Human Gene 40 (2024) 201290

Contents lists available at ScienceDirect

Human Gene

journal homepage: www.journals.elsevier.com/human-gene

# Genetic biomarkers for diagnosis and treatment of spondyloarthropathies in Moroccan population

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ARTICLE INFO

*Keywords:*
Spondyloarthropathies
CTLA-4
FCGR3A
SBT
Morocco

ABSTRACT

*Background:* The spondyloarthropathies (SpA) are inflammatory rheumatologic diseases whose pathogenesis comes from interaction between genetic and environmental factors.
*Methods:* This is a case-control analysis that aims to explore in Moroccan population the relationship of some allelic polymorphisms of the CTLA-4 CT60, FCGR3A-158G/T SNPs and HLA-B locus with SpA disease. Eighty-four Spa patients grouped according to pain site and 95 healthy individuals from Morocco were typed by sequence-based-typing methods.
*Results:* The CTLA-4 CT60 investigation showed a significantly increased frequency of rs11571319 A allele in male patients with sacroiliitis ($p_{corr.}$=0.048, OR=2.756) and a protective role of A/G genotype in SPA patients with thoracic spine (pcorr.=0.016, OR=0.055) and peripheral joints pain (pcorr.=0.036 OR=0.068), compared to controls. Furthermore, a significant increase of A/A in rs3087243 was shown in male patients with peripheral joints pain ($p = 0.035$ OR = 4.45). Data from FCGR3A-158 analysis evidenced a reduction of T/T (F/F) genotype in patients with thoracic spine pain ($p = 0.030$, OR = 0.09) and a higher frequency of G/T (F/V) ($p = 0.038$ OR = 5.045) towards controls. With regard to HLA-B locus, we noticed a protective role of HLA-B*45:01 (pcorr. = 0.033,OR = 0.041) and an increased frequency of HLA-B*51 alleles (51:01, 51:02, 51:08, 51:09, 51:29) and B*52:01:01 ($p = 0.041$,OR = 2.714), in addition to HLA-B*27 alleles ($p = 0.028$, OR = 2.593) commonly associated with AS pathologies.
*Conclusions:* Our data suggest the role of some additional CTLA-4, FCGR3A and HLA-B gene polymorphisms in SPA pathogenesis of Moroccan population, useful as new diagnostic biomarkers and targets for immunotherapy.

## 1. Introduction

The seronegative spondyloarthropathies (SpA) are inflammatory rheumatologic diseases that cause arthritis and are characterized by the absence of rheumatoid factor. The histopathological trait characteristic of SPA is the enthesitis that involves the sites where ligaments and tendons attach to bones. Symptoms present in two main ways: the first is inflammation causing pain and stiffness, most often of the spine, but also affecting the hands and feet or arms and legs; the second type is bone destruction, causing deformities. Ankylosing spondylitis (AS), is the most common type which affects mainly the spine, making it stiff and also causing difficulty in movement. Axial spondyloarthritis (AxSpA), which affects mainly sacroiliac joints and spine, peripheral spondyloarthritis (pSPA), affecting the arms and legs, Reactive arthritis (ReA), psoriatic arthritis (PsA) and enteropathic arthritis/spondylitis (EA) associated with inflammatory bowel diseases (IBD) are other forms of the disease.

The prevalence of SPA diseases is ranging from 0.20% in South-Eastern Asian populations to 1.61% in Northern Arctic communities [Olivieri et al., 2002]. The prevalence of ankylosing spondylitis from Europe is between 0.1% and 1.4% and in mid-Europe of 0.3–0.5% for ankylosing spondylitis [Braun et al., 1998] and 1–2% for the group of

*Abbreviations:* SpA, Spondyloarthropathies; AxSpA, Axial spondyloarthropathies; AS, Ankylosing Spondylitis; HLA, Human leukocyte antigen; TCR, T cell receptor; CTLA-4, Cytotoxic T-lymphocyte antigen-4; SNP, Single-nucleotide polymorphisms; sCTLA-4, soluble CTLA-4; 3'UTR, 3′ untranslated region; FcγR, Fc-gamma receptors; ADCC, Antibody-dependent cellular cytotoxicity; FHC, free-heavy chain; Mtb, *Mycobacterium tuberculosis*.

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https://doi.org/10.1016/j.humgen.2024.201290
Received 19 January 2024; Received in revised form 30 April 2024; Accepted 3 May 2024
Available online 5 May 2024

spondyloarthritides, which is similar to that for rheumatoid arthritis [Saraux et al., 1999].

Interplay of sex and gender with diseases evidenced in AxSpA. Generally, in Morocco men tend to have more radiographic damage and major inflammation, while women have more severe symptoms, mainly at the peripheral joints [Stovall et al., 2022; Ibn Yacoub et al., 2012; Hajjaj-Hassouni and Burgos-Vargas, 2008]. These differences play a role in immune responses, disease presentation and adverse effects associated with medical treatments [Rusman et al., 2020]. Mean age at onset of symptoms is 20–40 years, especially in Caucasian patients.

The pathogenesis of SpA is attributed to the interaction between genetic and environmental factors. Human leukocyte antigen (HLA)-B27, identified as the strongest susceptibility factor of SpA, is present in 80–95% of White (western Europeans) patients with AS [Reveille et al., 2001] and in 60–80% of those with ReA, compared to only about 8% of the general healthy population. However, the strength of this association varies markedly depending on the kind of SpA and also ethnicity. The prevalence of HLA-B27 in normal population from Middle Eastern and Arab countries is significantly lower than that in Western Countries [Ziade, 2017]. These data have been also confirmed by our genetic studies on Moroccan Chaouya ethnical group [Canossi et al., 2010] and Central and South areas of Africa [Reveille et al., 2001; Tikly et al., 2014], that have shown a frequency of HLA-B27 allele of 1%. Nonetheless, the link existing between HLA-B27 and AS has been evidenced also in North African populations, where HLA-B27 prevalence is lower but it also represents 29–64% in individuals from Algeria, Egypt, Morocco and Tunisia with the disease [Slimani et al., 2021; Atouf et al., 2012; El Mouraghi et al., 2015; Akassou et al., 2015; Rachid et al., 2012].

Moreover, as already demonstrated, there is a different clinical manifestation of the disease among patients of different ethnic groups. Patients from Morocco (where AS represents the second most common rheumatic disease) present a more severe pattern of hip involvement, coxitis mainly in males and a younger age of onset [Hajjaj-Hassouni et al., 1993; Claudepierre et al., 1995; Essouiri et al., 2018]. Therefore, a deeper understanding of SpA features in non-Caucasian population is an interesting task.

