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Association of the HLA-B27 antigen and the *CTLA4* gene *CT60/rs3087243* polymorphism with ankylosing spondylitis in Algerian population: A case–control study

C. A. Dahmani[1] | A. Benzaoui[2] | H. Amroun[3] | F. Mecabih[3] | F. Z. Sediki[1] | F. Zemani-Fodil[1] | M. Fodil[1] | W. Boughrara[1] | B. Mecheti[3] | N. Attal[3] | N. Mehtar[1] | E. Petit-Teixeira[4] | A. Boudjema[1]

[1]Laboratoire de Génétique Moléculaire et Cellulaire (LGMC), Université des Sciences et de la Technologie d'Oran-Mohamed Boudiaf (USTO-MB), Oran, Algeria

[2]Service de Rhumatologie, Centre Hospitalo-Universitaire (CHU) d'Oran, Oran, Algeria

[3]Laboratoire d'Immunogénétique et de Transplantation, Département d'Immunologie, Institut Pasteur d'Algérie à, Alger, Algeria

[4]Laboratoire Européen de la Polyarthrite Rhumatoide, Université d'Evry-Val D'Essonne, Evry, France

Correspondence
Chahinez Amira Dahmani, Université des Sciences et de la Technologie d'Oran, Mohamed-Boudiaf (USTO-MB), Oran, Algérie.
Emails: chahinez.dahmani@univ-usto.dz and chahinez.dahmani23@gmail.com

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Summary

Ankylosing spondylitis (AS) is a complex inflammatory disease that represents a major health problem both in Algeria and worldwide. Several lines of evidence support that genetic risk factors play a role in AS etiology and the *CTLA4* gene has attracted a considerable attention. In this study, we were interested in evaluating the *HLA-B27* frequency and in exploring the *CTLA4* gene in a sample of the North African population. The dataset of the current study is composed of 81 patients with AS and 123 healthy controls. All samples were genotyped by TaqMan®allelic discrimination assay. The genetic risk of the *HLA-B27* specificity and the *CTLA4/CT60* polymorphism were assessed by odds ratios (OR) with 95% confidence intervals (CI). High spondylitis risk was detected for *HLA-B27* allele (OR= 14.62, $p = 10^{-6}$) in addition to a significant association of the *CT60**G allele (OR= 1.89, $p = .002$). After gender and age stratifications, the association of the *CT60**G allele was still significant in females sample (OR= 2.10, $p = .001$) and when age up to 30 years (OR = 2.21, $p = .008$). Interestingly, the *CT60**G allele revealed an increased spondylitis risk in the B27 negative group (OR= 2.81, $p = .006$). The present work showed in West Algerian population that the *HLA-B27* antigen and the variation in the *CTLA4* 3′UTR region played an important role in the ankylosing spondylitis susceptibility. The heterogeneity of this disease is deduced by genetic difference found between B27+ and B27− groups.

KEYWORDS
Algerian population, ankylosing spondylitis, *CT60* polymorphism, *CTLA4*, *HLA-B27*

1 | INTRODUCTION

The ankylosing spondylitis (AS) is a prototype of inflammatory diseases group formerly known as spondyloarthropathies (SpA). It has been shown that the AS disease is more frequently present in men than in women at a ratio of 2:1. It often occurs between 20 and 30 years, but sometimes in adolescence or later in life (Feldtkeller, Khan, van der Heijde, van der Linden, & Braun, 2003). It is a progressive disease that affects 0.5% of the European population, 0.2% of the Asian, and between 0.2%–0.5% in American, but it is uncommon among Africans (Ng et al., 2007; Reveille et al., 2010). It appears that the spondylitis frequencies difference depends mostly on the *Human Leucocyte Antigen-B27* (HLA-B27) frequency in populations. Indeed, the study of Piazza, Menozzi, and Cavalli-Sforza (1980) showed that the HLA-B27 allele frequency follows a decreasing north–south geographic gradient (Piazza et al., 1980). In fact, it

is true that for a long time several hypotheses have been issued to explain the role of the *HLA-B27* gene in the onset of the spondylitis disease (Rysnik et al., 2016). Otherwise, the studies suggested the involvement of other genes not belonging to the HLA system.

