLA-B27* in individuals with AS compared to those with nr-axSpA (87.8% *vs* 38.5%) **(Table 2)**. In the patients who did not have allele *HLA-B27* (N=14), the sequencing revealed the presence of alleles *HLA-B40* (N=5), *HLA-B55* (N=4), *HLA-B56* (N=2), *HLA-B07* (N=1), *HLA-B39* (N=1) and *HLA-B47* (N=1), and no statistically significant differences were found between AS and nr-axSpA patients, since 4 of these alleles were detected in patients with AS [*HLA-B40* (N=3), *HLA-B55* (N=2), *HLA-B56* (N=1), *HLA-B07* (N=1)), and 5 in those with nr-axSpA (*HLA-B40* (N=2), *HLA-B55* (N=2), *HLA-B56* (N=1), *HLA-B39* (N=1), *HLA-B47* (N=1)].

Characteristic	AS (N = 49)	Nr-axSpA (N=13)	p_{value}
Current age, mean ± S.D. (years)	51.3 ± 13.5	47.5 ± 12.2	0.360
Male gender (N, %)	38 (77.6%)	7 (53.9%)	0.176
Female gender (N, %)	11 (22.5%)	6 (46.2%)	0.176
Family history (N, %)	15 (30.6%)	5 (38.5%)	0.838
Inclusion age mean ± S.D. (years)	49.7 ± 13.4	45.9 ± 12.0	0.355
Age at diagnosis mean ± S.D. (years)	33.3 ± 13.0	42.2 ± 13.4	0.039*
Age at symptom onset mean ± S.D. (years)	28.6 ± 12.5	32.7 ± 9.9	0.276
Disease duration mean ± S.D. (years)	17.9 ± 13.3	4.4 ± 4.3	2E-07*
Diagnostic delay mean ± S.D. (years)	5.00 ± 5.3	9.7 ± 11.1	0.180
HLA-B27(+) (N, %)	43 (87.8%)	5 (38.5)	0.0007*
CRP mean ± S.D. (mg/L)	21.7 ± 43.1	9.7 ± 10.1	0.367
ESR mean ± S.D. (mm/h)	22.2 ± 21.7	24.1 ± 19.2	0.795
BMI mean ± S.D. (kg/m^2)	26.7 ± 4.4	27.0 ± 5.4	0.395
Enthesitis (N, %)	2 (4.1%)	2 (15.4%)	0.401
Dactylitis (N, %)	6 (12.2%)	0 (0%)	0.424
Peripheral arthritis (N, %)	12 (24.5%)	1 (7.7%)	0.348
Uveitis (N, %)	14 (28.6%)	2 (15.4%)	0.542
Psoriasis (N, %)	3 (6.1%)	3 (23.1%)	0.190
IBD (N, %)	2 (4.1%)	3 (23.1%)	0.096
Obesity (N, %)	11 (22.5%)	4 (30.8%)	0.796
Smoker (N, %)	34 (69.4%)	7 (53.9%)	0.470
Presence of syndesmophytes (N, %)	28 (57.1%)	1 (7.7%)	0.004*
Cardiovascular disease (N, %)	12 (24.5%)	3 (23.1%)	0.796
BASDAI mean ± S.D. (score)	2.5 ± 2.1	3.53 ± 2.5	0.138
BASFI mean ± S.D. (score)	2.6 ± 2.4	3.16 ± 2.7	0.448
BASMI mean ± S.D. (score)	1.6 ± 0.5	1.25 ± 0.3	0.0005*

Table 2. Clinical and laboratory parameters of patients diagnosed with AS and nr-axSpA included in the present study, as well as the p_{value} of the comparison between the two patient groups (*p_{value}<0.05). AS, ankylosing spondylitis; nr-axSpA, non-radiographic axial spondyloarthritis; *HLA-B27*, human leukocyte antigen-B27; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; BMI, body mass index; IBD, inflammatory bowel disease; BASDAI, bath ankylosing spondylitis disease activity index; BASFI, bath ankylosing spondylitis functional index; BASMI, bath ankylosing spondylitis metrology index.

The analysis of the 28 risk SNPs located in genes involved in the pathogenesis to develop AS according to the existing GWAS studies, showed that over 75% of the patients of this study shared 60% of the risk alleles **(Figure 1)**. However, no statistically significant differences were found in the frequencies of the risk alleles of these 28 SNPs between the individuals with AS and those with nr-axSpA, except for allele *HLA-B27*, whose frequency was significantly higher in the AS group (p_{value}<0.05) **(Table 2)**.

Considering that some SNPs showed a dominance relationship, thus the existence of a single copy of that allele increases or decreases the risk of developing AS, we analysed the different genotypes of the 28 SNPs associated with AS.

Regarding the genotypic frequencies of these SNPs, no statistically significant differences were found in this study between the patients with AS and those with nr-axSpA. However, Figure 2 shows that some risk alleles in homozygous or heterozygous patients (in the case of showing a dominance relationship) relative to genes involved in important pathogenic routes, such as *ERAP1*, *ERAP2, GPR25,* 2p15 or *IL-23R*, appear at high frequencies in all the individuals of this study. Nevertheless, there is no defined pattern in any of the two pathological entities in terms of risk genotypes, which shows the great genetic heterogeneity underlying these two entities **(Figure 2)**.

A PCA was carried out considering the genotypic frequencies of such markers in the individuals with AS and those with nr-axSpA, obtaining two principal components that explain 19.42% of the variance **(Figure 3A)**. In the first component (10.20% of the variance), the genetic markers with greater correlation were: HLA-B27 (HLA-B27, 0.582), rs7282490 (rs7282490-ICOSLG, -0.316) and rs1801274 (rs1801274-FCGR2A, 0.3). In the second component (9.22% of the variance), the variables with greater correlation were: rs30187 (rs30187-ERAP1, 0.531), rs1128905 (rs1128905-CARD9, -0.383), HLA-B27 (HLA-B27, 0.314) and rs6600247 (rs6600247, RUNX3, -0.3).