It's known that AS is characterized by an imbalance of the peripheral tolerance. The T cell-mediated immunological response requires co-stimulation of T cell receptor (TCR) by CD28 and cytotoxic T-lymphocyte antigen-4 (CTLA-4). While the former provides positive signals to the T cell activation, CTLA-4 acts as an inhibitor, by competing for the B7 ligands with its homologue receptor CD28 [van der Merwe et al., *1997*]. CTLA-4 induces anergy, inhibiting NF-kB signaling pathway and IL-2 production, and stimulates T regulator lymphocytes to immunosuppression [Ueda et al., 2003]. Several autoimmune diseases, such as type 1 diabetes, systemic lupus erythematous and rheumatoid arthritis have been associated with single-nucleotide polymorphisms (SNPs) in the CTLA-4 gene [Spoletini et al., *2013*; Torres et al., 2004; Farago et al., 2010]. It consists of four exons mapping on chromosome 2q33 and the alternative splicing of CTLA-4 transcript generates three forms, one transmembrane (exons 1–4), one soluble (sCTLA-4, without exon 3) and a short form encoded by exons 1 and 4. Three SNPs have been associated with high AS risk (−318C/T, +49 A/G, CT60 UTR) [Kristiansen et al., 2000]. The CT60 SNP is located downstream of the 3′ polyadenylation site (3' UTR) and is responsible of the soluble CTLA-4 splicing and different production. Several studies indicated that polymorphisms within CTLA-4 gene that lead to reduction of molecule expression might cause autoimmune T cell proliferation and contribute to the pathogenesis of autoimmune diseases [Karabon et al., 2009]. In addition, as shown by Toussirot et al., higher levels of soluble form of CTLA-4 (sCTLA-4) were observed in SpA patients compared to RA group and healthy individuals [Toussirot et al., 2009]. These findings suggest that the sCTLA-4 could play a role in the activation and the regulation of the T cells. Also Dahmani et al., showed in West Algerian population that the HLA-B27 antigen and variation in CTLA-4 3'UTR played a role in the susceptibility to AS [Dahmani et al., 2018].

Regarding the therapy for such pathology, an early diagnosis and personalized treatment would be useful to reduce SpA activity and control joint damage and extra-articular manifestations. In the past decades, the development of biological therapies has changed the therapeutic approach to SpA. TNF antagonists, able of specifically binding to cell-surface Fc-gamma receptors (FcγR) [Tracey et al., 2008] and mediate induction of antibody-dependent cellular cytotoxicity (ADCC), are greatly used for immune mediated inflammatory diseases treatment [Mitoma et al., 2008]. In humans, three major classes of FcγR have been described, comprising 8 genes (FCGR1A, B and C; FCGR2A, B and C; FCGR3A, B), mapping on chromosome 1 [Nimmerjahn and Ravetch, 2008]. Several genetic polymorphisms of such receptors have been reported, some of which leading to known functional modifications [Gillis et al., 2014]. The FCGR3A allelic polymorphism at position 559 generates receptors containing either a phenylalanine (F) or a valine (V) at amino acid position 158, and this variability results in a higher binding affinity to NK cells of human IgG1 by homozygous FCGR3A-158 V (*V*/V) genotype, compared with homozygous FCGR3A-158F (F/F) [Bowles and Weiner, 2005; Wu et al., 1997; Koene et al., 1997].

Since the population of Morocco is a melting pot of indigenous Berber, Arab, African and European ethnicities, the identification of genetic polymorphisms, inside and outside HLA system, related to the susceptibility to SpA, may result fundamental. In this work, combining the HLA-B alleles and CTLA-4 gene polymorphisms with FCGR3A-158G/T variability data, might improve outcome prediction in SpA.

## 2. Materials and methods

### 2.1. Study population

This case-control study has been performed on 84 unrelated Moroccan patients (63 males and 21 females) and a diagnosis of SpA made by different rheumatology departments of the University Hospital Center of Rabat, based on the modified international criteria of New York [van der Linden et al., 1984]. The diagnosis was confirmed by clinical manifestations. Assessment of Spondyloarthritis International Society (ASAS) criteria have been used for non-radiographic axial SpA (nr-axSpA) diagnosis. Patients were grouped according to pain site: 34 individuals with sacroiliitis, 15 patients with thoracic spine pain, 13 patients with peripheral joint pain, 10 patients with pleuro-pulmonary complications and 12 with extra-articular disease manifestations (sciatica, uveitis). Controls used as a reference were a group of 46 Moroccan healthy subjects with mixed ethnic origin, from clinical departments of the University Hospital Center of Rabat, without any symptoms related to SpA, and a group of historical healthy controls, composed of Arabic individuals from Settat area (Chaouya ethnic group), previously studied for HLA class I variability [Canossi et al., 2010].

The sample size was calculated for genic variability using a confidence interval of 0.1 (10%) and a confidence level of 95%. The sample size for this analysis was determined considering a prevalence of SPA in Africa of 7.4 individuals on 10.000 peoples. In AS disease the HLA—B27, the most frequent allele associated, shows a prevalence range from 29% to 64% in Moroccan AS patients.

### 2.2. Editorial policies and ethical considerations

The research study was approved by the local Institutional Review Board (the National Center for Scientific and Technical research of Morocco, code 103212) with respect to the ethical principles of the Ministry of Health of Morocco and informed consent was obtained from all subjects.

### 2.3. DNA isolation and sequence-based typing (SBT)

#### 2.3.1. DNA extraction

Peripheral blood samples (5 ml) were collected in EDTA tubes and buffy coats were stored at −20 °C until analysis. The genomic DNA was extracted by a column-based nucleic acid purification method (*QIamp DNA Blood Midi kit, Qiagen*), according to the manufactures' protocol. DNA yield was measured by UV/visible spectrophotometer.

#### 2.3.2. Sequence-based typing

Genotyping of the different gene polymorphisms was performed by Sanger sequencing –based typing (SBT), using big dye terminator chemistry. Polymerase chain reaction (PCR) products were purified by ExoSAP digestion and sequence reactions purified and processed using an ABI 3130 Genetic Analyzer (*Applied Biosystems, Foster City, CA*).

*2.3.2.1. HLA-B allele-specific typing.* HLA-B typing was performed by using the AlleleSEQR HLA-B sequence-based typing kit (*Abbot Molecular, Des Plaines, Illinois, USA*), which analyzes the allelic polymorphisms in exons 2–4 of HLA-B gene. The exons were analyzed in both forward and *reverse* directions to determine the allelic polymorphisms. Allele assignments were evaluated using Assign™ SBT software (*Conexio Genomics, Fremantle, Western Australia)*.

*2.3.2.2. FCGR3A-158 typing.* The FCGR3A G/T gene polymorphisms were detected by PCR, using primers previously described [Koene et al., 1997]. Briefly, PCR amplification was carried out in a 50 ul reaction volume containing 50 ng genomic DNA. Amplified products were purified and then sequenced on an ABI 3130 Genetic Analyzer. Finally, the chromatograms were aligned with the reference human FCGR3A gene.

*2.3.2.3. CTLA-4 3'UTR typing.* Four SNPs in CTLA-4 CT60 region (rs182533364 C/T, rs345031880 T/C, rs3087243 G/A, rs11571319 G/A) were analyzed by the SBT technique, using primers already described in literature [Azarpira et al., 2010; Canossi et al., 2013], Fig. 1. Typing was obtained by alignment of the processed sequences with exon 4 sequences of the human CTLA-4 gene reference from the Genebank by using ABI Prism SeqScape software (Applied Biosystems, Foster City, CA).