The balance between stimulatory and inhibitory co-signals determines the ultimate nature of T-cell responses. Ankylosing spondylitis is an autoimmune disease and the imbalance of peripheral tolerance is identified in its pathogenesis. Many studies have shown that the patients with AS experienced higher numbers of circulating CD4+ T cells and CD8+ T cells than the healthy subject (Schirmer et al., 2002). The T-cell activation mediated by the T-cell receptor (TCR) complex after antigen recognition requires co-stimulation by co-receptors such as CD28 and *cytotoxic T-lymphocyte antigen-4* (CTLA-4) (Alegre, Frauwirth, & Thompson, 2001). The CD28 receptor provides positive signals that promote T-cell response while CTLA-4 transmits inhibitory signals to attenuate T-cell activation by competing for the B7 ligands with its homologue CD28 (van der Merwe, Bodian, Daenke, Linsley, & Davis, 1997).

Genome-wide scans have implicated regions contributing in the spondylitis genetic risk on chromosomes 2q, 6p, 6q, 10q, 11q, 16q, 17q and 19q (Brown et al., 1998; Laval et al., 2001). The *CTLA4* gene is located on chromosome 2q33 and encodes a protein actively involved in regulating T-cell activation (Dariavach, Mattei, Golstein, & Lefranc, 1988). *CTLA4* has become one of the main genes of research interest for association studies and has been considered as a target for immunotherapy because it has a crucial role in immunological homeostasis (Scalapino & Daikh, 2008). It consists of four exons, the alternative splicing of the CTLA-4 transcript generates three forms, a complete trans-membrane form (exons 1–4), a soluble CTLA-4 (sCTLA-4) (lacking exon 3) and a short form encoded only for exons 1 and 4 (Dariavach et al., 1988; Oaks et al., 2000). Up to now, among hundreds of *Single Nucleotide Polymorphisms* (SNPs) tested in *CTLA4* gene, three of them have been associated with high AS risk (*−318*C/T*, *+49*A/G*, and *CT60*) (Kristiansen, Larsen, & Pociot, 2000). The *CT60 (rs3087243)* polymorphism leads to a transition from A to G at the position 60 of the 3′UTR (Song, Kim, & Lee, 2013) located 279 base pairs downstream of the 3′ major polyadenylation site (Malquori, Carsetti, & Ruberti, 2008). The Ueda's report indicated the influence of the *CT60* SNP in the soluble CTLA-4 splicing and production (Ueda et al., 2003). The association of the *CT60**G allele with lower mRNA levels of the sCTLA-4 isoform was also observed (Ueda et al., 2003). Furthermore, the study of Toussirot et al. (2009) showed that the sCTLA-4 isoform rate was found higher in a SpA group compared to controls (Toussirot et al., 2009). These findings suggest that the soluble CTLA-4 plays an immune-genetic role in the activation and the regulation of the T cell. Also, the *CT60* SNP was reported to confer risk for autoimmune diseases in numerous studies such as Type 1 Diabetes (T1D) (Spoletini et al., 2013), systemic lupus erythematous (SLE) (Torres et al., 2004), Grave's disease (GD) (Kavvoura et al., 2007) and rheumatoid arthritis (RA) (Farago, Kisfali, Magyari, Polgar, & Melegh, 2010). It was recently associated with the AS risk in a Chinese study (Wang, Wang, Tan, Wang, & Yuan, 2015). However, results are controversial, some findings could not reveal any association (Barton et al., 2004; Pincerati, Dalla-Costa, & Petzl-Erler, 2010) such as a recent meta-analysis (Chen et al., 2016). In this case–control study, we investigated the *CTLA4* CT60 polymorphism and the *HLA-B27* allele genotyping. This is the first time that such association study was performed in spondylitis patients living in the North African population.

