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ORIGINAL RESEARCH ARTICLE

HLA class II antigen (DRB1 and DQB1) and rheumatoid arthritis in a Tunisian population: Relation to autoantibodies and disease severity

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ABSTRACT

We aimed to evaluate the association of HLA DRB1 and DQB1 alleles with rheumatoid arthritis (RA), and to investigate their relationship with anti-citrullinated peptide antibodies (ACPA) and RA severity. We performed a case-control study of 81 RA patients compared with 100 healthy controls. Sociodemographic data, disease activity scores, functional impact, structural damage, ACPA, rheumatoid factor (RF), and HLA DRB1 and DQB1 alleles were determined. Forty-six patients expressed HLA DRB1, predominantly DRB1*04. These alleles were more frequent in RA patients than in controls. DRB1*04:05 was the most associated allele with RA susceptibility, 54.5% of RF-positive patients expressed DRB1*04:05 with a significant correlation. Similarly, a highly significant association with DRB1*15:01 and RF positivity was found. DRB1*01:01, 04:05, 11:01, and 15:01 were associated with the presence of ACPA. Patients with DRB1 alleles had more extraarticular manifestations (EAM). Multivariate analysis concluded that DRB1*04:05 was only associated with ACPA positivity. Regarding DQB1, DQB1*03:02 was the most associated with RA. DQB1*02:01, 03:01, 03:02, 05:01, and 06:01 were associated with RF-positive RA. DQB1*06:01 was associated with ACPA. Only HLA DQB1*02:01 and 06:01 were associated with the presence of EAM. DAS28 was reduced in the presence of DQB1. HAQ > 0.5 were correlated with DQB1 alleles. In addition, there was more structural damage in RA encoding for DQB1. In linear regression, DQB1*06:01 were associated with RF positivity, but there was no association with the other parameters. Our study confirmed that DRB1 and DQB1 expression was significantly associated with ACPA, increased HAQ, increased sharp score, and the presence of EAM.

Keywords: HLA antigen; rheumatoid arthritis; ACPA; autoantibodies; disease severity

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1. Introduction

Rheumatoid arthritis (RA) is the most common chronic inflammatory rheumatic disease[1]. Its prevalence is estimated at 0.46% of the global population[2]. This complex autoimmune disease is multifactorial and involves hormonal, environmental, immunological, and genetic factors[3].

Currently, studies are increasingly focusing on the close interaction between genetic factors and epigenetic mechanisms[4]. These genetic and environmental factors are currently considered to contribute to the susceptibility and severity of the disease. Among genetic factors, HLA genes have been associated with one-third to one-half of RA cases. Notably, encryption of the shared epitope (SE) region: HLA DRB1*01; 04; 10; 14 alleles[5].

Furthermore, it is well known that anti-citrullinated peptide antibodies (ACPA) and rheumatoid factor (RF) are very specific markers of RA[6], these antibodies can be detected even years before the onset of RA[7] and are widely used for its diagnosis.

ACPA are considered produced on a genetic basis involving HLA DRB1 alleles and environmental factors such as periodontal disease and smoking[8]. However, its pathogenic role in RA has not yet been fully clarified[9].

Some studies have focused on the association between HLA DRB1 and ACPA, however, studies evaluating its association with HLA DQB1 are scarce. Moreover, studies evaluating the impact of HLA DRB1/DQB1 alleles on extra-articular manifestations (EAM) are rare. To our knowledge, only three studies have investigated the association between HLA and RA in Tunisia without focusing on the relationships of ACPA and EAM with HLA DRB1/DQB1 alleles[10–12]. Therefore, we conducted this study to evaluate the association of HLA DRB1/DQB1 alleles with RA, and to investigate their relationship with ACPA and RA severity.

2. Materials and methods

2.1. Patients and study design

We conducted a case-control study of 81 patients meeting the ACR/EULAR 2010 classification criteria for RA[13]. RA associated with any other autoimmune diseases were excluded.