### 2.4. Statistical analysis

The distribution of the CT60 and FCGR3A variants was analyzed according to Hardy-Weinberg equilibrium (HWE), using the Guo and Thomson exact test. Associations with clinical outcome were calculated using allelic and genotypic frequencies. Differences in genotype frequencies between cases and controls were performed by the Pearson X2 test with Yates correction or by Fisher exact tests. The Bonferroni correction was applied to correct for multiple testing. A *p*-value *of* 0.05 or lower was considered statistically *significant*. The genotype and allele distributions of the CT60 polymorphism were assessed by the odds ratios (OR) and 95% confidence intervals (CI). The predictive ability of several variables for the risk of SPA pathogenesis was assessed also by logistic regressions for simply dichotomous variables. The crude OR, 95% CI and *p* value were reported for each predictor in the univariable analysis. Only statistically significant variables in the univariable analysis were entered into multiple logistic regression analysis to predict the final independent factors. The model fit was assessed by chi-square, degrees of freedom and p-value. Spearman's r-test was used to calculate correlations between the CT60 and FCGR3A-158 variability and SPA subgroup. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) software version 13.0 for Windows (*SPSS Inc., Chicago, IL*). Haplotypes were calculated by the Expectation Maximum algorithm. Relative linkage disequilibrium between 2 alleles at 2 different loci evaluated using Arlequin v.3.5 software [Excoffier and Lischer, 2010].

## 3. Results

### 3.1. HLA-B allele frequencies in SPA patients and healthy controls

To determine the association between HLA-B locus gene polymorphism and increased or reduced susceptibility to develop SpA disease, the frequency of HLA-B alleles of SPA patients were evaluated and compared to historical healthy controls (Chaouya group, $n = 73$), already examined from our team in a previous study **(**Table 1**).** First of

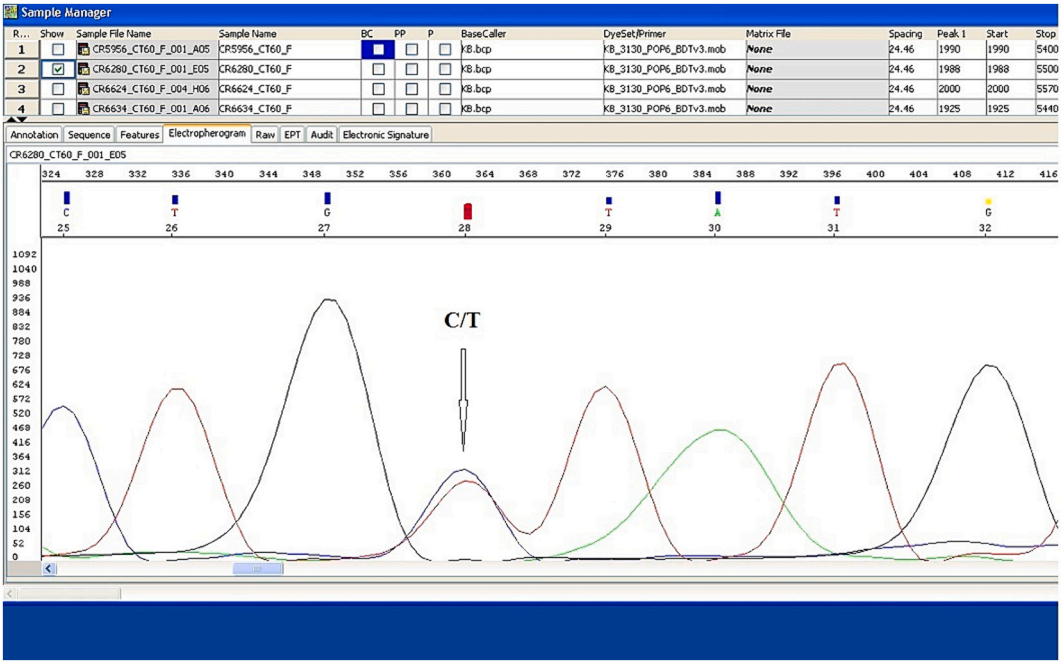


**Fig. 1.** An example of electropherogram from capillary gel electrophoresis of the nucleotide sequence in the 3'UTR, rs3087243C/T SNP in the CTLA-4 gene, showing a C/T heterozygosity.

**Table 1**
HLA-B allelic frequencies in SPA patients and Moroccan healthy controls.

| HLA-B* | SPA GROUP (2*n* = 158) | | | CHAOUYA[1] (2n = 146) | | | P value= | OR= | Pcorr. |
|---|---|---|---|---|---|---|---|---|---|
| | N= | % AF | | N= | % AF | | | | |
| 07:02:01 | 5 | 3.2 | | 5 | 3.4 | | 1.000 | | |
| 07:05,06 | 6 | 3.8 | | 2 | 1.4 | | 0.285 | | |
| 08:01:01 | 9 | 5.7 | | 9 | 6.2 | | 0.914 | | |
| 13:02:01 | 1 | 0.6 | | 3 | 2.1 | | 0.353 | | |
| 14:01:01 | 2 | 1.3 | | 1 | 0.7 | | 1.000 | | |
| 14:02:01 | 7 | 4.4 | | 6 | 4.1 | | 1.000 | | |
| 14:03 | 0 | 0 | | 1 | 0.7 | | 1.000 | | |
| 15:03:01 | 4 | 2.5 | | 1 | 0.7 | | 0.372 | | |
| 15:10 | 0 | 0 | | 2 | 1.4 | | 0.229 | | |
| 15:17 | 0 | 0 | | 4 | 2.7 | | 0.052 | | |
| 18:01:01 | 7 | 4.4 | | 6 | 4.1 | | 1.000 | | |
| **§27:02** | 1 | **0.6** | | 1 | **0.7** | | 1.000 | | |
| **27:03** | 1 | **0.6** | **2.5** | 0 | **0.0** | **0.7** | 1.000 | | |
| **27:05** | 2 | **1.3** | | 0 | **0.0** | | 0.499 | | |
| 35:01:01 | 8 | 5.1 | | 5 | 3.4 | | 0.576 | | |
| 35:02:01 | 2 | 1.3 | | 2 | 1.4 | | 1.000 | | |
| 35:03:01 | 2 | 1.3 | | 0 | 0.0 | | 0.499 | | |
| 35:08:01 | 2 | 1.3 | | 3 | 2.1 | | 0.673 | | |
| 37:01 | 0 | 0 | | 0 | 0.0 | | 1.000 | | |
| 38:01:01 | 4 | 2.5 | | 1 | 0.7 | | 0.372 | | |
| 39:01 | 2 | 1.3 | | 3 | 2.1 | | 0.673 | | |
| 39:05 | 0 | 0 | | 1 | 0.7 | | 1.000 | | |
| 39:06:02 | 1 | 0.6 | | 1 | 0.7 | | 1.000 | | |
| 39:10 | 1 | 0.6 | | 2 | 1.4 | | 0.609 | | |
| 39:20 | 1 | 0.6 | | 0 | 0.0 | | 1.000 | | |
| 40:01:01 | 0 | 0 | | 2 | 1.4 | | 0.229 | | |
| 40:02:01 | 2 | 1.3 | | 2 | 1.4 | | 1.000 | | |
| 40:06 | 0 | 0 | | 1 | 0.7 | | 1.000 | | |
| 41:01 | 1 | 0.6 | | 3 | 2.1 | | 0.353 | | |
| 41:02.01 | 1 | 0.6 | | 2 | 1.4 | | 0.609 | | |
| 42:01 | 0 | 0 | | 4 | 2.7 | | 0.052 | | |
| 42:02 | 1 | 0.6 | | 4 | 2.7 | | 0.198 | | |
| 44:02:01 | 6 | 3.8 | | 5 | 3.4 | | 1.000 | | |
| 44:03:01 | 8 | 5.1 | | 10 | 6.8 | | 0.677 | | |
| 44:05:01 | 2 | 1.3 | | 0 | 0.0 | | 0.499 | | |
| 44:27 | 1 | 0.6 | | 0 | 0.0 | | 1.000 | | |
| **45:01** | 0 | **0** | | 10 | **6.8** | | **0.0006** | **0.041** | **0.0336** |
| 47:01:01 | 0 | 0 | | 1 | 0.7 | | 1.000 | | |
| 49:01 | 4 | 2.5 | | 5 | 3.4 | | 0.742 | | |
| 50:01:01 | 14 | 8.9 | | 16 | 11.0 | | 0.674 | | |
| 50:02 | 5 | 3.2 | | 2 | 1.4 | | 0.450 | | |
| **§51:01:01*** | 11 | **6.9** | | 6 | **4.1** | | | | |
| **51:02:01** | 1 | **0.6** | **11.8** | 0 | **0.0** | **4.8** | **0.041** | **2.714** | |
| **51:08** | 1 | **0.6** | | 0 | **0.0** | | | | |
| **51:09** | 1 | **0.6** | | 0 | **0.0** | | | | |
| **51:29** | 1 | **0.6** | | 0 | **0.0** | | | | |
| **52:01:01** | 4 | **2.5** | | 1 | **0.7** | | | | |
| 53:01.01 | 6 | 3.8 | | 3 | 2.1 | | 0.504 | | |
| 55:01:01 | 0 | 0 | | 1 | 0.7 | | 1.000 | | |
| 56:01:01 | 2 | 1.3 | | 0 | 0.0 | | 0.499 | | |
| 57:01:01 | 3 | 1.9 | | 4 | 2.7 | | 0.714 | | |
| 57:03 | 0 | 0 | | 2 | 1.4 | | 0.229 | | |
| 58:01:01 | 9 | 5.7 | | 5 | 3.4 | | 0.418 | | |
| 58:02 | 0 | 0 | | 0 | 0.0 | | 1.000 | | |
| 78:01 | 1 | 0.6 | | 0 | 0.0 | | 1.000 | | |
| 78:02:02 | 1 | 0.6 | | 0 | 0.0 | | 1.000 | | |