2 | MATERIALS AND METHODS

2.1 | Study population

A total of 81 patients diagnosed with AS (37 males and 44 females) were randomly selected and treated in the Rheumatology Service at the Oran University Hospital Center (CHU) in the diagnosis age range of 10–72 years. The diagnosis of spondylitis patients was made based on the Amor and the European Spondylarthropathy Study Group (ESSG) criteria (Amor, Dougados, & Mijiyawa, 1990; Dougados et al., 1991). Clinical characteristics including the age at the initial symptom, the HLA-B27 status, the SpA family history and the medication history were summarized in Table 1. The control group selected in the same geographical areas as cases was composed of 123 healthy subjects (51 males and 72 females) in the age range of 20–50 years, which have never suffered from any rheumatologic or inflammatory diseases.

All enroled patients and controls in this study were unrelated. Informed consent was obtained from all participants before sampling and the experiment was supported by the Oran University Hospital Center's Ethics Committee. The patients and control groups were stratified in according to age and gender (Tables 3 and 4). For age stratification, two groups were set as follow: under 30 years and over 30 years, as the AS disease occurs often before 30 years (Feldtkeller et al., 2003). Furthermore, we have stratified the CT60 allele's distribution into two groups, HLA-B27 negative (HLA-B27–) and HLA-B27 positive (HLA-B27+) individuals in Table 5.

2.2 | DNA isolation

Approximately 35 ml of peripheral blood samples were collected in EDTA tubes and stored at −20°C until analysis. The DNA was isolated from peripheral white blood cells by a standard manual salting-out method (Miller, Dykes, & Polesky, 1988).

2.3 | SNP genotyping

Firstly, the HLA-B generic typing was performed by real-time polymerase chain reaction (GeneFinder HLA-B27 RealAmp kit). All samples underwent at least two reactions to confirm the genotypes. Then, the *CTLA4 CT60* SNP (rs3087243) was genotyped using the TaqMan SNP 5′-Allelic Discrimination (AD) assay (Applied Biosystems, Foster City, CA). Two reference samples (from CEPH "Centre d'Etude du Polymorphisme Humain" families, France) were

co-genotyped for each experiment to a reproductive data. Moreover, 10% of randomly chosen samples were genotyped a second time in an independent experiment to avoid compliance issues.

2.4 | Statistical analysis

Statistical description of continuous variables was indicated by a mean and a standard error (SE); frequencies and percentages were used for categorical data. The distribution of the *CTLA4 CT60* SNP among controls was analysed according to Hardy–Weinberg equilibrium (HWE). The distribution of the demographic variables (age, gender) and the polymorphism frequencies distribution between cases and controls were performed by the Pearson's chi-square (χ^2) test and *p* values were considered significant when $p < .05$. The genotypic and allelic distributions of the *CT60* polymorphism were assessed by the odds ratios (ORs) and 95% confidence intervals (CIs).

The multivariate analysis of age of diagnostic (> and ≤30 years), gender (female/male), HLA-B27 status (presence or absence) and the AS clinical characteristics (including the CRP test, the SPA family history, and the Uveitis status) stratifications were performed using the Statistical Package for Social Sciences (SPSS) software version 22.0 for Windows (SPSS Inc., Chicago, IL).

3 | RESULTS

The demographical and clinical characteristics of 81 patients and 123 controls are summarized in Table 1. In this study, the sex ratio was 44 females per 37 males in patients with AS while it was 72 females per 51 males in the controls. The mean age of the cases was 39.80 ± 1.6; while as mean age of the healthy group was 30.02 ± 0.95. There was no statically significant difference in mean age and gender distributions between cases and controls, indicating a well-matched study population ($p > .05$). Interestingly, AS frequency was higher among individuals more than 30 years compared to those less than 30 years. It was a statically significant difference between the two age groups ($p < .05$). Comparing the AS frequency between males and females, females were more commonly affected than males (70% vs. 30%). As expected, the *HLA-B27* allele frequency distribution was higher in our patients than controls ($p = 10^{-6}$) (results not shown). The clinical syndromes of patients with AS were presented, which 42% of patients were diagnosed with uveitis, 63% with peripheral arthritis and the majority (63%) of patients have the mixt form (axial and peripheral). We have observed that the frequency of positive CRP test (C-reactive protein) in patients with AS was very higher (73%).