Patients were compared with 100 healthy controls, matched for age and sex unrelated, and with no family or personal history of chronic inflammatory arthritis or any other disease known to be associated with HLA class II alleles.

All patients and controls were Tunisian. Tunisia's population is relatively heterogeneous, considered to be ethnically Arab, North African, Berber and Caucasian[14].

All patients and controls agreed to participate in this study. This study was approved by our local ethics committee at the Habib Thameur University Hospital in Tunis.

2.2. Outcome

The primary outcome was the association of HLA alleles DRB1 and DQB1 with RA. Secondly, the links with ACPA and RA severity.

2.3. Data gathering

For each patient, sociodemographic data including age, gender, age at RA onset and disease duration were assessed at inclusion.

Disease activity was assessed with the 28-joint disease activity score using C-reactive protein (DAS28-CRP)[15]. The health assessment questionnaire (HAQ) and sharp score were used to assess functional impact and structural damage, respectively[16,17].

We also looked for extra-articular manifestations of RA, including rheumatoid nodules, vasculitis, ocular, cardiac, pulmonary, renal, neurological, hematological, and gastrointestinal involvement, secondary Sjögren's syndrome and osteoporosis. The treatments used for each patient were also detailed.

The severity of rheumatoid arthritis was assessed on the basis of the disease activity score (DAS), functional impact using the HAQ score, structural damage according to the sharp score, and the presence of extra-articular manifestations[18–20].

RF isotypes were measured using a latex agglutination test according to the manufacturer's instructions (Biosystems SA, Barcelona, Spain). RF was considered positive above 20 IU/ml. ACPA were determined by

enzyme-linked immunosorbent assay (ELISA) (EuroImmun Medizinische Labordiagnostika, Germany). Serum samples tested at 5 IU/mL were considered positive[21].

Genomic DNA was extracted from peripheral venous blood using the QIAampR DNA Blood Mini *Kit* (Qiagen GmbH, Hilden, Germany). Low-resolution HLA class II DRB1/DQB1 genotyping was performed via the single-specific primer polymerase chain reaction (SSP-PCR) method, using *Micro SSP™ DRB/DQB DNA Typing Trays* (One Lambda, Canoga Park, LA). High-resolution specific genotyping was performed in subjects carrying the homozygous or heterozygous HLA DRB1*04 allele, using the *Micro SSP™ Specific DRB1*04 DNA Typing Tray* (One Lambda).

3. Statistical analysis

Frequency calculations were performed using a filtering machine available in Excel 2010 for Windows. All statistical analyses were performed using the Statistical Analysis System (SAS) software.

Comparison of quantitative variables was performed using the Student-t-test, and the Mann-Whitney test when the data were not normally distributed.

Percentage comparisons of independent series were performed by the Pearson chi-square test, and in case of invalidity, by the Fisher test.

Logistic regression analysis including variables with $p < 0.2$ in univariate analysis was performed to identify factors associated with HLA DRB1/DQB1 alleles.

The significance level for all statistical tests was set at $p < 0.05$.

4. Results

A total of 81 RA patients were evaluated. The sex ratio (F/M) was 4.78. The main characteristics of these patients and controls are presented in **Table 1**.

Table 1. Main characteristics of RA patients.

	RA patients (81 patients)	**Controls (100 patients)**
Age (years ± SD)	49.17 ± 11.21	46.2 ± 11.8
Women (%)	82.71	77.5
Mean disease duration (years ± SD)	7.44 ± 2.12	-
Seropositive RA (%)	80.24	-
ACPA positive RA (%)	71	-
DAS28 CRP (mean ± SD)	3.9 ± 1.27	-
HAQ (mean ± SD)	0.82 ± 0.67	-
Sharp score (mean ± SD)	20.90 ± 13	-
Extra-articular manifestations (N)	51	-
Secondary Sjögren's syndrome (N)	41	-
Pulmonary involvement (N)	8	-
Rheumatoid nodules (N)	3	-
Osteoporosis (N)	19	-
Renal involvement (N)	2	-

RA: rheumatoid arthritis, ACPA: anti-citrullinated peptide antibodies, DAS: disease activity score, HAQ: health assessment questionnaire, N: number, SD: standard deviation.