[1] *Canossi A.* et al*, 2010.* *HLA-B*51:01, *51:02, *51:08, *51:09, *51:29 (n = 15) and HLA-B*52:01 (n = 4): 11.8% vs. 4.8%, p = 0.041 OR = 2.714, 95% CI: 1.1058–6.6622. §HLA-B*27 and HLA-B*51,52 alleles together (p = 0.028, OR = 2.593, 95% CI:1.1578 to 5.8091).

all, a statistically significant lower incidence of the HLA-B*45:01 allele has been reported in SPA patients, also confirmed by Bonferroni correction (0.0% vs. 6.8%, $p = 0.0006$ OR = 0.041; $p_{corr.} = 0.033$). On the other hand, HLA-B*51, B*52 alleles (HLA-B*51:01:01, B*51:02:01, B*51:08, B*51:09, B*51:29 and B*52:01:01) were more frequent in patients, particularly with thoracic spine pain than in controls (11.9% vs. 4.8%, $p = 0.041$ OR = 2.714 95%CI:1.1058–6.6622). Univariate regression confirmed this positive association with SpA pathogenesis ($p = 0.045$ OR = 3.750). In detail, the correlation with B*51, B*52 alleles was positive in this group of patients with thoracic spine pain (Spearman's r test $p = 0.024$, $r = 0.263$), while negative in patients with sacroiliitis ($p = 0.028$ $r = -0.255$). With regard to HLA-B*27 alleles, the HLA-B*27:02, *27:03 and *27:05 variants came only from AS patients and their frequencies were not significantly higher compared with controls (2.5% vs. 0.7%, p = ns).

### *3.2. Genetic predisposition of CTLA-4 CT60 single-nucleotide polymorphisms (SNP) to SPA disease*

The distribution of genotype and allele frequencies of the CTLA-4/CT60 polymorphisms between SPA patients ($n = 84$) and two healthy controls (specific HCTR $n = 46$, Chaouya group $n = 49$) is shown in

**Table 2**
– Distribution of CT60 gene polymorphisms and SPA susceptibility in Moroccan populations.

| Variable CTLA-4 CT60 | SPA patients (n = 84) | Specific HCTR ($n$ = 46) | P= | OR= | Chaouya group (n = 49) | P = | OR= |
|---|---|---|---|---|---|---|---|
| **rs182533364 C/T** | **Genotypes, n (%)** | | | | **Genotypes, n (%)** | | |
| CC | 81 (96.4) | 44 (95.7) | 1.000 | | 47 (95.9) | 1.000 | |
| CT | 3 (3.6) | 2 (4.3) | 1.000 | | 1 (2.0) | 1.000 | |
| TT | 0 (0) | 0 (0.0) | 1.000 | | 1 (2.0) | 0.368 | |
| **rs345031880 T/C** | | | | | | | |
| TT | 83 (98.8) | 44 (95.7) | 0.285 | | 47 (95.9) | 0.554 | |
| TC | 1 (1.2) | 0 (0.0) | 1.000 | | 2 (4.1) | 0.554 | |
| CC | 0 (0) | 2 (4.3) | 0.123 | | 0 (0.0) | 1.000 | |
| **rs3087243 G/A** | | | | | | | |
| AA | 27 (32.1) | 11 (23.9) | 0.432 | | 13 (26.5) | 0.627 | |
| AG | 36 (42.9) | 21 (45.7) | 1.000 | | 27 (55.1) | 0.236 | |
| GG | 21 (25.0) | 14 (30.4) | 0.697 | | 9 (18.4) | 0.504 | |
| *rs11571319 G/A | | ($n$ = 41) | | | ($n$ = 48) | | |
| **AA** | **9 (10.7)** | 2 (4.9) | 0.326 | | **1 (2.1)** | 0.092 | |
| AG | 18 (21.4) | 15 (36.6) | 0.236 | | 12 (25.0) | 1.000 | |
| GG | 57 (67.9) | 24 (58.5) | 0.409 | | 35 (72.9) | 0.680 | |
| **Allele** | **Allele freq, n (%)** | | | | **Allele freq., n (%)** | | |
| **rs182533364 C/T** | | | | | | | |
| C | 165 (98.2) | 90 (97.8) | 1.000 | | 95 (96.9) | 0.672 | |
| T | 3 (1.8) | 2 (2.2) | | | 3 (3.1) | | |
| **rs345031880 T/C** | | | | | | | |
| **T** | **167 (99.4)** | **88 (95.7)** | **0.054** | **7.590** | 96 (98.0) | 0.556 | |
| C | 1 (0.6) | 4 (4.3) | | | 2 (2.0) | | |
| **rs3087243 G/A** | | | | | | | |
| G | 78 (46.4) | 49 (53.3) | 0.355 | | 45 (45.9) | 1.000 | |
| A | 90 (53.6) | 43 (46.7) | | | 53 (54.1) | | |
| **rs11571319 G/A** | | | | | 2 $N$ = 96 | | |
| G | 132 (78.6) | 63 (76.8) | 0.881 | | 82 (85.4) | 0.229 | |
| A | 36 (21.4) | 19 (23.2) | | | 14 (14.3) | | |

* rs11571319 SNP genotype AA in males SPA patients: 14.3% vs. 2.1%, p = 0.041 OR = 7.833.