Genotyping experiments are reproducible as the 10% randomly samples genotyped twice revealed no discrepancy. The distribution of allele and genotype frequencies of the *CT60* SNP between patient and control groups is presented in Table 2. At first, allelic distribution was consistent with the assumption of the HWE in controls ($p > .05$). There were significant differences in the frequencies distribution of the *CT60**G allele (OR = 1.89 [1.26–2.84], $p = .002$) and also of

TABLE 1 Demographic and clinical characteristics of AS patients and healthy controls

Subject characteristics	AS patients (*N* = 81)	Healthy control (*N* = 123)
Gender*		
Females, *n* (%)	44 (54)	72 (58)
Males, *n* (%)	37 (46)	51 (42)
Age* (mean ± SE), years	39.80 ± 1.6	30.02 ± 0.95
≤30, *n* (%)	24 (30)	86 (70)
>30, *n* (%)	57 (70)	37 (30)
HLA-B27*		
HLA-B27+, *n* (%)	42 (52)	7 (5)
HLA-B27–, *n* (%)	39 (48)	116 (95)
Family history		
Presence, *n* (%)	59 (73)	
AS forms		
Axial form, *n* (%)	22 (27)	
Peripheral form, *n* (%)	8 (10)	
Mixt form, *n* (%)	51 (63)	
Clinical features		
Age of symptom (mean ± SE), years	31.40 ± 1.6	
Disease duration (mean ± SE), years	8.61 ± 0.9	
Diagnosis delay (mean ± SE), years	4.16 ± 0.7	
Clinical syndromes		
Uveitis, *n* (%)	34 (42)	
Peripheral arthritis, *n* (%)	51 (63)	
Deformation (kyphosis), *n* (%)	14 (17)	
Laboratory test		
BASDAI (mean ± SE), cm	3.6 ± 0.2	
BASFI (mean ± SE), cm	3.9 ± 0.25	
SGOT (mean ± SE), UI	22.74 ± 1.87	
SGPT (mean ± SE), UI	23.24 ± 2.3	
ESR (mean ± SE), mm/hr	35.95 ± 2.4	
CRP >6 mg/L, *n* (%)	59 (73)	
Medication history		
NSAIDs use, *n* (%)	65 (80)	
NSAIDs sensibility, *n* (%)	55 (84)	
DMARDs use, *n* (%)	35 (43)	

The data are presented as the mean ± standard error; *n*, number; %, frequency; *$p < .05$ considered as statistically significant.
AS, ankylosing spondylitis; HLA-B27, human leukocyte antigen-B27; BASDAI, bath ankylosing spondylitis disease activity index; BASFI, bath ankylosing spondylitis functional index; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; SGOT, serum glutamooxaloacetate transferase; SGPT, serum glutamopyruvate transferase; NSAIDs, non-steroidal anti-inflammatory drugs; DMARDs, disease-modifying anti rheumatic drugs.

TABLE 2 The distribution of allele and genotype frequencies of the *CT60* SNP (*rs3087243*) between ankylosing spondylitis (AS) patients and controls

	AS patients *N* = 81 (%)	Controls *N* = 123 (%)	OR [CI]	*p* value
Genotypes				
*CT60**AA	8 (10)	35 (30)	–	–
*CT60**AG	41 (50)	55 (50)	–	–
*CT60**GG	32 (40)	33 (20)	1.77 [0.98–3.21]	.004
*CT60**AG+GG	73 (90)	88 (70)	**3.46 [1.54–7.79]**	**.001**
Alleles				
*CT60**A	57 (30)	125 (51)	–	–
*CT60**G	105 (70)	121 (49)	**1.89 [1.26–2.84]**	**.002**

The values are presented as genotypes and alleles number (*N*) and frequency in percentage (%). *p*, significance; OR, odds ratio; CI, 95% confidence interval. Bold *p* values mean a significant association with $p < .05$ and OR > 1 [CI 95%].