RF-positive RA patients accounted for 80.3% of the study population. ACPAs were positive in 71% of patients, 66.1% of whom had polyarticular involvement.

4.1. HLA DRB1 allele assessment

Forty-six patients (79%) expressed SE alleles (33 patients expressed HLA DRB1*04, 3 HLA DRB1*10, and 16 HLA DRB1*01). These alleles were significantly more frequent in RA patients than in controls (5.28 vs. 3.15%, $p = 0.03$).

HLA DRB1*04:05 was the most strongly associated allele with RA susceptibility (20.37% vs. 10% in controls, $p = 0.01$). However, the frequency of DRB1*16:01 was significantly lower in RA patients compared to controls (0.61% vs. 4%, $p = 0.04$) (**Table 2**).

Table 2. Comparison of frequencies of HLA DRB1/DQB1 alleles in patients and controls.

Alleles		RA patients (81 patients)		Controls (100 patients)		*p*
		Number of alleles	Frequency	Number of alleles	Frequency	
DRB1 alleles	DRB1*01:01	16	9.87	21	10.5	0.18
	DRB1*03:01	19	11.72	24	12	0.75
	DRB1*03:02	10	6.16	9	4.5	0.57
	DRB1*04:01	33	20.37	20	10	0.01
	DRB1*07:01	15	9.25	24	12	0.29
	DRB1*08:01	2	1.23	4	2	0.51
	DRB1*09:01	4	2.47	1	0.5	0.12
	DRB1*10:01	3	1.85	2	1	0.53
	DRB1*11:01	12	7.40	24	12	0.24
	DRB1*11:02	1	0.61	0	0	-
	DRB1*12:01	3	1.85	6	3	0.42
	DRB1*13:01	16	9.87	25	12.5	0.19
	DRB1*14:01	4	2.47	5	2.5	0.83
	DRB1*15:01	22	13.58	27	13.5	0.82
	DRB1*15:02	1	0.61	0	0	0.46
	DRB1*16:01	1	0.61	8	4	0.04
DQB1 alleles	DQB1*02:01	40	24.69	52	26	0.2109
	DQB1*03:01	30	18.51	45	22.5	0.0833
	DQB1*03:02	30	18.51	17	8.5	0.044
	DQB1*03:03	2	1.23	5	2.5	0.2568
	DQB1*04:01	8	4.94	14	7	0.2008
	DQB1*05:01	22	13.58	28	14	0.1790
	DQB1*05:02	1	0.62	0	0	-
	DQB1*06:01	28	17.28	39	19.5	0.179

RA: rheumatoid arthritis.

Among the 64 RF-positive patients, 35 (54.5%) expressed the DRB1*04:05 allele with a highly significant correlation (20.37 vs. 10%, $p < 10^{-3}$). Four patients expressing this allele were homozygous. Similarly, a highly significant association with DRB1*15:01 allele ($p < 10^{-3}$) and RF positivity was found, followed by DRB1*03:01, 13:01, and 11:01.

There was no statistically significant difference between patients encoding for SE alleles and those who didn't, regarding disease activity, HAQ score, and sharp score. However, patients with SE alleles had more

EAM ($p < 10^{-3}$). Most of them had single doses for the SE allele (48.14%). Considering each EAM separately, we found no statistically significant associations with SE alleles (**Table 3**).

Table 3. RA severity and shared epitope.

	SE+	SE−	*p*
Sjogren's syndrome+	23	21	0.1
Pulmonary involvement	6	1	0.3
Osteoporosis	9	3	0.6
FR+	40	26	0.24
ACPA+	33	25	0.69
DAS28 ≥ 5.1	33	25	0.34
HAQ ≥ 0.5	13	5	0.13

SE: shared epitope, RF: rheumatoid factor, ACPA: anti-citrullinated peptide antibodies, DAS: disease activity score, HAQ: health assessment questionnaire.