Table 2.

We have focused investigation of the CT60 variability in rs3087243 G/A and rs11571319 G/A polymorphisms but we also noticed some variants on other SNPs inside this region (rs182533364 C/T and rs345031880 T/C, Table 2).

We observed an increase of the T allele of the rs34500318880 (99.4% vs. 95.7%, $p = 0.05$ OR = 7.833) and a higher frequency of rs11571319 A/A genotype in SPA patients compared with controls, mainly Chaouya individuals. This difference became significant when males patients were considered (14.3% vs. 2.1%, $p = 0.041$ OR = 7.833) and its predisposing effect was expressed in the recessive model.

Our analysis showed that only the rs3087243G/A and rs11571319G/A SNPs had the minor allele with af > 5%, suggesting which SNP was more statistically informative for disease association.

Allele frequencies in these SNP sites were in Hardy-Weinberg equilibrium (HWE) in controls (rs3087243 $p = 0.833$; rs11571319 $p = 1.000$) and in SPA patients (rs3087243 $p = 0.196$), with the exception of rs11571319 ($p = 0.002$), possibly due to gene drift on this gene (spHCTR: allele G af% = 76.8 vs. allele A af% = 23.2) or to the effect of the limited number of cases.

### 3.3. Analysis of predisposing biomarkers to different SPA pathologies

The patients included in the study were first examined according to the specific reported symptoms and they were stratified in two main groups of SPA patients, one affected by sacroiliac joints/thoracic spine/peripheral joints pain ($n = 62$), typical symptoms of ankylosing spondylitis, and one by extra-articular disease manifestations, represented by pleuro-pulmonary abnormalities ($n = 10$) and other extra-articular pathologies (uveitis, skin, bowel and sciatica, $n = 12$).

Examining the subgroup 1 of SPA patients, a higher incidence of A/A genotype of rs11571319 CT60 SNP was evidenced in comparison to two controls, mainly CH group (11.3% vs. 2.1%, $p = 0.075$ OR = 6.109), difference which was significant considering only males (47/62, 14.9% vs. 2.1%, $p = 0.030$ OR = 8.225), but result after Bonferroni's correction lost significance. On the contrary we did not evidence any significant difference in the frequency of CT60 allelic variants between patients with extra-articular manifestations and controls.

Considering in detail the patients according to specific symptoms, we evidenced in males patients with sacroiliitis **($n = 25/34$)**, a significantly increased frequency of CT60 *rs11571319 A allele* towards Chaouya controls (32.0% vs. 14.6%, $p = 0.024$ $p_{corr.} = 0.048$, OR = 2.756), Table 3. In SPA patients with thoracic spine pain ($n = 15$) we detected that the A/G genotype of rs11571319 was protective compared with both the controls (0.0% vs. spHCTR 36.6%, $p = 0.0054$ $p_{corr.} = 0.0162$ OR = 0.055; vs. CH group 24.5% $p = 0.05$) (Table 4). In the third group of patients with peripheral joints pain (pSPA, $n = 13$), we noticed that there was a significant increase in the A/A genotype of the rs3087243 SNP in males when compared with both control groups (58.3%vs. specific HCTR: 23.9% $p = 0.035$, OR = 4.455; vs. Chaouya group: 26.5%, $p = 0.046$, OR = 3.877). The significance was lost in case of Bonferroni's correction. In addition, noteworthy the A/G genotype of rs11571319 was protective towards disease (0.0% vs 36.6% $p = 0.0121$, $p_{corr.} = 0.036$ OR = 0.068), probably according a codominant model (Table 5). Finally, patients with extra-skeletal manifestations (pleuro-pulmonary, iris or mixed type pain) didn't show any significant CT60 differences respect of controls.

### 3.4. Distribution of FCGR3A polymorphisms in SPA patients and controls

FCGR3A-158G/T (F/V) genotype was increased in patients with thoracic spine pain (86.7% vs. 57.0%, $p = 0.064$). This genotype is an independent variable for this pathology in a univariate regression ($p = 0.038$ OR = 5.045). In contrast, the FCGR3A-158 T/T (F/F) genotype was significantly reduced in this type of patients (0.0% vs. 26.0%, p = 0.03 OR = 0.092) (**Supplemental Table 1**). FCGR3A-158G/T allele frequencies were in Hardy-Weinberg equilibrium (HWE, Guo and Thompson 1992) in controls ($p = 0.403$,sd = 0.00048), and in SPA

**Table 3**
- Association between CT60 gene polymorphisms and AS susceptibility in males.

| Variable<br>CTLA-4 CT60 | Sacro-iliac joint pain, males<br>(n=25) | Chaouya group<br>(n=49) | P value | OR= | 95% CI |
|---|---|---|---|---|---|
| **Genotypes** | | | | | |
| **rs182533364 C/T** | **n (%)** | **n (%)** | | | |
| CC | 24 (96.0) | 47 (95.9) | 1.000 | | |
| CT | 1 (4.0) | 1 (2.0) | 1.000 | | |
| TT | 0 (0.0) | 1 (2.0) | 1.000 | | |
| **rs345031880 T/C** | | | | | |
| TT | 25 (100.0) | 47 (95.9) | 0.546 | | |
| TC | 0 (0.0) | 2 (4.1) | 0.546 | | |
| CC | 0 (0.0) | 0 (0.0) | 1.000 | | |
| **rs3087243 G/A** | | | | | |
| AA | 6 (24.0) | 13 (26.5) | 1.000 | | |
| AG | 11 (44.0) | 27 (55.1) | 0.510 | | |
| GG | 8 (32.0) | 9 (18.4) | 0.304 | | |
| **rs11571319 G/A** | | (N=48) | | | |
| ***AA** | 3 (12.0) | 1 (2.1) | 0.113 | | |
| ***AG** | 10 (40.0) | 12 (25.0) | 0.290 | | |
| GG | 12 (48.0) | 35 (72.9) | 0.064 | | |
| | | | | | |
| **Allele** | | | | | |
| **rs182533364 C/T** | **Allele frequencies** | **Allele frequencies** | | | |
| | **n (%)** | **n (%)** | | | |
| C | 49 (98.0) | 95 (96.9) | 1.000 | | |
| T | 1 (2.0) | 3 (3.1) | | | |
| **rs345031880 T/C** | | | | | |
| T | 50 (100.0) | 96 (98.0) | 0.549 | | |
| C | 0 (0.0) | 2 (2.0) | | | |
| **rs3087243 G/A** | | | | | |
| G | 27 (54.0) | 45 (45.9) | 0.449 | | |
| A | 23 (46.0) | 53 (54.1) | | | |
| **rs11571319 G/A** | | 2N=96 | | | |
| G | 34 (68.0) | 82 (85.4) | | | |
| **A** | **16 (32.0)** | **14 (14.6)** | **0.024** | **2.756** | **1.212-6.265** |