TABLE 3 The distribution of the allele and genotype frequencies of the *CT60* SNP (*rs3087243*) after gender and age stratifications

	Female group		Statistical analysis		Age >30 years old group		Statistical analysis	
	Patients *N* = 44 (%)	Controls *N* = 72 (%)	OR [CI]	*p* values	Patients *N* = 57 (%)	Controls *N* = 37 (%)	OR [CI]	*p* values
Genotypes								
*CT60**AA	3 (7)	21 (29)	–	–	4 (7)	12 (32.5)	–	–
*CT60**A G	21 (48)	34 (46)	–	–	30 (53)	15 (40.5)	–	–
*CT60**GG	20 (45)	18 (25)	**2.51 [1.14–5.52]**	**.006**	23 (40)	10 (27)	1.78 [0.74–4.32]	.006
*CT60**AG+GG	41 (93)	52 (71)	**4.85 [1.46–16.1]**	**.004**	53 (93)	25 (67.5)	**5.82 [1.80–18.89]**	**.001**
Alleles								
*CT60**A	27 (30)	77 (52)	**–**	**–**	38 (33)	39 (53)	–	–
*CT60**G	61 (70)	71 (48)	**2.42 [1.39–4.21]**	**.001**	76 (67)	35 (47)	**2.21 [1.22–4.01]**	**.008**

The values are presented as genotypes and alleles number (*N*) and frequency in percentage (%). *p*, significance; OR, odds ratio; CI, 95% confidence interval. Bold *p* values mean a significant association with $p < .05$ and OR > 1 [CI 95%].

the *CT60**(AG+GG) combined genotypes (OR = 3.46 [1.54–7.79], $p = .001$) between cases and controls.

To better study the effect of the difference observed between female and male patients (Table 1), we explored age and gender stratification analysis; results were summarized in Table 3. Firstly, there was a significant difference in the allelic and genotypic distributions between female cases and controls [*CT60**G allele (OR= 2.42 [1.39–4.21], $p = .001$) and *CT60**(GG+AG) genotypes (OR= 4.85 [1.46–16.13], $p = .004$)] (Table 3). Secondly, we also showed in the group of age >30 years that the *CT60**G allele and the *CT60**(GG+AG) genotypes frequencies were significantly higher (OR= 2.21 [1.22–4.01], $p = .008$ and OR= 5.82 [1.80–18.89], $p = .001$, respectively) (Table 3). Interestingly, when we combined the results observed in Table 3, we showed a significant association between the *CT60**G* allele and the AS susceptibility in women with age >30 years (OR = 3.38 [1.40–8.19], $p = .005$) (Table 4).

Later to consider association of the HLA-B27 allele and the CT60 polymorphism, we analysed the CT60 allele's distribution into the HLA-B27− and HLA-B27+ individuals (Table 5). A significant association of the CT60*G allele with AS risk is restricted to HLA-B27− sample (OR = 2.10 [1.23–3.59], $p = .006$) while it has no effect in the B27+ sample ($p > .05$). Subsequently, the *CT60* allelic and genotypic distributions results according to the AS clinical characteristics were presented in Table 6. We observed a significant difference of the *CT60**(AG+GG) genotypes considering AS family history status (OR = 4.85 [1.46–16.13], $p = .02$) and of the *CT60**GG genotype considering the CRP test (OR = 3.72 [1.20–21.48], $p = .02$). However, no difference was found considering the uveitis status ($p > .05$).