Furthermore, HLA DRB1*01:01, 04:05, 11:01, and 15:01 were strongly associated with the presence of ACPA ($p < 10^{-3}$). HLA DRB1*07:01 and 13:01 were also associated with ACPA positivity ($p = 0.002$) (**Table 4**). In addition, all 4 homozygous DRB1*04:05 carriers were ACPA positive.

Table 4. HLA DRB1 and DQB1 alleles and ACPA status in rheumatoid arthritis patients.

	Number of alleles	ACPA−	ACPA+	*p*
DRB1	162	46	116	-
01:01	16	3	13	0.0018
03:01	19	10	9	< 0.0001
03:02	4	2	2	0.333
04:05	39	12	27	< 0.0001
07:01	15	3	12	0.002
08:01	2	2	0	-
09:01	4	0	4	-
10:01	3	1	2	0.333
11:01	13	4	9	0.0014
11:02	1	-	1	-
12:01	3	0	3	-
13:01	16	3	13	0.002
14:01	4	1	3	0.25
15:01	22	5	17	< 0.0001
16:01	1	-	1	-
DQB1	162	49	113	-
02:01	40	12	28	< 0.0001
03:01	31	8	23	< 0.0001
03:02	25	8	17	< 0.0001
03:03	4	4	-	-
04:01	7	5	2	0.047
05:01	27	6	21	< 0.0001
06:01	28	6	22	< 0.0001

ACPA: anti-citrullinated peptide antibodies.

Multivariate analysis concluded that the HLA DRB1*04:05 allele was only associated with ACPA positivity (OR = 1.851, $CI_{95\%}$ [0.586–5.842]). Twenty ACPA-positive patients expressed the HLA DRB1*04:05 allele. Their mean DAS28 was 3.49 ± 1.12 [1.2–5.8]. Compared with ACPA-negative patients, they had predominantly polyarticular involvement (68.75% vs 48.48%, $p < 10^{-3}$), more bone involvement (29.16% vs 15.15%, $p < 10^{-3}$), and more impaired HAQ (0.9 ± 0.65 vs 0.86 ± 0.31, $p < 10^{-3}$). However, there was less structural progression considering the sharp score: 20.14 ± 0.41 vs 21.27 ± 25.13, but with no significant difference ($p = 0.16$).

Conventional synthetic antirheumatic drugs (csDMRAD) were used in all patients. The most effective was methotrexate (MTX). Thirty patients were treated with biologics in combination with MTX. Among these patients, 16 expressed SE alleles: 12 expressed DRB1*04:05, including one homozygous patient, and four DRB1*01:01.

4.2. HLA DQB1 allele assessment

Regarding HLA DQB1, HLA DQB1*03:02 was significantly the most associated allele with RA (63.83% vs 36.17% in controls, $p = 0.044$) (**Table 2**).

HLA DQB1*02:01, 03:01, 05:01, and 06:01 were associated with RF-positive RA. HLA DQB1*03:02 was also associated with RF-positive RA, but with a less significant association. HLA DQB1*06:01 was associated with the presence of ACPA ($p = 0.0588$) (**Table 5**).

Table 5. RA severity and HLA-DQB1 alleles.