When the 62 individuals were represented on the first two components of the PCA **(Figure 3B)**, there was no significant spatial distribution of the individuals of the two entities regarding the genotypic frequencies. On the other hand, there were some common genotypes of SNPs among the analysed individuals. Likewise, no common genetic pattern was found in the patients of each group, which indicates the existence of great intragroup variability.

Given that the presence of SNPs associated with the susceptibility to develop Ps and IBD has been described in patients with AS, which suggests the existence of a common pathogenetic mechanism, we analysed in the patients of this study, 6 SNPs associated with the susceptibility to develop Ps and PsA and 9 SNPs associated with IBD. The analysis of the genotypic frequencies of these SNPs did not reveal any statistically significant difference between the two patient groups analysed in the present study.

A second PCA was conducted considering the genotypes of the SNPs associated with the susceptibility to develop AS, Ps, PsA and IBD, as well as the alleles found for the *HLA-B* gene, obtaining two principal components that explain 15.61% of the variance **(Figure 5A)**. In the first component of this PCA (8.13% of the variance), the genetic markers with greater correlation were: rs27222427 (rs27222427-NOD2, 0.648), rs30187 (rs30187-ERAP1, -0.474), HLA-B27 (HLA-B27, -0.366) and rs2076756 (rs2076756-NOD2, 0.335). In the second component (7.48% of the variance), the variables with greater correlation were: rs1801274 (rs1801274-FCGR2A, -0.409), rs10865331 (rs10865331-2p15, 0.384), rs6600247 (rs6600247, RUNX3, -0.355) and rs1128905 (rs1128905-CARD9, 0.320).

When the 62 individuals were represented on the first 2 components **(Figure 5B)**, there were no patterns based on the genotypic frequencies of the SNPs associated with AS, Ps, PsA an IBD. Figure 5 shows what was already observed in the first PCA, regarding the genetic heterogeneity underlying these two entities. However, it is worth highlighting two variables that showed a higher correlation values with respect to axis 1; these are two SNPs of the *NOD2* gene (rs27221427 and rs2076756), which is a gene that shows a stronger association with CD and whose association with AS has not been described to date **(Figure 5A)**. Moreover, the combination of the risk genotypes of these two SNPs of the *NOD2* gene is present in 16 individuals, almost exclusively in the AS group (N=15, 30.61%), appearing only in one nr-axSpA individual (7.69%). These results suggest an association of these alleles with a greater radiographic progression of the disease, although no statistically significant differences were detected between the two groups (p_{value}>0.0001) due probably to the sample size.

Discussion

There is current debate on whether nr-axSpA is a different disease from AS [8,9], an early form of AS [10,11] or whether both are two expressions of the same disease [12,13]. In the present study, we analysed the clinical and demographic characteristics, laboratory markers and SNPs associated with the susceptibility to develop AS, Ps, PsA and IBD in 62 patients of the University Hospital of Basurto (Bilbao, Spain), with the aim of determining whether these two entities, apart from sharing clinical characteristics, had a common genetic background.

It was observed that the patients with AS had a lower symptom onset age, shorter diagnostic time and longer duration of the disease compared to the patients with nr-axSpA **(Table 2)**. This could be due to the fact that AS can be more aggressive than nr-axSpA, with a faster radiographic progression, which would imply an earlier symptom onset and diagnosis compared to nr-axSpA. Regarding peripheral

manifestations (arthritis, enthesitis, dactylitis), our data confirm those obtained in other studies related to the prevalence of enthesitis in the two patient groups [10-12]. However, the prevalence of peripheral arthritis and dactylitis was greater in AS patients [10-12] **(Table 2)**. With respect to extra-articular manifestations, we found a greater prevalence of uveitis among the patients with AS, as has been reported in other studies [19], which could be due to the fact that uveitis is associated with the duration of the disease, which is longer in AS [62] **(Table 2)**. Nevertheless, the prevalence of Ps and IBD is greater among individuals diagnosed with nr-axSpA, which is in contrast to what has been reported in the literature to date [10-12] **(Table 2)**.

The BASMI index is statistically higher among individuals diagnosed with AS [11,12,25,32] **(Table 2)**, due to the decreased mobility of the axial skeleton as a consequence of the formation of syndesmophytes and even bone bonds and changes in the SIJs [26]. The BASFI and BASDAI indices, which measure the clinical activity, damage and functional deterioration, would be expected to be higher in individuals with AS, given their greater severity and progression [10,11,32]; however, in the present study, higher values of these indices were found in those individuals with nr-axSpA **(Table 2)**. In this study, high levels of CRP were more frequent in patients with AS **(Table 2)**, which could be due to the fact that axSpA patients with high CRP values tend to progress more rapidly to radiographic sacroiliitis [28].

Regarding the gender of the patients, our results confirmed the predominance of males among the individuals diagnosed with AS (77.55%) **(Table 2)**, which could be due to the fact that these patients have a faster progression than females, as well as more structural changes, which in turn, may cause a more severe disability [28]. Another possible influencing factor is physical activity, which, in the case of men usually involves greater mechanic stress, which would increase the inflammatory activity [63]. However, in the group of nr-axSpA patients, the proportion of women (46.15%) was very similar to that of men (53.85%) **(Table 2)**, as has been described in other studies conducted in European and North American cohorts [8,11,12]. The explanation suggested for the differential prevalence between the two entities in terms of gender is that women have a lower radiographic damage and a slower progression to a radiographic state, remaining in the non-radiographic form for longer periods of time [64], suggesting the possible differential role of hormones in the formation of new bone material in patients with SpA.