*rs11571319 dominant form: **AA+AG vs. GG**: 52.0% vs. 27.1%, p=0.064 OR=2.916 95%CI: 1.0619-8.0112 Z=2.076

**Table 4**
- Correlation between CT60 gene polymorphisms and susceptibility to SPA disease (thoracic spine pain).

| Variable<br>CTLA-4 CT60 | Thoracic spine pain<br>(n = 15) | Specific HCTR<br>(n = 46) | P value | OR= | 95% CI |
|---|---|---|---|---|---|
| **rs182533364 C/T** | **Genotypes, n (%)** | | | | |
| CC | 14 (93.3) | 44 (95.7) | 1.000 | | |
| CT | 1 (6.7) | 2 (4.3) | 1.000 | | |
| TT | 0 (0.0) | 0 (0.0) | 1.000 | | |
| **rs345031880 T/C** | | | | | |
| TT | 15 (100.0) | 44 (95.7) | 1.000 | | |
| TC | 0 (0.0) | 0 (0.0) | 1.000 | | |
| CC | 0 (0.0) | 2 (4.3) | 1.000 | | |
| **rs3087243 G/A** | | | | | |
| AA | 4 (26.7) | 11 (23.9) | 1.000 | | |
| AG | 8 (53.3) | 21 (45.7) | 0.826 | | |
| GG | 3 (20.0) | 14 (30.4) | 0.523 | | |
| **rs11571319 G/A** | | (N = 41) | | | |
| AA | 2 (13.3) | 2 (4.9) | 0.288 | | |
| **AG*** | **0 (0.0)** | **15 (36.6)** | **0.0054** | **0.055** | **0.0031–0.9875** |
| GG | 13 (86.7) | 24 (58.5) | 0.061 | | |
| | | | | | |
| **Allele** | **Allele frequencies** | **Allele frequencies** | | | |
| | **n (%)** | **n (%)** | | | |
| **rs182533364 C/T** | | | | | |
| C | 29 (96.7) | 90 (97.8) | 1.000 | | |
| T | 1 (3.3) | 2 (2.2) | | | |
| **rs345031880 T/C** | | | | | |
| T | 15 (100.0) | 88 (95.7) | 1.000 | | |
| C | 0 (0.0) | 4 (4.3) | | | |
| **rs3087243 G/A** | | | | | |
| G | 14 (46.7) | 49 (53.3) | 0.676 | | |
| A | 16 (53.3) | 43 (46.7) | | | |
| **rs11571319 G/A** | | 2 *N* = 82 | | | |
| G | 26 (86.7) | 63 (76.8) | 0.302 | | |
| A | 4 (13.3) | 19 (23.2) | | | |

* rs11571319 AG genotype is protective probably in a codominant model, $p_{corr.} = 0.0162$. Chaouya controls: **rs11571319 AG genotype**: 12/48 = 25.0%, p = 0.05; **GG genotype**: 35/48 = 72.9%, **AA genotype**: 1/48 = 2.1%. Allele A: 14.6% G = 85.4%.

**Table 5**
- Correlation between CT60 gene polymorphisms and susceptibility to SPA disease with peripheral joints pain.

| Variable CTLA-4 CT60 | Peripheral joints, males (n = 12) | Specific HCTR (n = 46) | P value | OR= | 95% CI |
|---|---|---|---|---|---|
| **Genotypes** | | | | | |
| **rs182533364 C/T** | **n (%)** | **n (%)** | | | |
| CC | 12 (100.0) | 44 (95.7) | 1.000 | | |
| CT | 0 | 2 (4.3) | 1.000 | | |
| TT | 0 | 0 (0.0) | 1.000 | | |
| **rs345031880 T/C** | | | | | |
| TT | 11 (91.7) | 44 (95.7) | 1.000 | | |
| TC | 1 (8.3) | 0 (0.0) | 0.206 | | |
| CC | 0 (0.0) | 2 (4.3) | 1.000 | | |
| **rs3087243 G/A** | | | | | |
| **AA** | **7 (58.3)** | **11 (23.9)** | **0.035** | **4.455** | **1.174–16.889** |
| AG | 3 (25.0) | 21 (45.7) | 0.324 | | |
| GG | 2 (16.7) | 14 (30.4) | 0.479 | | |
| **rs11571319 G/A** | | (N = 41) | | | |
| AA | 2 (16.7) | 2 (4.9) | 0.217 | | |
| **AG*** | **0 (0.0)** | **15 (36.6)** | **0.012** | **0.068** | **0.0038–1.2372** |
| GG | 10 (83.3) | 24 (58.5) | 0.189 | | |
| | | | | | |
| **Allele** | **Allele frequencies, n (%)** | **Allele frequencies, n (%)** | | | |
| **rs182533364 C/T** | | | | | |
| C | 24 (100.0) | 90 (97.8) | 1.000 | | |
| T | 0 | 2 (2.2) | | | |
| **rs345031880 T/C** | | | | | |
| T | 23 (95.8) | 88 (95.7) | 1.000 | | |
| C | 1 (4.2) | 4 (4.3) | | | |
| **rs3087243 G/A** | | | | | |
| G | 7 (29.2) | 49 (53.3) | 0.061 | | |
| **A** | 17 (70.8) | 43 (46.7) | | | |
| **rs11571319 G/A** | | 2 N = 82 | | | |
| G | 20 (83.3) | 63 (76.8) | 0.584 | | |
| A | 4 (16.7) | 19 (23.2) | | | |

* AG genotype, rs11571319 SNP: pcorr. = 0.036.

patients where equilibrium was instead doubtful ($p$ = 0.049, sd = 0.00021 allele G: 48.8%,$T$ = 51.2%).

### 3.5. Association of CT60 haplotypes with different SPA pathologies

Four-sites CT60 haplotypes (rs182533364 C/T, rs345031880 T/C, rs3087243 G/A, rs11571319 G/A) were estimated for SPA patient population and the whole group of healthy controls (Table 6). Noteworthy, the CTGA haplotype had an increased incidence in patients with sacroiliitis (Table 7) in comparison with controls (27.9% vs. 15.7% $p$ = 0.046 OR = 2.077), while the CTAA haplotype was more frequent in pSpA patients compared with controls (7.1% vs. 1.1%, $p$ = 0.079 OR = 7.33) Table 8**.** The Expectation Maximum algorithm showed a linkage disequilibrium between the rs3087243 and rs11571319 SNPs in SPA patients ($p$ = 0.0178, Delta = 32.59%, $X^2$ = 5.616).