4 | DISCUSSION

We conducted a case–control study to investigate a relationship between the HLA-B27 status, the *CTLA4 CT60* polymorphism and the AS susceptibility in a Western Algerian population. Ankylosing spondylitis is associated with the HLA system, particularly the HLA-B27 antigen. Nevertheless, the strength of this association may vary in different ethnic populations (Gonzalez-Roces et al., 1997). Our study was conducted to assess the prevalence of HLA-B27 antigen

TABLE 4 The distribution of allele and genotype frequencies of the *CT60* SNP (*rs3087243*) only in female group with age >30 years

	Females with age >30 years		Statistical analysis	
***CT60* SNP**	**Patients *N* = 39 (%)**	**Controls *N* = 14 (%)**	**OR [CI]**	***p* values**
Genotypes				
*CT60**AA	2 (5)	6 (43)	–	–
*CT60**AG	20 (51)	5 (36)	–	–
*CT60**GG	17 (44)	3 (21)	2.55 [0.66–9.86]	.003
CT60* AG+GG	37 (95)	8 (57%)	**11.47 [2.23–58.99]**	**.0007**
Alleles				
*CT60**A	24 (31)	17 (61)	–	–
*CT60**G	54 (69)	11 (39)	**3.38 [1.40–8.19]**	**.005**

The values are presented as genotypes and alleles number (*N*) and frequency in percentage (%). *p*, significance; OR, odds ratio; CI, 95% confidence interval. Bold *p* values mean a significant association with $p < .05$ and OR > 1 [CI 95%].

TABLE 5 The *CT60-CTLA4* (*rs3087243*) and *HLA-HLA-B27* alleles' distribution among patients and controls

HLA-B27 status	**HLA-B27− group**		**HLA-B27+ group**	
CTLA4 alleles	***CT60**A**	***CT60**G**	***CT60**A**	***CT60**G**
AS patients	25	53	32	52
Controls	115	115	6	10
Statistical analysis		**$p = .006$ OR = 2.10 CI = [1.23–3.59]**	NS	

The values are presented as genotypes number, *p*, significance; OR, odds ratio; CI, 95% confidence interval. Bold *p* values mean a significant association with $p < .05$ and OR > 1 [CI 95%].

TABLE 6 The *CT60* (*rs3087243*) allele and genotype distributions in the ankylosing spondylitis (AS) clinical characteristics

	SpA family history		**Uveitis status**		**CRP test**	
	Presence *N* = 59 (%)	**Absence *N* = 22 (%)**	**Presence *N* = 34 (%)**	**Absence *N* = 47 (%)**	**Positive (>6 mg/L) *N* = 59 (%)**	**Negative (<6 mg/L) *N* = 22 (%)**
Genotypes						
*CT60**AA	3 (05)	5 (23)	3 (9)	5 (11)	6 (10)	2 (9)
*CT60**AG	33 (56)	8 (36)	16 (47)	25 (53)	25 (42)	16 (73)
*CT60**GG	23 (39)	9 (41)	15 (44)	17 (36)	28 (48)	4 (18)
*CT60**AG+GG	56 (95)	17 (77)	31 (91)	42 (89)	53 (90)	20 (91)
Alleles						
*CT60**A	39 (33)	18 (41)	22 (32)	35 (37)	37 (31)	20 (45)
*CT60**G	79 (67)	26 (59)	46 (68)	59 (63)	89 (69)	34 (55)
Statistical analysis	**OR [CI] *p* values**		**OR [CI] *p* values**		**OR [CI] *p* values**	
*CT60**GG	NS		NS		**OR = 3.72 [1.18–11.72] $p = .02$**	
*CT60**(AG+GG)	**OR = 4.85 [1.46–16.13] $p = .02$**		NS		NS	
*CT60**G allele	NS		NS		NS	

The values are presented as genotypes and alleles number (*N*) and frequency in percentage (%). *p*, significance; OR, odds ratio; CI, 95% confidence interval; SpA, spondylarthropathies; CRP, C-reactive protein. Bold *p* values mean a significant association with $p < .05$ and OR > 1 [CI 95%].