Some studies carried out in relatives and twins indicate that the genetic component has a fundamental role in the immunopathogenesis of SpA, although the primary trigger is still unknown [34]. Allele *HLA-B27*, which has been traditionally granted considerable relevance, could contribute to up to 25% of the total inheritance of AS [35]. In the present study, *HLA-B27* showed a significantly greater frequency among the patients with AS compared to those with nr-axSpA (87.8% *vs* 38.5%), which is in line with the results obtained in other studies [12,28,53-55] **(Table 2)**. Furthermore, this greater frequency of *HLA-B27* in the AS group confirms the hypothesis that associates the presence of *HLA-B27* with a lower onset age that characterize the AS and an earlier diagnosis [30] **(Table 2)**.

Apart from allele *HLA-B27*, 6 additional alleles of the *HLA-B* gene were found in the patients of this study (*HLA-B39*, *HLA-B40*, *HLA-B47*, *HLA-B55*, *HLA-B56* and *HLA-B07*). Alleles *HLA-B39*, *HLA-B40* and *HLA-B47*

have been previously associated with AS [65-67] and, in the case of *HLA-B39*, with PsA [68]. However, allele *HLA-B07* is associated with a decrease in the risk of developing AS [67] and alleles *HLA-B55* and *HLA-B56* are not associated with AS. Although the proportion of individuals with other alleles of the *HLA-B* gene different from *HLA-B27* is greater in the group with nr-axSpA with respect to the AS group (61.5% *vs* 12.2%), both entities share some alleles (*HLA-B40*, *HLA-B55* and *HLA-B56*). According to these data, it can be stated that there is a predominance of allele *HLA-B27* in patients with AS (87.8%); however, the patients with nr-axSpA showed a greater heterogeneity regarding the alleles of the *HLA-B* gene.

GWAS studies have revealed a considerable number of genes or gene regions that contribute to the susceptibility to develop AS. In our study, we analysed the allele frequencies of 28 risk SNPs, and found no statistically significant differences between the AS and nr-axSpA patients. It was observed that 75% of the individuals of both groups shared a large number of risk alleles (60%) **(Figure 1)**. These results indicate that both pathological entities have a common genetic background, at least at the level of risk SNPs.

At the genotype level, no statistically significant differences were found between the two groups of patients for risk SNPs located in genes involved in the pathogenesis of AS. Some SNPs showed similar genotypes in most of the individuals of the two groups, e.g., *ERAP1* (rs30187), *ERAP2* (rs10045403), *IL-23R* (rs11209026), *GPR25* (rs41299637), and in intergenic region 2p15 (rs10865331) **(Figure 2)**. Outside of the MHC, it is worth highlighting the *ERAP1* gene for its strong association with the susceptibility to develop AS. *ERAP1* is an aminopeptidase involved in the cleavage of peptides to a length of 8-9 amino acids, in order for the new peptide segments to be presented by MHC class I molecules, such as *HLA-B27* [47]. The SNPs located in the *ERAP1* gene only appear to be associated with AS in *HLA-B27*(+) and *HLA-B40*(+) patients [65,67]. The *ERAP2* is another aminopeptidase, whose association with AS has been described in *HLA-B27*(+) and *HLA-B27*(-) patients [49]. The high frequency of risk SNPs of the *ERAP-1* and *ERAP-2* genes found in the analysed patients could suggest the alteration of the correct functioning of both genes [69], thus influencing the supply of optimum peptides for MHC class I molecules, such as *HLA-B27*, which could explain their relationship with the development of SpA [48,70].

The *IL-23R* gene encodes for *IL-23* receptor. *IL-23* is a key cytokine involved in the differentiation of naive $CD4^+$ T-lymphocytes into Th17 lymphocytes, which produce *IL-17*, *IL-6*, *IL-22*, *TNF-α* and other similar proinflammatory cytokines. Furthermore, other genes of the *IL-23* route have been associated with AS, which shows that this route is an important pathway in the pathogenesis of AS [46,48,49]. There are polymorphisms of *IL-23R*, such as rs11209026, associated with AS [46], IBD [71] and Ps [72]. This SNP has been found with a very high frequency among the patients of this study **(Figure 2)**, which confirms the evidence that *IL-23* and its entire pathogenic route are involved in the susceptibility to develop axSpA, granting inflammation a main role in the triggering of the disease.

GWAS studies have shown the association of SNPs of intergenic region 2p15 (rs10865331) with AS, although their role in the pathogenesis of AS is unknown. It has been hypothesised that this region has non-coding RNA species or protein-coding genes unknown to date, which could be involved in the

susceptibility to develop AS [47]. G-protein coupled receptor 25 (*GPR25*), which is associated with AS [49] is strongly expressed in memory T-cells and NK-cells and involved in the positive regulation of B-cell proliferation [73], suggesting a potential mechanism in different autoimmune diseases. Although it is still unknown how the SNPs located in 2p15 (rs10865331) and *GPR25* (rs41299637) may influence the pathogenesis of AS, their high prevalence in our study could suggest a key role in the onset and development of axSpA **(Figure 2)**.

The results obtained for SNPs located in genes (*ERAP1*, *ERAP2*, *IL-23R*, *GPR25)* and gene regions (2p15), along with the different *HLA-B* gene subtypes, demonstrate their importance in the pathogenesis of axSpA, probably with an essential role in the onset of both AS and nr-axSpA. Despite the fact that the individuals who suffer from different axSpA have common genotypes for some SNPs of genes that are important in the pathogenesis of AS, we observed great genetic heterogeneity within each group and between the two pathological entities, which demonstrates the complex nature of this type of diseases **(Figure 3)**.