DQB1	Without EAM (%)	With EAM (%)	*p*	RF− (%)	RF+ (%)	*p*	ACPA− (%)	ACPA+ (%)	*p*	DAS28 G1 (%)	DAS28 G2 (%)	*p*	HAQ G1 (%)	HAQ (%)	*p*
02:01	27.5	72.5	**0.0044**	12.5	87.5	**<0.0001**	40	60	0.2059	85	15	**< 0.0001**	40	60	0.2059
03:01	40	60	0.2733	16.67	83.33	**0.0003**	43.33	56.67	0.4652	93.33	6.67	**< 0.0001**	30	70	**0.0285**
03:02	53.33	46.67	0.7150	30	70	0.0285	60	40	0.2733	70	30	0.0285	16.67	83.33	**0.0003**
03:03	50	50	1	50	50	1	100	0	-	100	0	-	0	100	-
04:01	25	75	0.1573	37.50	62.50	0.4795	25	75	0.1573	75	25	0.1573	0	100	-
05:01	40.91	59.09	0.3938	13.64	86.36	**0.0006**	36.36	63.64	0.2008	72.73	27.27	0.0330	4.55	95.45	**< 0.0001**
05:02	0	100	-	0	100	-	0	100	-	100	0	-	0	100	-
06:01	25	75	**0.0082**	25	75	**0.0082**	32.14	67.86	**0.0588**	78.57	21.43	**0.0025**	21.43	78.57	**0.0025**

EAM: extraarticular manifestations, RF: rheumatoid factor, ACPA: anti-citrullinated peptide antibodies, DAS: disease activity score, DAS28 G1 = DAS28 < 5.1, DAS28 G2 = DAS28 ≥ 5.1, HAQ: health assessment questionnaire, HAQ G1 = HAQ < 0.5, HAQ G2 = HAQ ≥ 0.5.

Furthermore, only HLA DQB1*02:01 and 06:01 alleles were significantly associated with the presence of EAM ($p = 0.004$ and 0.008 respectively). DAS28 was significantly reduced in the presence of DQB1*02:01, 03:01 and 06:01 alleles ($p < 10^{-3}$). This association was less significant for DQB1*0302 ($p = 0.029$) and 05:01 ($p = 0.033$). As for HAQ scores, scores >0.5 were significantly correlated with HLA alleles DQB1*03:01, 03:02, 05:01, and 06:01 (**Table 5**).

In addition, there was more structural damage in RA encoding for HLA DQB1*03:01($p = 0.01$), 03:02 ($p < 10^{-3}$), 04:01 ($p = 0.033$), and 06:01($p < 10^{-3}$).

In linear regression, HLA DQB1*06:01 ($B = 0.313$, $p = 0.016$, $IC_{95\%}$ [0.06–0.567]) and 03:03 ($B = -0.711$, $p = 0.014$, $IC_{95\%}$ [−1.274–0.147]) were associated with RF positivity, but there was no significant association with the other parameters.

5. Discussion

Despite the small number of patients involved, our study is a preliminary one, enabling us to identify certain risk factors for the susceptibility and severity of RA in Tunisia. We looked for associations between HLA DRB1 alleles and clinical manifestations, immunological status, and structural damage in RA. We also sought associations between HLA DQB1 alleles and disease severity.

Genetic associations between genes located in the HLA zone and susceptibility to RA were suspected as early as 1976[22,23]. A few years later, the existence of an association between the regional genes encoding the HLA DRB1 alleles DR4 and DR1 was demonstrated[24,25]. Since then, this association has been reported in multiple studies in several different regions, including the SE alleles: DRB1*04, DRB1*01, DRB1*10, and DRB1*14[26–30]. Furthermore, over 100 additional alleles contributing to disease risk and involving immune pathways have been identified[3].

In the current study, we were able to correlate RA with the presence of SE alleles ($p = 0.036$); the HLA DRB1*04:05 was the most frequent allele (24%) ($p = 0.01$). Our findings were in line with three previous Tunisian studies[10–12]. In addition, Bizzari et al. showed in a meta-analysis that there was an association between the presence of HLA DRB1 alleles (DRB1*04, *10) and an increased risk of RA in the Arab ethnic group (OR > 2; $p < 0.0001$)[31]. In these populations, DRB1*04:05 and 04:04 are also involved in RA predisposition. Furthermore, their frequency is intermediate between southern Mediterranean and northern European populations.