## 4. Discussion

This study is a preliminary investigation on some polymorphic genes involved in the pathogenesis of rheumatic diseases on Moroccan population, little studied until now. Despite recent studies reported a predominant influence of HLA-B*27 alleles in patients with AS from North Africa, our case-control genetic analysis on Moroccan patients with spondyloarthropathies highlighted also the role of other HLA-B alleles on the disease risk. Particularly, a protective role of HLA-B*45:01 allele and a higher risk from some HLA-B*51 variants and the B*52:01:01 allele were observed in our patients cohort, in addition to the effect of HLA-B*27 alleles. We have considered the HLA-B*51 and B*52 alleles together because they differ only by two amino acids in the helical region of the alpha 1 domain (in codon 63, B*51 alleles Asn vs. B*52 Glu; codon 67 B*51 alleles Phe vs. B*52 Ser) and because the B*52 antigen is also involved in other inflammatory immune diseases, such as Takayasu arteritis and enteropathic colitis in adult patients[Zaldivar Villon et al., 2019; Ino et al., 2022]. A possible explanation of the positive genetic

**Table 6**
– Haplotype frequencies of CT60 gene polymorphisms (CTLA-4) in Moroccan SPA patients (n = 84) and healthy controls.

| CT60 Haplotype 4 SNPs | | | | SPA PATIENTS (2n = 168) | | MOROCCAN HCTRs (2n = 178) | | P= | OR= |
|---|---|---|---|---|---|---|---|---|---|
| rs182533364 (C/T) | rs345031880 (T/C) | rs3087243 (G/A) | rs11571319 (G/A) | N | HF° | N | HF | | |
| **C** | **T** | **A** | **G** | 85 | 0.5036 | 89 | 0.5001 | 0.997 | |
| **C** | **T** | **G** | **G** | 43 | 0.2582 | 48 | 0.2669 | 0.867 | |
| **C** | **T** | **G** | **A** | 34 | 0.2000 | 29 | 0.1630 | 0.4172 | |
| **C** | **C** | **G** | **G** | 1 | 0.0059 | 4 | 0.0193 | 0.372 | |
| **T** | **T** | **A** | **G** | 3 | 0.0178 | 4 | 0.0199 | 1.000 | |
| **C** | **C** | **G** | **A** | 0 | 0 | 2 | 0.0143 | 0.499 | |
| **C** | **T** | **A** | **A** | 2 | 0.0142 | 1 | 0.0080 | 0.613 | |
| **T** | **T** | **G** | **G** | 0 | 0 | 1 | 0.0081 | 1.000 | |

°HF = Maximum-likelihood haplotype frequencies.

**Table 7**
– Haplotype frequencies of CT60 gene polymorphisms (CTLA-4) in Moroccan SPA patients with sacroiliitis (AS, $n = 34$) and healthy controls (HCTR, $n = 89$).

| CT60 Haplotype 4 SNPs | | | | AS (2n = 68) | | MOROCCAN HCTRs (2n = 178) | | P= | OR= | 95%CI |
|---|---|---|---|---|---|---|---|---|---|---|
| rs182533364 (C/T) | rs345031880 (T/C) | rs3087243 (G/A) | rs11571319 (G/A) | N | % HF° | N | % HF | | | |
| C | T | A | G | 33 | 48.5 | 89 | 50.0 | 0.949 | | |
| C | T | G | G | 15 | 22.1 | 48 | 27.0 | 0.531 | | |
| | | | | 11 | 21.2 | | | 0.506 | | |
| **C** | **T** | **G** | **A** | 19 | **27.9** | 28 | 15.7 | **0.046*** | **2.077** | 1.0673–4.0428 |
| | | | | M:16 | **30.8** | | | **0.014** | **2.521** | 1.229–5.170 |
| C | C | G | G | 0 | 0 | 3 | 1.7 | 0.563 | | |
| T | T | A | G | 1 | 1.5 | 4 | 2.2 | 1.000 | | |
| C | C | G | A | 0 | 0 | 3 | 1.7 | 0.563 | | |
| C | T | A | A | 0 | 0 | 2 | 1.1 | 1.000 | | |
| T | T | G | G | 0 | 0 | 1 | 0.6 | 1.000 | | |

*$Chi^2 = 3.990$; M: male individuals. °HF = Maximum-likelihood haplotype frequencies.

**Table 8**
– Haplotype frequencies of CT60 gene polymorphisms (CTLA-4) in Moroccan SPA patients with peripheral joint pain (pSPA, n = 13) and healthy controls.

| CT60 Haplotype 4 SNPs | | | | pSPA (2n = 26) | | MOROCCAN HCTRs (2n = 178) | | P= | OR= | 95%CI |
|---|---|---|---|---|---|---|---|---|---|---|
| rs182533364 (C/T) | rs345031880 (T/C) | rs3087243 (G/A) | rs11571319 (G/A) | N | % HF° | N | % HF | | | |
| C | T | A | G | 15 | 53.6 | 89 | 50.0 | 1.000 | | |
| C | T | G | G | 5 | 17.9 | 48 | 27.0 | 0.480 | | |
| C | T | G | A | 3 | 10.7 | 28 | 15.7 | 0.773 | | |
| C | C | G | G | 1 | 3.6 | 3 | 1.7 | 0.423 | | |
| T | T | A | G | 0 | 0 | 4 | 2.2 | 1.000 | | |
| C | C | G | A | 0 | 0 | 3 | 1.7 | 1.000 | | |
| **C** | **T** | **A** | **A** | 2 | **7.1** | 2 | **1.1** | **0.079** | **7.333** | 0.9867–54.5051 |
| T | T | G | G | 0 | 0 | 1 | 0.6 | 1.000 | | |

°HF = Maximum-likelihood haplotype frequencies.

association of HLA-B*27 and HLA-B*51,52 alleles with the pathogenesis of such autoimmune diseases might be due, at least in part, to the presence of particular P97 amino acids on the altered HLA-B free heavy chain (FHC) expression and the different interaction between various HLA-B alleles and the rs30187-T variant of ERAP1 (Endoplasmatic Reticulum Aminopeptidase) locus. ERAP1 is able to trim peptides in ER in the correct length for binding to MHC class I molecules on APC and activate $CD8^+$ T cells [Cortes et al., 2015]. *Chen* et al. provided findings about a possible association of P97 amino acids and AS by altering the free heavy chain (FHC) expression, a soluble form of HLA-class I molecule [Chen et al., 2017]. In particular, the pathogenic role of HLA-B*51,52 antigens would be linked to presence of the threonine in position 97 (P97) that, like the asparagine in the HLA-B*27 molecule, lies in the floor of the HLA-B peptide-binding groove and increases cell surface FHC expression. The P97 residues might contribute to SpA pathogenesis through altering the strength of association of molecule with b2-microglobulin, favoring the dissociation of HLA class I molecules. The protective role of HLA-B*45:01 allele towards SpA would be correlated on the contrary to the neutral presence of an arginine, not altering this FHC expression.

However, the biological mechanisms by which HLA-B27 alleles (and also HLA-B51,52) confer risk of disease remain uncertain. Two ways to act are known, a canonical mechanism, based on their proprieties into the adaptive immune system of presenting arthritogenic peptides found in the inflammation site in AS to CD8+ T lymphocytes, and a non-canonical mechanism in which HLA-B27 misfolding is associated with a reduced gut immunity, which permits a migration of bacteria across the intestinal mucosa and that induces the production of pro-inflammatory cytokines and development of AS [Sorrentino et al., 2014].