Moreover, given that AS, IBD and Ps could have a common pathogenic mechanism, we analysed 15 SNPs associated with the susceptibility to develop IBD, Ps and PsA, finding no statistically significant differences for any of these SNPs between the two groups of patients (AS and nr-axSpA). However, the combination of the risk genotypes of 2 SNPs located in the *NOD2* gene associated with CD was found mainly in individuals with AS (AS: 30.7% ***vs*** nr-axSpA: 7.7%), which could indicate that these two SNPs are associated with a greater progression of this disease **(Figures 4 and 5)**. The *NOD2* gene was identified as the most relevant risk factor of CD [74], although its association with AS had not been described to date. *NOD2* is an intracellular pathogen recognition receptor [75] and plays a role in the immune response to bacterial lipopolysaccharides, since it regulates the response of Th17 cells for the elimination of bacteria by inducing the secretion of cytokines *IL-23* and *IL-1B*, which appear in individuals with CD who have a mutation in *NOD2* [76]. The results suggest that individuals with these two SNPs of the *NOD2* gene will be at greater risk of developing intestinal lesions characteristic of IBD throughout their lives **(Figure 4)**. Furthermore, the presence of these and other SNPs located in genes associated with AS and IBD would confirm the hypothesis about the relevance of intestinal dysbiosis in the genesis of axSpA [20].

Between 20-25% of the known inheritance of AS is attributed to allele *HLA-B27*, and 3-7% to SNPs identified in GWAS studies [35,36], thus approximately 70% of the inheritance of this pathology could be related to genetic variants that have not been described to date. Missing heritability is a common issue in complex genetic diseases, and it may be caused by multiple factors. It has also been suggested that general heritability is overestimated, with the probability that epigenetic factors, especially environmental factors (smoking and obesity), may be more relevant in the susceptibility to develop AS.

Smoking is an important environmental factor in the inflammatory process of rheumatic diseases, including SpA, and it is a risk factor of cardiovascular disease. In the present study, 66.1% of the patients were smokers or ex-smokers, with a higher proportion of smokers in the individuals diagnosed with AS (69.4%) with respect to those diagnosed with nr-axSpA (53.9%) **(Table 2)**. This suggests that smoking

could contribute to increasing the progression to the radiographic form of SpA [29], promoting spine damage and an earlier onset of the inflammatory back pain, which characterises AS [29].

Obesity and overweight are an increasing issue worldwide due to their influence on the development of metabolic, cardiovascular and rheumatic diseases, which increases the mortality and morbidity of patients [77,78]. In our study, it was observed that 61.3% of the patients were obese (BMI>29.99 kg/m^2) or overweight (BMI>24.99 kg/m^2), with BMI and the prevalence of obesity being similar between patients with AS and those with nr-axSpA **(Table 2)**. Excess adipose tissue in overweight and obese individuals may have immunomodulating properties that affect the course of the disease [79], which could be associated with an increase in the production of proinflammatory cytokynes [78]. Previous studies have reported a greater prevalence of obesity and overweight [80] and a significantly higher BMI in AS patients compared to the healthy population [81]. It has been described that overweight and obese patients with AS have greater functional limitations, greater activity of the disease and a lower response to anti-*TNF-α* therapies [79,82], which would contribute to increasing physical inactivity and, thus, gaining weight [80,82]. The high prevalence of smoking and obesity or overweight among the individuals analysed in the present study would suggest the relationship between these life habits and the development of these diseases, with inflammation playing a key role in their triggering.

Conclusions

In the SNPs analysed in the present study, we observed that AS and nr-axSpA have a common genetic background associated with the pathogenic development of these diseases; therefore, from the genetic perspective, it could be suggested that the two entities constitute two different expressions of the same disease. However, the pathogenesis of autoimmune diseases, such as SpA, is very complex, and it is the result of intricate mechanisms interaction where the genetic and environmental factors are involved. Among the genetic factors, this study shows the importance of genes and intergenic regions involved in the pathogenesis of AS, such as *HLA-B27*, *ERAP1*, *ERAP2*, *IL-23R*, *GPR25* and 2p15, whose role may influence the onset, development and severity of the disease. Nevertheless, we also observed great genetic heterogeneity among individuals who had the same clinical diagnosis, which could indicate the involvement of exogenous factors in the triggering of the disease, thus patients with different genotypes would have the same pathogenic phenotype. The most relevant of these external factors are: infections by pathogens that alter the intestinal microbiota, endocrine alterations such as overweight and obesity, and unhealthy life habits (e.g., smoking). Numerous studies have granted great relevance to the genetic component in the development of AS; however, this relevance may be overestimated, since environmental factors could be more important than reported to date in the triggering of the disease. Our results should be confirmed in larger observational studies with a larger nr-axSpA population.

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Abbreviations

AS: Ankylosing spondylitis

ASAS: Assessment of Spondyloarthritis International Society

axSpA: Axial spondyloarthritis

BASDAI: Bath Ankylosing Spondylitis Disease Activity Index

BASFI: Bath Ankylosing Spondylitis Functional Index

BASMI: Bath Ankylosing Spondylitis Metrology Index

BMI: Body mass index

CD: Crohn disease

CRP: C-reactive protein

ERAP1: Endoplasmic reticulum aminopeptidase 1

ERAP2: Endoplasmic reticulum aminopeptidase 2

ESR: Erythrocyte sedimentation rate

GPR25:

GWAS: Genome-wide association study

HLA-B27: Human leukocyte antigen B27

IBD: Inflammatory bowel disease

IL-23R: G protein-coupled receptor 25

MHC: Major histocompatibility complex

MRI: Magnetic resonance imaging

NOD2: Nucleotide-binding oligomerization domain 2

nr-axSpA: non-radiographic axial spondyloarthritis

NSAID: Non-steroidal anti-inflammatory drug

PCA: Principal component analysis

Ps: Psoriasis

PsA: Psoriatic arthritis

SIJ: Sacroiliac joint

SNP: Single nucleotide polymorphism

SpA: Spondyloarthritis

TNF: Tumor necrosis factor

UC: Ulcerative colitis

Declarations

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Contributions

All authors were involved in drafting the manuscript or revising it critically for important intellectual content, and all authors approved the final version to be published. IML, MH, JMBM, EG, NAR, MLGV and CdR had full access to all of the data in the study and take responsibility for the integrity of the data. IML, MH and CdR carried out the statistical analysis and the accuracy of the data analysis. IML, MH, MLGV and CdR contributed to the study conception and design. IML, MH, JMBM, EG, NAR, MLGV and CdR contributed to the analysis and interpretation of data.