On the other hand, unlike patients from Northern Europe and the Mediterranean, HLA DRB1*01 was not associated with RA in our study, nor in the study by Dhaouadi et al.[10,32,33]. This could be attributed to the low prevalence of this allele in the Tunisian population.

Additionally, Liu et al, in a systemic review and meta-analysis of 40 Asian studies involving 5470 RA patients and 5837 controls, found that the frequencies of HLA DRB1*04 and *10 were higher in RA patients, and that the frequency of DRB1*14 was lower in RA patients than in controls. Pooled odds ratios revealed associations of *01:01 ($OR = 1.58$), *04:01 ($OR = 2.17$), *04:10 ($OR = 2.24$) and *10:01 ($OR = 1.78$) with RA. However, HLA subtype DRB1*14 showed no association with RA[34].

The incidence of DRB1*16:01 in our study was significantly lower in the RA group ($p = 0.015$). This result agreed with that of Castro-Santos et al, where no patient in the RA group expressed this allele[35]. This suggests the protective effect of this allele in RA.

In the same context, some authors have studied the protective role of HLA DRB1 allele in RA. The protective effect was mainly linked to the DERAA sequence (D = aspartic acid, E = glutamic acid, R = arginine, A = alanine) of amino acids in position 70–74 of DRB1, this sequence was identified in several alleles (*01:03, *11:02, *11:03, *13:01, *13:02, *13:04 and *04:02). The mechanism of this protection is not well

elucidated[36–38]. A meta-analysis of four European trials highlighted the protective impact of the DRB1*13 allele, and that of all DRB1*13 alleles, only DRB1*13:01 was identified as protective against ACPA-positive RA[39]. A protective role for DRB1*13:02 was confirmed in the Japanese study by Oka et al.[40]. Similarly, in a recent study by Maurits et al., DRB1*13:01 and *13:02 were more frequent in healthy controls and increasingly less frequent in RA patients[41]. Moreover, a recent Latin American study showed that DRB1*07:01 and *08:02 were associated with a reduced risk of ACPA-positive RA ($p < 10^{-3}$ and $p < 0.01$, respectively)[35]. On the other hand, the importance of the DRB1*04:02 and DRB1*03 alleles remains controversial. The latter two had been identified as having a protective effect on RA by some authors, but others denied this effect[36].

ACPA is a prognostic indicator for the development of aggressive RA[42]. In our study, HLA DRB1*04:05, *03:01, *15:01 were significantly associated with the presence of ACPA in RA. Furthermore, ACPA-positive RA was predominantly polyarticular, with more bone involvement and a more impaired HAQ. Literature data concerning this association are contradictory, as genetic and environmental backgrounds differ from one population to another. While some studies have shown an association between HLA DRB1 and ACPA positivity[43–48], others have shown that ACPA-negative RA is also associated with HLA DRB1 alleles[49–51]. Recently, an Egyptian case-control study of 157 RA patients and 150 controls investigated the association between the SE encoding HLA DRB1 and ACPA and found that this association involved both positive and negative ACPA[52]. The risk of developing positive ACPA isotypes was higher in the presence of 2 copies of SE alleles[52]. Similarly, other studies had confirmed this association[46,53,54]. Kissel et al even reported that SE was associated with glycosylation of the V domain of ACPA-IgG in pre-symptomatic individuals, reinforcing the link between HLA SE and the development of ACPA-positive RA[55].

Moreover, the study by Charpin et al showed that there was no significant difference in ACPA between SE-negative patients and patients expressing at least one SE ($p = 0.140$). Similarly, no statistical difference in ACPA was found between patients expressing one or two SE[56]. This suggests that SE may play a role in the development of ACPA-positive RA rather than in the development of ACPA itself.