among patients with AS disease living in the West of Algeria. The HLA-B27 frequency's distribution between cases and controls (OR = 14.62 [6.43–33.20]) (results not presented) suggests that this allele is involved in the predisposition to the spondylitis in the West of Algeria. The frequency of the HLA-B27 antigen already described in Algerian population was 5%. This value is similar in several Maghreb countries (3% to 5%) but is lower than observed in the Caucasian population (6% to 8%) (Sieper, Rudwaleit, Khan, & Braun, 2006). In our study, the HLA-B27 was found to be positive in 52% of the spondylitis patients, at a lower percentage compared to Caucasian of European ancestry patients (90%) (Reveille, Ball, & Khan, 2001), to Asian (61%) (Abdelrahman et al., 2012) and to Arabian (74%) (Abdelrahman et al., 2012). A study on 100 AS Algerian patients reported a frequency of 63% (Amroun et al., 2005). Equivalent HLA-B27 frequencies have been described in the North African countries: Egypt (59% (Tayel et al., 2012)), Morocco (58% (Atouf et al., 2012)) and Tunisia (43%, Sakly et al., 2009). Various ancestral genetic components in the northern Africa have been described because of a complex history of demographic events (Bekada et al., 2015). One of the most relevant ancestors of West Algerian region were the Berbers but, historical events testified to numerous invasions and migrations by the Phoenicians, the Romans, the Arabs, the Spanish, the Ottomans and the French (Bosch et al., 1997; Henn et al., 2012).

CTLA-4 (CD152) is an important co-stimulatory molecule, which can inhibit the function of T lymphocytes. Indeed, *CTLA4* gene variations might participate in genetic susceptibility to autoimmune diseases by modifying the inhibitory effect on T-cell activity. Importantly, the study of Maier and colleagues demonstrated that the *CT60*G* allele alters the signal pattern of CD4_T cells in the autoimmune diseases, suggesting a direct mechanistic link with T-cell function (Maier, Anderson, De Jager, Wicker, & Hafler, 2007). The *CT60**G allele has been extensively studied, but its frequency changes with ethnicity. In our population, the minor allele frequency (MAF) of this variant in controls group (MAF ≤50%) was found similar to those reported in European (Farago et al., 2010) Tunisian (Benmansour et al., 2010) and Chinese Han (Wang et al., 2015) populations, whereas it was different (MAF >50%) in Asian (Lei et al., 2005; Tsukahara et al., 2008) and American populations (Pincerati et al., 2010; Torres-Carrillo et al., 2013).

Considering the present genetic association study, the *CT60**G allele may be associated with the AS risk in Algerian population (Table 2). In contrast, a case–control study in Chinese Han population reported that the major allele *CT60**A was involved in the AS susceptibility (Wang et al., 2015). One reason for this discrepancy is the difference observed in the MAF of *CT60**G between populations. It was lower (MAF <50%) in the Chinese Han controls (Wang et al., 2015), but it was so higher in other Chinese and Japanese populations (MAF >50%) (Lei et al., 2005; Tsukahara et al., 2008). One reason for this discrepancy between these Asian populations could be the difference in haplotype frequencies, as the haplotype *+49*G/CT60*A* was rarely found in Japanese population whereas it was more frequent in the Chinese one (Tsukahara et al., 2008). It seems that the biological impact of genetic markers on the risk for common diseases may usually be consistent with different origins (Ioannidis, Ntzani, & Trikalinos, 2004). The ethnic differences and the genetic heterogeneity in our population may be another major reason to explain the controversial findings between the present Algerian study and the Chinese findings (Wang et al., 2015).