Ethics declarations

Ethics approval and consent to participate

This study was conducted with the approval of the Drug Research Ethics Committee of Euskadi (CEImE, PI2017141). All patients gave their signed informed consent to participate in the study.

Consent for publication

No individual person´s data are present in this manuscript. All data are completely anonymized. All patients gave their signed informed consent to participate in the study.

Competing interests

The author declares that they have no competing interests.

Figures

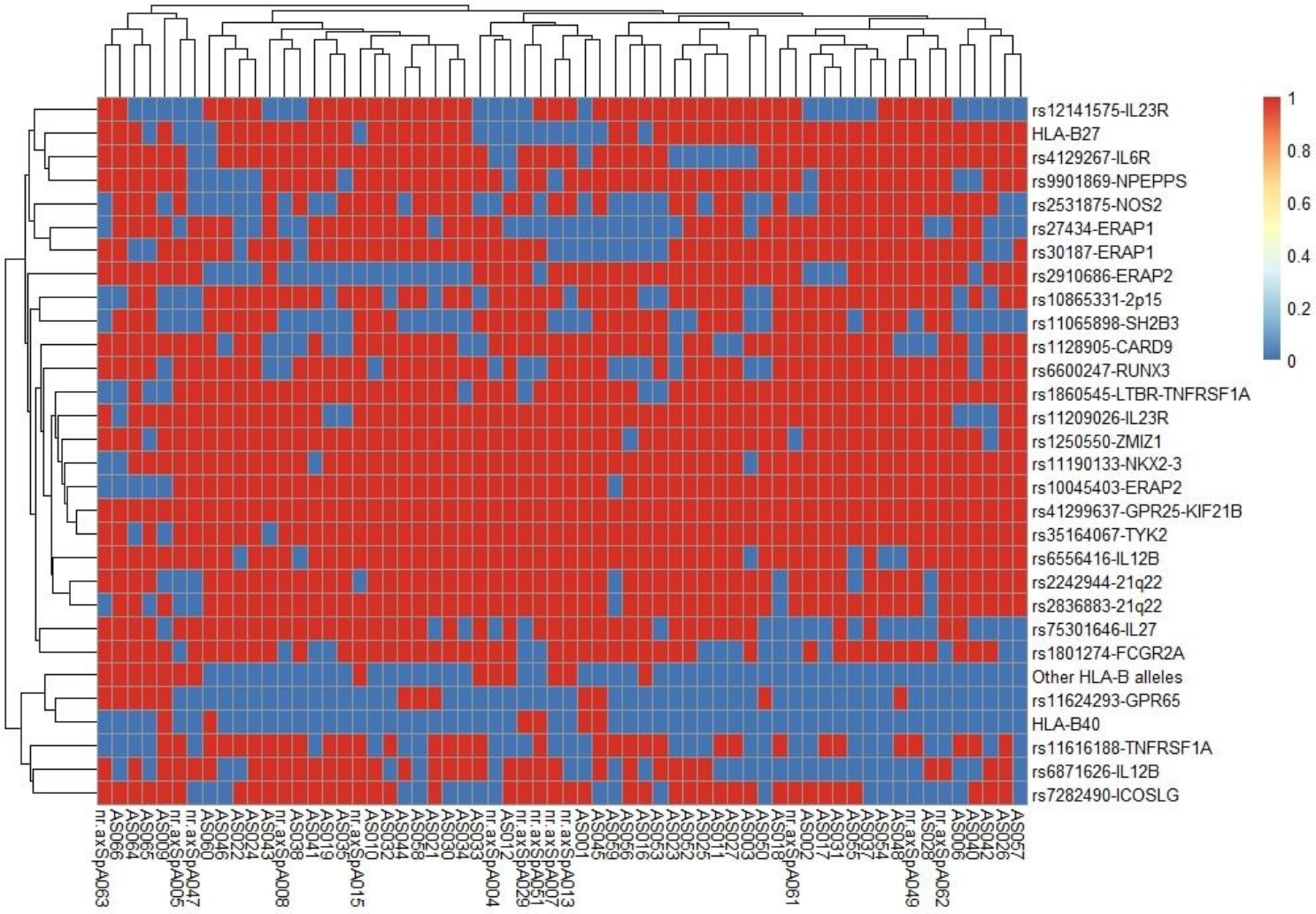

Figure 1

Heatmap of the allele frequencies of the SNPs associated with the susceptibility to develop AS in patients diagnosed with AS and nr-axSpA analysed in the present study. Red: presence of the risk allele; blue: absence of the risk allele. AS, patients diagnosed with ankylosing spondylitis; nr.axSpA, patients diagnosed with non-radiographic axial spondyloarthritis.