The anomalous association of SPA diseases in Morocco with other HLA-B alleles is also explainable by the fact that there is a different geographic distribution of the HLA-B27 molecule worldwide linked to a latitude-related gradient inverse to that of malaria endemic, probably due to a negative selective pressure exerted by P. falciparum. The frequency of HLA-B27 is low in those areas where malaria is endemic and high in those preserved [Mathieu et al., 2008]. Particularly, in our study the higher frequency of these B*51,52 alleles was detected in patients with thoracic spine pain and sacroiliitis. The Spearman's r-test showed that B*51,52 alleles are negatively related to ankylosing spondylitis, but positively to AxSpA, maybe for their different contribution to inflammation in two SPA pathologies. HLA-B51 is a genetic marker strongly associated with other inflammatory diseases, such as Behçet's disease (BD) [Giza et al., 2018] or Reiter's syndrome [Siala et al., 2009], which share common features with polyarthritis. Recently, HLA-B51 positivity has been also described in patients with AS spondyloarthropathies negative for HLA—B27. In addition, Lim MJ presented a case report of familial AS occurrence in Korea related to HLA-B51 [Lim et al., 2022].

To further investigate the mechanisms linked to the aberrant immune response by T lymphocytes to bacterial or self-antigens observed in SpA diseases, often involving HLA-B27 molecule, we analyzed genetic polymorphisms in the CTLA-4, responsible for down-regulating immune responses, induction of apoptosis and immunological anergy. In the South Moroccan population an association of the CTLA4–1661 G allele sited in the promoter region was evidenced with type 1 diabetes mellitus T1D [Bouqbis et al., 2003].

Because a soluble form of CTLA-4 resulting from an alternative splicing was found increased in several autoimmune diseases [Toussirot et al., 2009; Simone et al., 2014], we evaluated the gene polymorphisms of 3′ untranslated region (UTR), responsible of the expression regulation of this molecule in order to examine their role in development of these SpA pathologies. Currently, there is a limited knowledge about the CT60 genetic variability data in SpA diseases in North African populations. Dahmani CA showed in Algerian population that the CTLA4 SNP

CT60*G allele increased susceptibility to SPA in HLA-B27-negative individuals [Dahmani et al., 2018]. Our choice to use the SBT technology for CT60 SNPs typing has provided a more detailed information than that achieved by previous studies. For this reason, we investigated genetic variability sited in this CT60 regulatory region in SPA patients with different rheumatologic symptoms, comparing it with that of Moroccan healthy population.

As for the CT60 SNPs examined in this study, it's known that rs3087243 polymorphism leads to a transition from A to G at the position 60 of the 3′UTR [Song et al., 2013], located 279 base pairs downstream of the 3′ major polyadenylation site [Malquori et al., 2008]. In North Africa, such variant was investigated in Algerian AS patients [Dahmani et al., 2018] and a significantly higher incidence of allele G was shown in Algerians, in comparison to Moroccans (70.0% vs 46.4%, $p = 0.0001$), while research on healthy populations showed a similar frequency.

Our study evidenced that A allele of rs11571319 SNP is a risk factor for SPA disease with sacroiliac pain (OR = 2.756), particularly in affected males. Instead, in patients with thoracic spine pain and with peripheral joints pain (AxSPA) the A/G genotype of the rs11571319 is protective ($p_{corr.} = 0.162$, OR = 0.055; $p_{corr.} = 0.036$, OR = 0.068, respectively), probably according a codominant model. Chen et al. showed similar correlations (AG genotype related to a lower risk of RA, AA genotype to an increased risk of developing it) in patients with rheumatoid arthritis [Chen et al., 2023]. From the literature it's known that such A allele of rs11571319 is also implicated in other autoimmune diseases, such as asthma [Choi et al., 2017]. CTLA4 might promote Th2 cell activation in this pathology.

In addition, we observed that the A/A genotype of the rs3087243 SNP is predisposing in peripheral joints pain patients, with a 4-fold increase in risk. Even if the A/A genotype of rs3087243 was found to be protective towards other autoimmune diseases, like in type 1 autoimmune hepatitis in the Tunisian population [Chaouali et al., 2017], however, it is associated with an increased level of solCTLA-4, that could cause increase cellular T reactivity by interfering with the B7-fl CTLA-4 interaction.

Regarding the different results of the same SNP on different populations, it might be explained by different genetic heterogeneity and interaction with environmental factors. Also the linkage disequilibrium might influence the different results of associations, these CTLA-4 SNPs being the cause of the risk of disease alone or in haplotype (for example CTGA haplotype more frequent in sacroiliitis group) or interacting with other genes, not yet identified. With this analysis we may confirm what already described in previous studies regarding a higher genetic diversity, lower LD values and higher heterogeneity in Africans than elsewhere [Tishkoff et al., 1996; Ramírez-Soriano et al., 2005].

Concerning the FCGR3A-158 G/T (V/F) polymorphism, we found a trend to a predisposing role of the heterozygous G/T genotype (V/F) in AxSPA Moroccan patients (thoracic spine symptoms) compared with controls, also confirmed by regression statistics, while the T/T (F/F) homozygous was protective. It's known that FCGR3A variants regulate the ADCC response, involved in such inflammatory diseases. In fact, Fc-gamma receptors play a key role in various infection diseases [Neisseria Meningitis (Fijen et al., 2000), Meningococcal disease (Domingo et al., 2002), Mycobacterium tuberculosis (Maglione et al., *2008*)], as wells as in autoimmune diseases [Kyogoku et al., 2004].

Our data also indicate that gender can influence these SPA pathologies, probably acting at the genetic level in a different manner on affected males and women in AS and peripheral joints SPA diseases.

This is also supported by Dahmani CA et al., who evaluated in the Algerian population the impact of copy number variations (CNVs) of associated AS genetic risk factors, such as FCG3A and FCGR3B. They found a different frequency distribution of FCGR3A CNVs between cases and controls after gender and age stratifications [Dahmani et al., 2019]. Regarding the robustness of the data analysis, we recognize the relatively smaller sample size in our study which may limit the statistical power to detect a small increase in the risk. However, the analysis for genic variability took into account the prevalence of various SPA pathologies in Africa.

## 5. Conclusions

This is a preliminary study that has investigated at *high resolution* a panel of genes in Moroccan patients suffering from various types of spondyloarthropathies and healthy controls. Our results encourage to further explore the role of these polymorphisms as new diagnostic biomarkers and targets for immunotherapy. The goal for the near future will be to arrive at a better understanding of the mechanisms of the disease, identifying more appropriate therapeutic approaches associated with a more favorable long-term prognosis for patients.

## Funding

Partial financial support was received from Bilateral Agreement CNR/CNRST "Role of genetic polymorphism in susceptibility and pathogenesis of celiac disease and autoimmune spondyloarthropathies in patients from Morocco", and by National Research Council ref. DSB. AD007.043.

## CRediT authorship contribution statement

**Angelica Canossi:** Writing – review & editing, Writing – original draft, Supervision, Investigation, Funding acquisition, Conceptualization. **Khadija Oumhani:** Writing – review & editing, Project administration, Methodology, Funding acquisition, Conceptualization. **Tiziana Del Beato:** Methodology, Investigation. **Pierluigi Sebastiani:** Methodology, Investigation. **Alessia Colanardi:** Methodology, Investigation. **Anna Aureli:** Writing – review & editing, Supervision, Project administration, Investigation, Conceptualization.

## Declaration of competing interest

The authors have no relevant financial or non-financial interests to disclose.

## Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request and also included as supplementary information files.

## Acknowledgements

This study was partly supported by CNR/CNRST Bilateral Agreement Italia-Morocco. We thank Dr. Khadija Oumhani for her technical and scientific support.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.humgen.2024.201290.

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