One of the highlights of our study was the assessment of the HLA DQB1 allele. We found that the DQB1*03:02 allele was the most associated with RA, RF, and ACPA positivity, DAS28, HAQ, and sharp score. Similarly, in a Moroccan study, HLA DQB1*03:02 was associated not only with RF-positive RA, but also with the clinical and radiological severity of the disease[57]. This similarity might be explained by the common ethnic origin of the two populations, which confirms our results despite the small sample size. In addition, some other authors have concluded that HLA alleles DQB1*03:02 and 05:01 are associated with ACPA positivity in RA[58,59].

Likewise, in our study, as in the aforementioned studies, we found a significant association between ACPA-positive RA and HLA DQB1*03:01; 03:02, and 05:01. HLA DQB1*02:01 and 06:01 were also correlated with ACPA-positive RA. Nevertheless, in some studies, the presence of DQB1*02:01 and 06:03 was inversely correlated with the presence of ACPA in RA[60]. A meta-analysis of 15 studies involving 1,250 cases and 1,621 controls also concluded that DQB1*02 and DQB1*06 may be negatively associated with RA[61]. In addition, a recent study showed that the HLA DQB1*03:02 allele was inversely related to the risk of developing ACPA-positive RA in Malaysians patients[47]. These discrepancies can be attributed to ethnic diversity.

Besides studying the impact of SE on ACPA positivity, we focused on the impact of SE on EAM. To our knowledge, studies dealing with this subject are scarce. Patients expressing SE alleles had more EAM in our series ($p < 10^{-3}$). However, when considering each EAM separately, we found no statistically significant correlation with SE alleles. In addition, patients with SE alleles had more severe RA requiring the use of biologics. Our results also suggest that SE allele, in particular the HLA allele DRB1*04:01, are a risk factor

for disease severity. Our results were not in agreement with other authors who found no correlation between HLA DRB1 and disease severity[45,58,62], particularly EAM[56,63].

Thirty patients required treatment with biologics in combination with MTX. Sixteen of these had SE alleles. The severity of the disease required more sustained treatment in these patients. This was in line with literature data stipulating, in addition to an association of SE alleles with disease susceptibility, an association with radiological severity, mortality, and even response to treatment[64–66]. A recent Korean study, similarly, showed that the presence of SE affected disease characteristics and prognosis in Korean RA patients. However, it had no significant impact on the survival rate of TNF inhibitors and abatacept[67].

In addition to ethnic and geographical diversity, these discrepancies might be explained by differences in sequencing techniques and methodologies, as well as by differences in patient selection. Further studies with larger samples of different ethnic origins are therefore recommended to support or refute our findings.

Our study was limited by the small size of the population and the fact that we had not assessed the response to the various DMARDs (especially biologic ones). Nevertheless, it might be considered a preliminary study that needs to be extended to a larger number of patients in order to confirm its results.

In this study, we confirmed that HLA DRB1 and DQB1 alleles were associated with increased severity of RA. SE allele expression was significantly associated with the presence of ACPA, increased HAQ score, increased sharp score, and the presence of EAM.

Author contributions

Conceptualization, SB and HT; methodology, SB; validation, SB, AL, EG, SK and SR; investigation, SB; resources, SB, AL and EG; writing—original draft preparation, SB and HT; writing—review and editing, SB and HT; visualization, SB, YM, MH, AL, EG, SK and SR; supervision, SB, AL, EG, SK and SR. All authors have read and agreed to the published version of the manuscript.

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Author agreement

I, Houssem TBINI, hereby certify that all named authors have seen and approved the final version of the manuscript being submitted. I on behalf of all authors warrant that the article is the authors' original work, hasn't received prior publication, and isn't under consideration for publication elsewhere.

I confirm that the order of authors listed in the manuscript has been approved by all of them. Authors understand that the Corresponding Author is the sole contact for the Editorial process. He is responsible for communicating with the other authors about progress, submissions of revisions, and final approval of proofs.

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethical approval

The local ethics committee at Habib Thameur University Hospital in Tunis has approved the study.

Informed consent

Informed consent was obtained from all individuals included in this study.

Conflict of interest

The authors declare no conflict of interest.

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