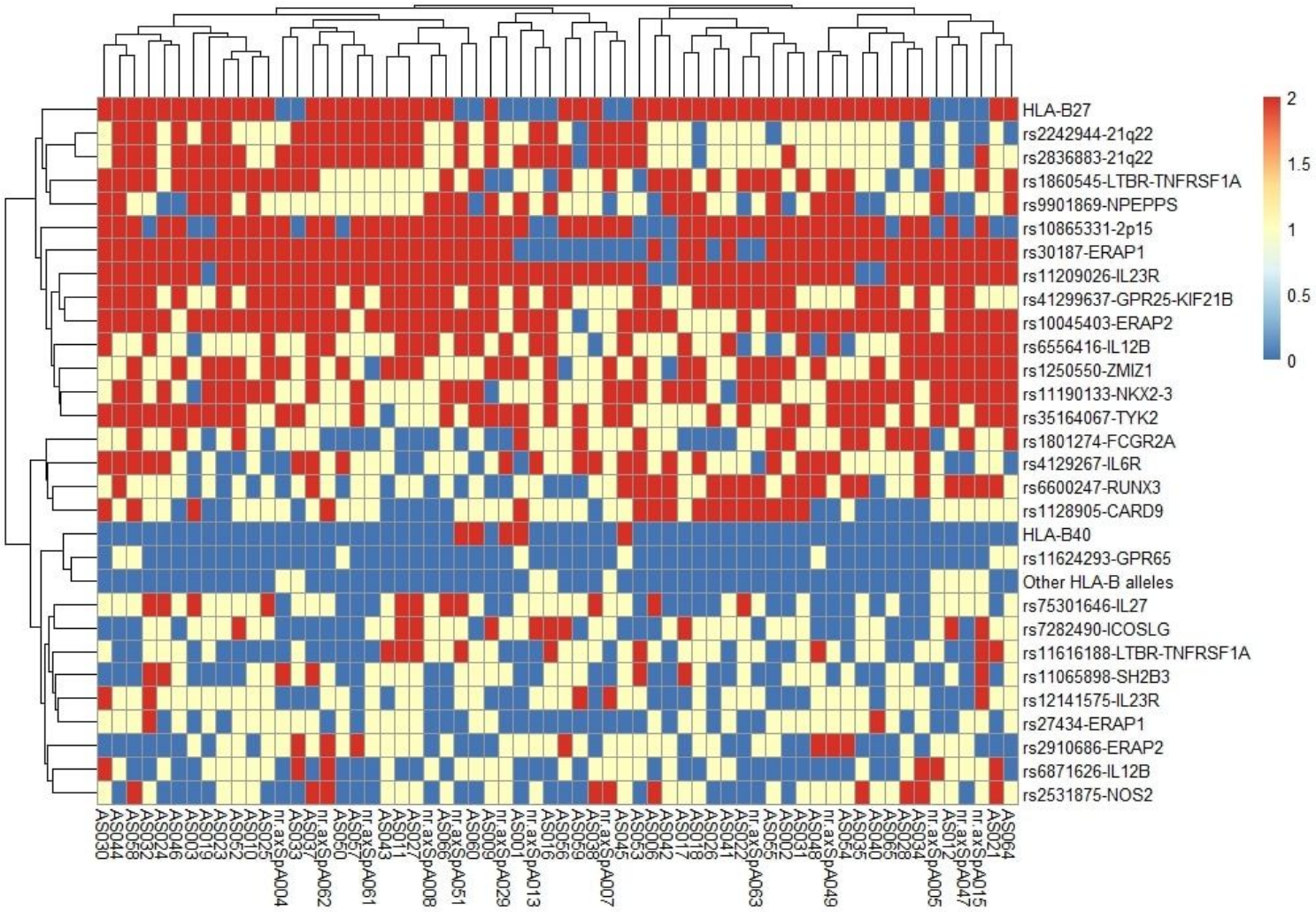

Figure 2

Heatmap of the genotypic frequencies of the selected SNPs in the patients diagnosed with AS and nr-axSpA in the present study. Red: presence of the risk allele in homozygous or in heterozygous patients when such allele is dominant; yellow: presence of the risk allele in heterozygous patients; blue: absence or presence of the risk allele in heterozygous patients when such allele is recessive. AS, patients diagnosed with ankylosing spondylitis; nr.axSpA, patients diagnosed with non-radiographic axial spondyloarthritis.