In this study, we observed that the AS cohort randomly recruited (n = 81) consists of slightly more women than men (54% vs. 46%). This observation is in accordance with the recent article on the Chinese population (55% vs. 45%) (Wang et al., 2015), but, it does not imply that women are more affected by the spondylitis in Algeria. Additionally, the *CT60**G allele was statistically associated in the female patients compared to females control after gender stratification in Table 3. Our result can be in accordance with the Lei's report in which it is supposed that the *CT60**G allele was associated with the RA risk in a Chinese population with a female predominance (65%) (Lei et al., 2005). The sex factor could play an important role in AS susceptibility in Algeria. On the other hand, the mean age of AS cases in our study was 39.8 years (Table 1). This observation is similar to findings from the Wang et al.'s (2015) study (36 years) (Wang et al., 2015). Interestingly, the mean age in our AS females was observed so higher (45.7 years). This observation is in accordance with findings from the Wang et al.'s (2015) among female patients with AS (49.6 years) (Wang et al., 2015). After the age-adjustment in our study, the *CT60*G* allele was associated with the AS risk in the group of age >30 years (Table 4). It seems that the age factor could play an important role in the occurrence of spondylitis disease. The involvement of the sexual hormones in the immune response has been extensively studied, particularly estrogens as much as a modulator of humoral immunity and progesterone as a natural suppressor of immunity. Indeed, a hormone-immune imbalance could contribute to the autoimmune disease's etiology (Cutolo et al., 2004). Curiously, we have observed in Table 4 that the *CT60**G allele was associated with the AS risk in the group of the females with older age (>30 years). It is also possible that AS development was declared in the oldest of females because of the hormonal disequilibrium during menopause, but further studies are required to confirm this hypothesis.

To evaluate whether the association of the *CT60* SNP is independent of the HLA B27-AS relation, we stratified the *CT60* allele's distribution into HLA-B27− and HLA-B27+ groups (Table 5). About 52% of our patients with spondylitis were HLA-B27+ whereas in Caucasian populations this value was estimated to be 90% (Reveille et al., 2001), this suggests that other genes such as *CTLA4* could have an important effect on the disease susceptibility in our population. Other studies found that several candidate gene polymorphisms are associated with AS development in HLA-B27− subgroup (Gonzalez et al., 2001; Popa et al., 2016) suggesting genetic factors with an independent effect on the overall contribution of this disease. Whether this set of genetic factors has a direct effect or represents an additive contribution in AS susceptibility remains to be determined.

For several decades, it has been known that AS disease inherited due to shared susceptibility factors. We have found in our study a significant association between the *CT60**AG+GG genotype and the SpA family history (Table 6), suggesting that the *CTLA4 CT60* SNP could be involved in AS heritability in Algerian population. Furthermore, we identified an increased risk between the *CT60**GG genotype and AS patients with a CRP up to 6 mg/L, the *CT60* SNP might therefore be associated in our population with AS disease progress. Nevertheless, our findings should be confirmed with similar studies performed on larger samples.

Ankylosing spondylitis genetic predisposition associated with *HLA-B27* antigen seems to be confirmed in our West Algerian sample. Various studies suggest that apart from of the strong association between *HLA-B27*, *ERAP*, *IL23R* genes and the AS susceptibility (Duan et al., 2012; Popa et al., 2016; Reveille et al., 2001), other genetic factors could be considered in the spondylitis disease development. To our best knowledge, this is the first time that the *CTLA4* gene association was analysed with AS risk in an Algerian population sample. Our findings suggest that the *CT60* polymorphism is involved in genetic AS susceptibility but, curiously this SNP was associated only in the female group and in older patients. Further stratification based on the HLA-B27 status showed that the *CT60*G* allele was also associated with the disease in the HLA-B27− group. This association study strengthens our understanding of the link between the inhibitory signal resulting from the CTLA-4/CD80–CD86 binding and the AS pathogenesis. However, such studies should be performed on larger populations to deal with the statistical limitations. Moreover, it is important to include other variants in *CTLA4* gene as the effect of some haplotype containing *CT60* polymorphism seems higher than the effect of this SNP alone. This has to be confirmed with haplotype study in Algerian population.

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ETHICS

The experiment was supported by the Oran University Hospital Center's Ethics Committee.

CONFLICT OF INTEREST

No conflict of interest is declared.

ORCID

C. A. Dahmani http://orcid.org/0000-0002-9529-2936

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