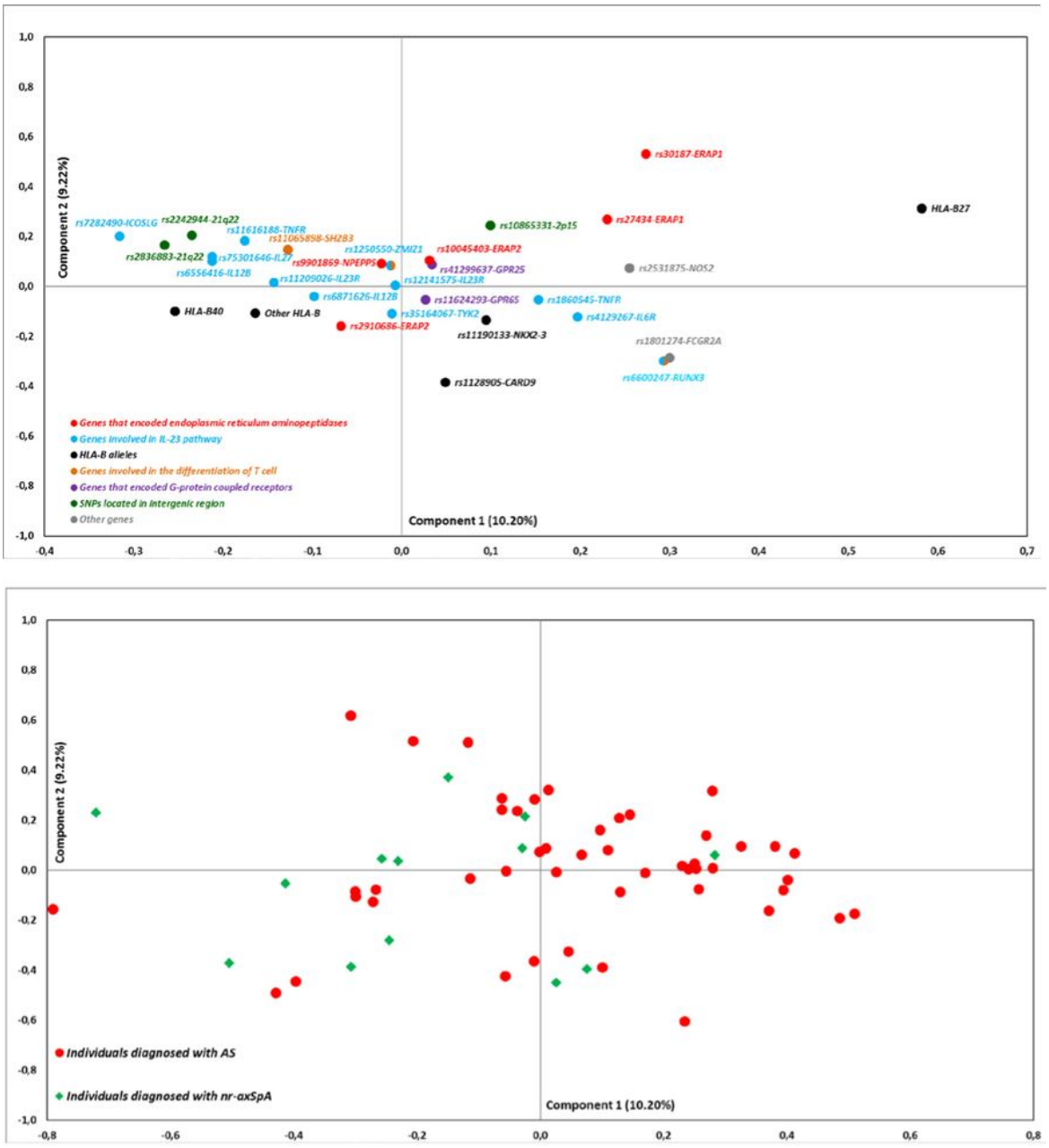

Figure 3. Principal component analysis of the genotypic frequency of the SNPs associated with the susceptibility to develop AS and of the alleles of gene *HLA-B* in the 62 individuals analysed. A) Distribution of the genetic variables analysed. B) Distribution of the patients. AS, ankylosing spondylitis; nr-axSpA, non-radiographic axial spondyloarthritis.

Figure 3

Principal component analysis of the genotypic frequency of the SNPs associated with the susceptibility to develop AS and of the alleles of HLA-B gene in the 62 individuals analysed. A) Distribution of the genetic variables analysed. B) Distribution of the patients. AS, ankylosing spondylitis; nr-axSpA, non-radiographic axial spondyloarthritis.

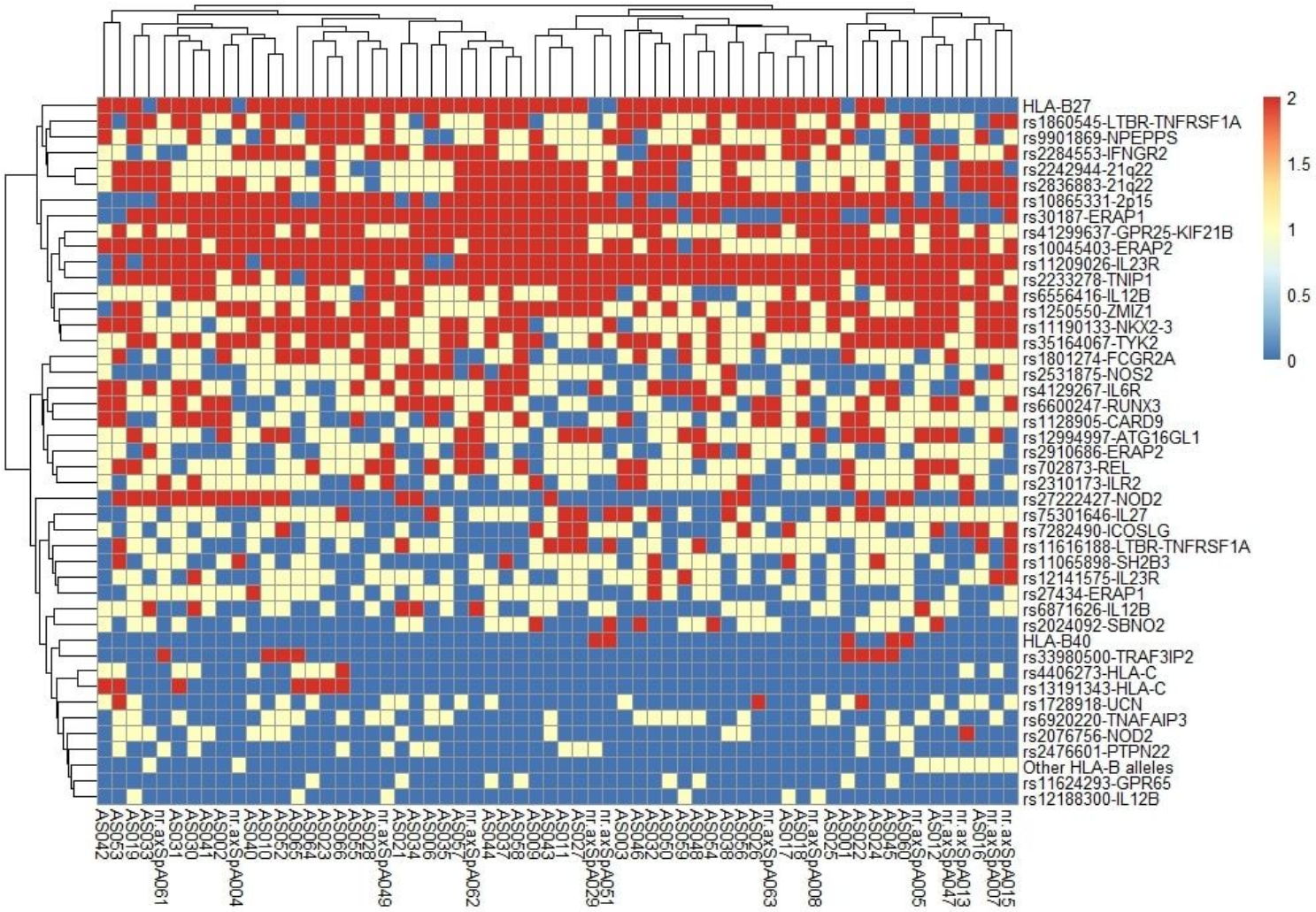

Figure 4

Heatmap of the genotypic frequencies of the SNPs associated with the susceptibility to develop AS, Ps, PsA and IBD in the patients diagnosed with AS and nr-axSpA analysed in the present study. Red: presence of the risk allele in homozygous or heterozygous patients when such allele is dominant; yellow: presence of the risk allele in heterozygous patients; blue: absence or presence of the risk allele in heterozygous patients when such allele is recessive. AS, patients diagnosed with ankylosing spondylitis; nr.axSpA, patients diagnosed with non-radiographic axial spondyloarthritis.

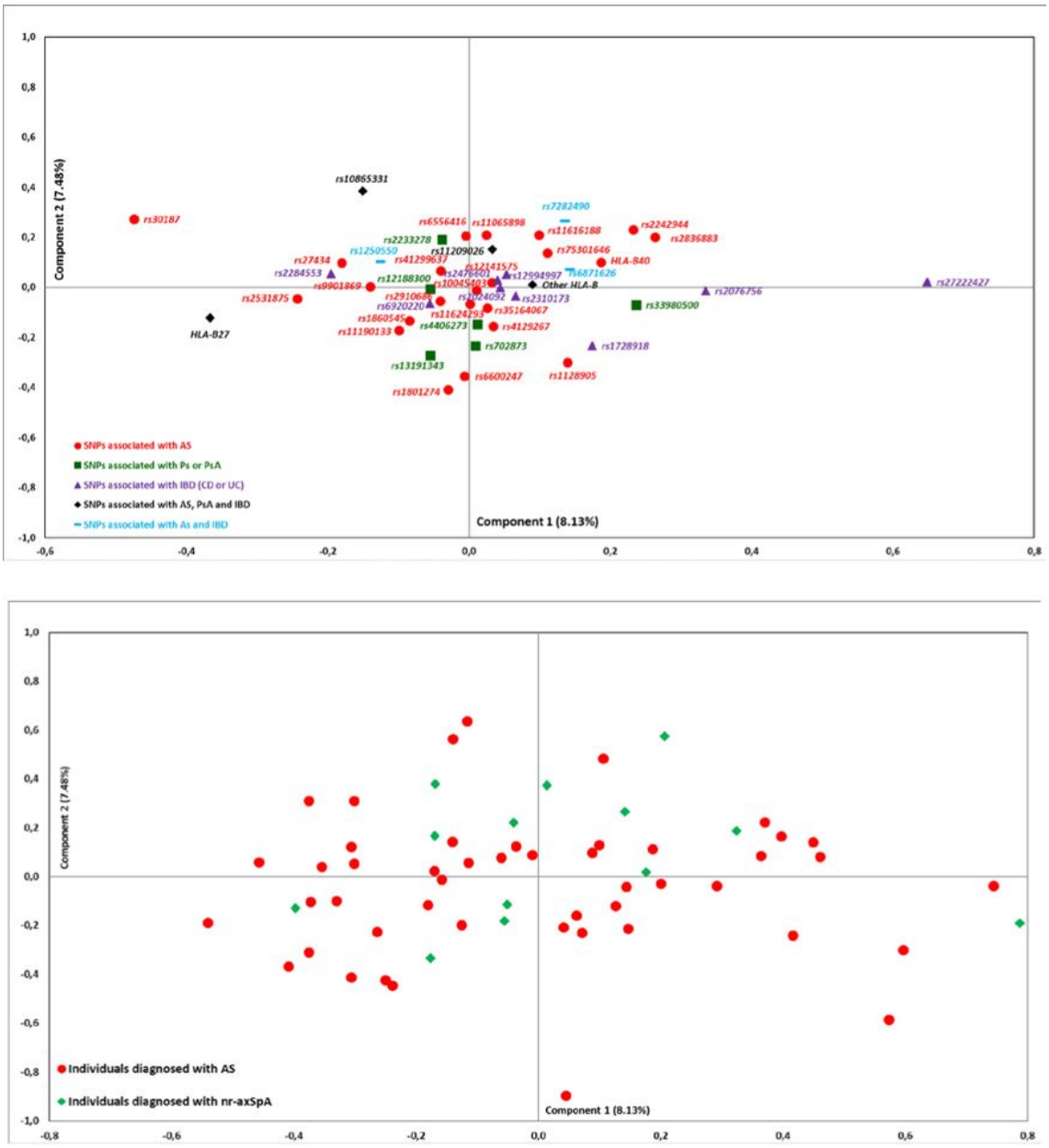

Figure 5. Principal component analysis of the genotypic frequency of the SNPs associated with the susceptibility to develop AS, Ps, PsA and IBD and the genotypic frequency of the alleles of gene *HLA-B* in the 62 individuals analysed. A) Distribution of the genetic variables. B) Distribution of the patients. AS, ankylosing spondylitis; nr-axSpA, non-radiographic axial spondyloarthritis.

Figure 5

Principal component analysis of the genotypic frequency of the SNPs associated with the susceptibility to develop AS, Ps, PsA and IBD and the genotypic frequency of the alleles of HLA-B gene in the 62 individuals analysed. A) Distribution of the genetic variables. B) Distribution of the patients. AS, ankylosing spondylitis; nr-axSpA, non-radiographic axial spondyloarthritis.

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